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PISONIA CALAFIA (NYCTAGINACEAE) SPECIES NOVA FROM THE BAJA CALIFORNIA PENINSULA, MEXICO

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ABSTRACT

Pisonia is a primarily American genus, distributed mainly in subtropical to tropical regions. In this paper a new species, *Pisonia calafia,* is described and documented. This species occurs as a small population on a coastal strand in the Cape Region of the Baja California Peninsula. The main characteristics that differentiate *P. calafia* from the rest of *Pisonia* is the lack of sulci and glands in the fruit. In addition to the new species description, we discuss the evolutionary affinities of this new taxon based on phylogenetic analysis of nrITS sequence data. The known population is limited to a few dozen individuals, now seriously endangered by the ongoing development of tourism projects.

Key words: East Cape Region, Nyctaginaceae, *Pisonia*, phylogeny.

RESUMEN

El género *Pisonia* es primordialmente americano, se distribuye principalmente en regiones subtropicales y tropicales. En este trabajo se describe y documenta la nueva especie *Pisonia calafia*. Este taxon consiste de una relativamente pequeña población en la costa de la región de Los Cabos en la península de Baja California. La principal característica que diferencia a *P. calafia* del resto de las especies de este género es la carencia de sulcos y glándulas en el fruto. En adición a la descripción morfológica, se documenta la afinidad evolutiva del nuevo taxon con base en el análisis filogenético de la secuencia de nrITS. Las poblaciones conocidas consisten de unas pocas docenas de individuos, seriamente amenazados por el desarrollo de proyectos turísticos en la zona.

Palabras clave: Cabo del Este, filogenia, Nyctaginaceae, *Pisonia.*

Personnel of the herbarium at CIBNOR have been collecting plants in several areas of the Baja California Peninsula as part of their mission to compile a catalogue of the coastal flora. In 1997, an isolated vegetative specimen of a shrub (*M. Domínguez 1694, 1771*) was found and identified tentatively as *Pisonia* sp., since the leaves were somewhat similar to plants of this genus; however, they did not match the two species of *Pisonia* that are known from Baja California: *P. flavescens* Brandegee and *P. capitata* (S. Wats.) Standl.

In the summer of 2009, a population of 30-40 plants of this still unknown species was located. This population was visited regularly until flowers and fruits were available for collecting and complete identification. Close morphological study revealed that both flower perianths, staminate (campanulate) and pistillate (urceollate), as well the inflorescences in headlike cymes, show affinity with *Pisonia*. However, compared to other described *Pisonia* species, the anthocarp anatomy is somewhat different, as the fruits are not ribbed (slightly sulcate when immature) and lack glands or warts. Another notable difference is that the fruit-supporting structures (peduncle and pedicels) are shorter than the same structures in the other peninsular species of *Pisonia.* Given these distinctive characteristics, the authors believe that this population should be considered a new species.

Pisonia calafia León de la Luz et Levin sp. nova (Fig. 1).

Frutex dioecius, confertus, spinescens, foliis ellipticis. Flores staminati 6-lobulati; flores pistillati in inflorescentiis densibus dispositi, 4-lobulati. Fructus dense velutinus, non glandularis, indehiscens, 1-seminatus, interne cotyledonibus plicatis.

Dense dioecious shrubs, erect, up to 4 m high, developing some stems from rootstocks, stems and branchlets grayish, secondary branchlets spreading in an opposite pattern at right angle; strong and straight spines 10 mm long, almost opposite in the new branchlets, absent in old stems. Leaves elliptic to broadly elliptic, 14 × 20 mm long, some cuneate at base, petioles 4-7 mm long in mature leaves, indumentum short velutinous when young, pubescent with age. Staminate inflorescences capitate, 10-12 flowered in a single raceme, peduncle to 10 mm long, pedicels up to 1 mm long; perianth campanulate, 6 mm long, 6-lobed, each lobe 1 mm long; stamens 6 alternate with perianth lobes, anthers bilocular, dorsifixed, elliptic < 1 mm diameter,

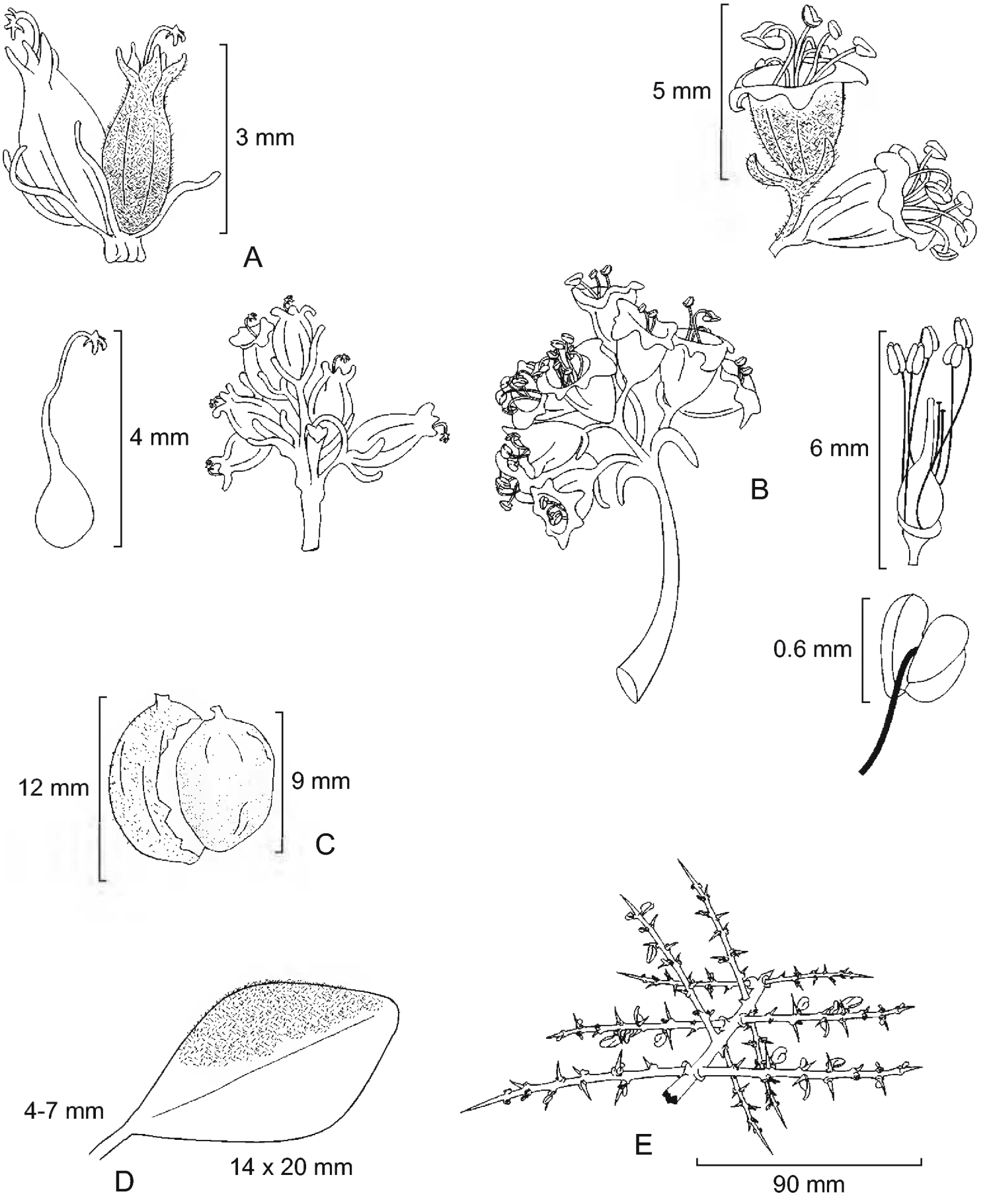

Fig. 1. *Pisonia calafia* León de la Luz et Levin. A. Female inflorescence and details; inflorescence raceme with six flowers, female flower showing pubescence pattern, ovary; B. Male inflorescence with eight flowers and details; flower detail and pubescence, stamen detail and pistillode, anther detail; C. Fruit and seed; D. Leaf and pubescence; E. Branching pattern in young stem. Illustrated by Danira León Coria.

extrorse, generally 1 or 2 reduced to staminodes; the fine filaments connate basally, borne at the base of a pistillode 3 mm long; lower part of the perianth reduced to 2 or 3 small involucral bracts; indumentum densely short velutinous. Pistillate inflorescences capitate, arranged in 2-5 racemes, each with 4-7 flowers, peduncles to 20 mm long, congested in anthesis, expanded after fertilization, pedicels short < 1mm long; involucre well differentiated 1-2 mm long, sacciform to urceolate in shape, very thick, with 4-5 lanceolated lobes < 1 mm long, bicolor, the basal half darker than the upper; stigma somewhat exserted, lobed and decurrent; lower part of the perianth consists of 4 lanceolate involucral bracts 1 mm long; indumentum densely short velutinous; no staminodes are present. Anthocarp, diclesium, or fruit elliptic, 10-12 × 7-8 mm, rounded at base, finely velutinous, coriaceous, indehiscent, with several parallel longitudinal lines, suggesting ancestral ribs; each fruit one-seeded, seed pisiform, 6-7 mm in diameter, black. In the plantlets, cotyledons unfold from a conduplicate position.

Type: Mexico. Baja California Sur: delegación La Ribera, Laguna Grande, Las Lagunas, municipio de Los Cabos. 2.7 km al W del faro de Punta Arena, por la carretera costera de terracería, km 11 entre La Ribera y Cabo Pulmo, 23°32'01.72" N, 109°29'40.88" W, 2 June 2011, *J. L. León de la Luz 11167* (holotype: HCIB 26596 (male), 26597 (female), isotypes to be distributed, CAS, ENCB, IEB, MEXU, RSA, SD, UC) .

Eponymy. The species epithet *calafia* was selected in honor of the beauty queen of the mythic California island, according to the book "Las sergas de Esplandián" written ca. 1510 by Garci Rodríguez de Montalvo, that supposedly inspired the conqueror Hernán Cortés to use the same name for the then recently discovered land (ca. 1535).

Related species. The mainly tropical genus *Pisonia* includes approximately 40 species, with the greatest species-richness in the Americas (Mabberley, 1997). Only two species of *Pisonia* are previously known to inhabit the Baja California Peninsula: *P. capitata* and *P. flavescens*. The former occurs in the Sierra de la Giganta, a hundred kilometers from the type location of this new taxon. However, this species is more common in the dry tropical forest of Sonora, mainland northwestern Mexico (Spellenberg, 2003). By contrast, *P. flavescens* is endemic to the tropical dry forest in the Cape Region of Baja California Sur. Table 1 shows the characteristics that can be used to differentiate

Table 1. Morphological traits that differentiate the *Pisonia* species of the Baja California Peninsula.

Species	*Pisonia flavescens*	*Pisonia capitata*	*Pisonia calafia*
Spines	Mainly spineless or rarely with stout straight spines 5-6 mm long.	Armed with recurved spines 7-14 mm long, rarely absent.	Armed with recurved spines 10 mm long, absent in old branches.
Leaves	Leaves oblanceolate, 30 × 50-60 mm long, narrowly acute to acuminate at base, mostly glabrous, slightly hairy beneath, mostly along veins.	Leaves broadly obovate, 30 × 50 mm long, rounded to broadly cuneate at base, sparsely short-villous above, densely villous below but becoming glabrate.	Leaves elliptic to broadly elliptic, 14 × 20 mm long, some cuneate at base, indumentum short velutinous when young, absent at age.
Staminate flowers	Staminate inflorescence of congested cymules appearing capitate, 10 mm diameter, densely puberulent, peduncle 8-10 mm long, many-flowered, stamens somewhat exserted.	Staminate inflorescence dense, puberulent, appearing capitate, 10 mm diameter, peduncle 10-15 mm long, many flowered, pedicels very short, and stamens well exserted.	Staminate inflorescence capitate, peduncle to 10 mm long, 10-12 flowered, short pedicellate; stamens exserted, indumentum densely short velutinous.
Pistillate flowers	Pistillate inflorescence capitate, 10 mm diameter, densely puberulent, peduncle stout to 20 mm long, 15-25 flowered, campanulate, each 2-3 mm long, subtended by small bractlets.	Pistillate inflorescence trumpet-shaped, densely puberulent, 10-20 flowered, each 2-2.5 mm long, subtended by a single bractlet.	Pistillate inflorescence capitate, short velutinous, 8-35 flowered in several racemes, subtended by 4 bracts, each 1 mm long, peduncles to 20 mm long.
Fruits	Fruit 8-10 mm long, angles with low, stout, stipitate glands.	Fruit 7-10 mm long, clavate, angles with rows of heavy stipitate glands, interspaces puberulent.	Fruit elliptic, to 10-12 mm, rounded at the base, finely velutinous, not ribbed nor having glands when mature.
Fruit supporting structure (peduncle and pedicels)	20-30 times longer than unpollinated flowers.	20-30 times longer than unpollinated flowers.	6-10 times longer than unpollinated flowers.

between the Baja California *Pisonia* species, including this new taxon. Images of *P. calafia* are shown in Fig. 2.

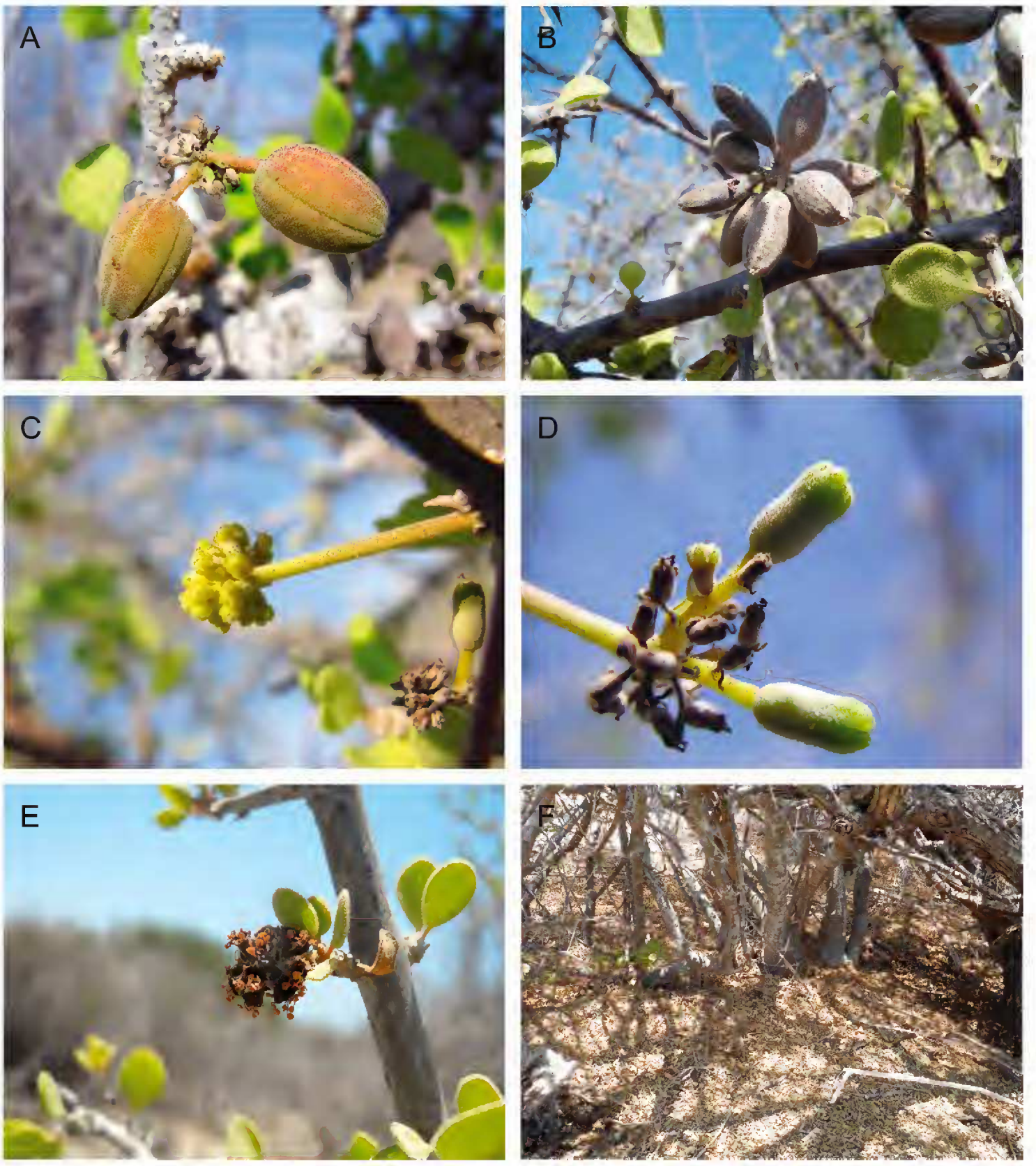

Fig. 2. *Pisonia calafia* León de la Luz et Levin. A. Immadure fruits showing vestigial sulci; B. Mature fruits without sulci; C. Immadure female inflorescence; D. Accrescent female flowers after fertilization; E. Male inflorescence; F. Basal stems.

Evolutionary affinities. To understand the evolutionary affinities of *Pisonia calafia*, we used the internal transcribed spacer region of nuclear ribosomal DNA, composed of ITS1, the 5.8S gene, and ITS2 (Baldwin, 1992; Baldwin et al., 1995). ITS is commonly employed in fine-scale studies of angiosperm relationships, and this region has been used for understanding relationships among closely-related species within Nyctaginaceae (Levin, 2000; Douglas and Manos, 2007). This region was amplified for one accession of *Pisonia calafia* (*J. L. León-de la Luz 11167*, type location), as well as an accession of *P. flavescens,* (León de la Luz s/n., Sierra Cacachilas).

Amplification was done using primers ITSleu1 (5′-GTC CAC TGA ACC TTA TCA TTT AG-3′; Bohs and Olmstead, 2001) and ITSc26a (5′- GTT TCT TTT CCT CCG CT-3′; Wen and Zimmer, 1996). Twenty-five microliter reactions contained 1X buffer, 3.0 mM $MgCl_2$, 0.20 mM dNTPs, 0.40 μM of each primer, 1X Qiagen Q-solution (Qiagen, Valencia, CA), 0.625 units Taq polymerase, and 1μL DNA. Thermal cycler conditions included an initial denaturation at 94 °C for 3 min; 8 cycles at 94 °C for 30 s, 58 °C down to 52°C (decreasing 2 °C every two cycles) for 1 min, 72 °C for 1 min; 25 cycles at 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min; ending with an extension at 72 °C for 10 min. PCR products were cleaned and sequenced following Levin et al. (2011) with the same primers used for amplification.

The two new sequences (*Pisonia calafia* and *P. flavescens*) were cleaned using Sequencher 4.7/4.8 (Gene Codes, 1991-2007). Consensus sequences from each of these species were manually aligned (SeAl v2.0a11 (Rambaut, 1996-2002)) to a larger data set of 18 taxa from within *Pisonia* and across Nyctaginaceae. The family-wide phylogenetic study of Douglas and Manos (2007) guided the choice of taxa to include. ITS sequences for these additional taxa were available from GenBank (accessions: EF079454-5, EF079463, EF079475-6, EF079484-6, EF079489-90, EF079493, EF079495-6, EF079498, EF079502, EF079505, DQ317077, AF212015).

The ITS dataset was analyzed using maximum likelihood, with substitution model parameters estimated using the Akaike Information Criterion in Modeltest 3.7 (Posada and Crandall, 1998). Maximum likelihood nonparametric bootstrap analysis was conducted using the estimated model parameters, 500 full heuristic bootstrap replicates, each with 10 random-addition sequence replicates and TBR branch-swapping; the MulTrees option was disabled. Maximum likelihood bootstrap analyses were conducted using PAUP* 4.0b10 for UNIX (Swofford, 2002) on the Condor (Anonymous, 2005) computer cluster at Amherst College. Bootstrap replicates were parsed for processing using RepMaker (Wilgenbusch, 2003). Given the results of Douglas and Manos (2007), *Salpianthus arenarius* was defined as the outgroup.

As shown in Fig. 3, our analysis strongly suggests that the new taxon belongs within *Pisonia*. The genus *Pisonia* (excluding *P. rotundata*) is well supported as monophyletic (Bootstrap (BS) = 99) . Within this clade, the pantropical *P. aculeata*

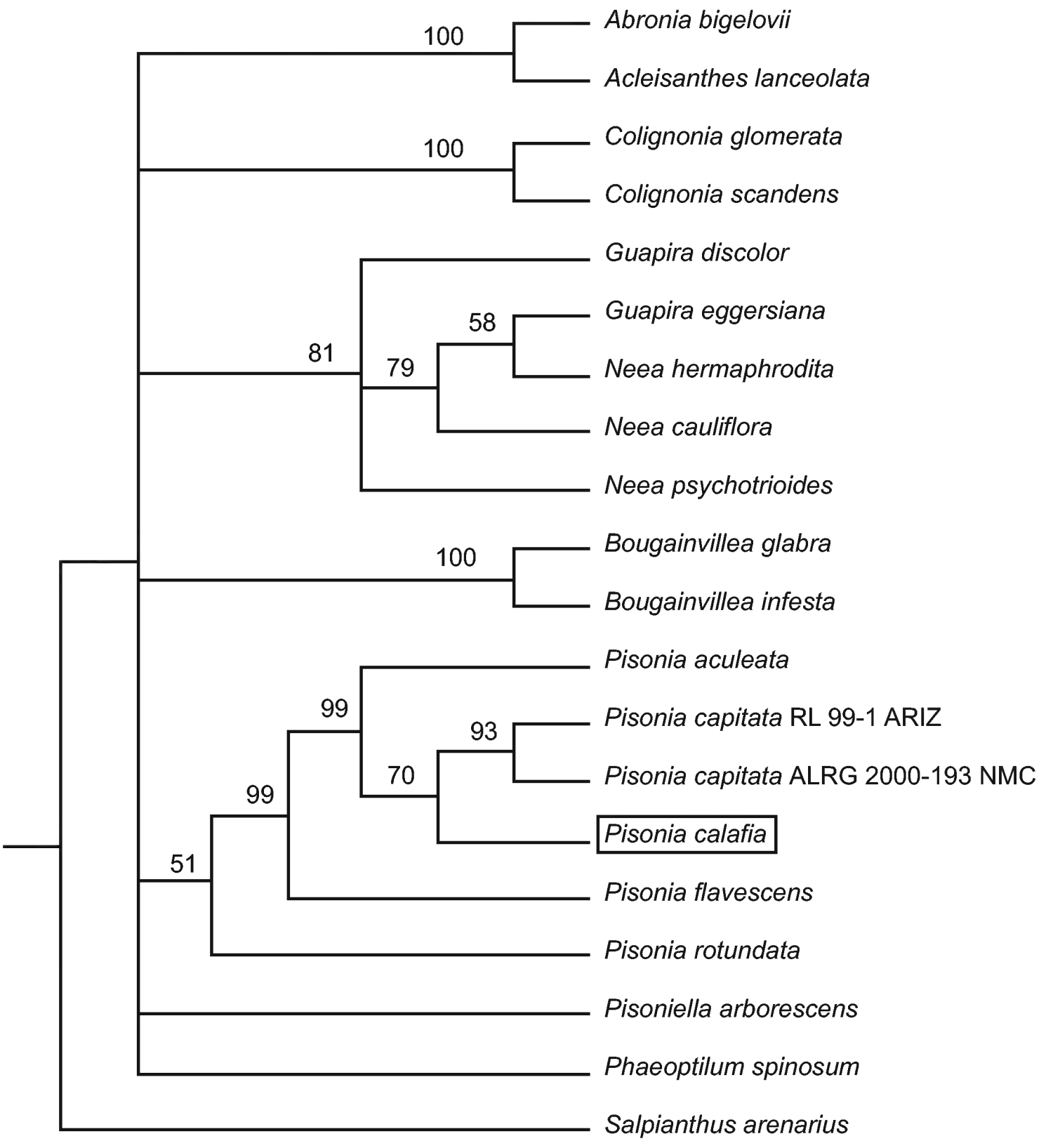

Fig. 3. The 50% majority-rule bootstrap consensus tree inferred from nrITS data to differentiate the two accessions of *Pisonia capitata*, herbarium voucher information is listed after the species name.

+ *P. capitata* + *P. calafia* are strongly supported as monophyletic (BS = 99), and *P. calafia* appears sister (BS = 70) to *P. capitata*. Although the new taxon is clearly within *Pisonia*, increased sampling of *Pisonia* species is needed to conclusively determine the closest relatives of *P. calafia*.

Ecology. From our field observations, some plants begin to develop flowers in June, usually the driest month, but most of the plants begin blooming just after the first heavy rain in August or September. Mature fruits were collected in November-January. At the location where 30-40 specimens were identified, no plantlet recruitment was observed. This location is at the end of a micro-basin; because there is no drainage to the sea, the micro-basin is temporarily inundated after heavy rains. A second population was found in the La Ribera arroyo, a few kilometers NW of the other site and has fewer plants than the first site. Both sites are at an elevation of 2-4 m above sea level on sandy and saline soil.

The type population grows in a coastal thorn scrubland; this vegetation type has not been previously documented in the Baja California Peninsula. Common species at the site were: *Phaulotamnus spinescens* A. Gray (Achatocarpaceae), *Lycium brevipes* Benth. (Solanaceae), *Condalia globosa* I.M. Jhtn. var. *globosa* (Rhamnaceae), *Parkinsonia florida* (Benth. ex A. Gray) S. Watson (Fabaceae), *Randia capitata* DC. (Rubiaceae), *Stenocereus gummosus* (Engelm.) A. Gibson et K.E. Horak (Cactaceae), and *Ximenia parviflora* Benth. var. *glauca* de Filipps (Ximeniaceae).

The Baja California flora (Wiggins, 1980) has almost 3000 species, subspecies, and varieties of vascular plants. The level of endemism is difficult to determine, given recent unpublished accounts (Rebman, pers. comm.). However, there are an estimated 800 endemic plant species on the Baja California Peninsula. Riemann and Ezcurra (2004), in an analysis of the spatial distribution of the peninsular endemics, stated that there are some "hot spots" of endemics in this region, and one of them is the Cape Region. It contains several plant communities, each with high endemism at generic, specific, and infraspecific levels (León de la Luz et. al., 1999). Peninsular endemism is attributed to historical vicariance events during its geological separation from the Mexican mainland and recurrent glacial-postglacial colonizations (Nason et al., 2002).

Given the limited number of individuals, low apparent level of recruitment, and restricted geographic range, the outlook for the continued persistence of *P. calafia* is not good. As numerous tourism projects are also under development in this area, this new taxon should be considered for IUCN and Mexican enviromental law in critically endangered status.

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