Universidad Michoacana de San Nicolás de Hidalgo
Facultad de Biología
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MAESTRÍA EN CIENCIAS BIOLÓGICAS
MAESTRÍA EN ECOLOGÍA Y CONSERVACIÓN

Diversity, taxonomy and phylogeny of *Aristolochia* subsection *Pentandrae* (Aristolochiaceae)

Thesis presented by:
Anna Paizanni Guillén

In fulfillment of the requirements to obtain the degree of:
Master in Biological Sciences

Tutor: Dr. Juan Carlos Montero Castro (U.M.S.N.H.)

Co-tutor: Dra. Marie-Stéphanie Samain (INECOL-Bajío)

Morelia, Michoacán, March 2016

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Diversidad, taxonomía y filogenia del género *Aristolochia* subsección *Pentandrae* (Aristolochiaceae)

Tesis que presenta:

Anna Paizanni Guillén

Como requisito para obtener el grado profesional de:

Maestra en Ciencias Biológicas

Tutor: Dr. Juan Carlos Montero Castro (U.M.S.N.H.)

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DR. HÉCTOR GUILLÉN ANDRADE
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P R E S E N T E

Por este conducto nos permitimos comunicarle que después de haber revisado el manuscrito final de la Tesis Titulada: "Diversidad, taxonomía y filogenia del género *Aristolochia* subsección *Pentandrae* (Aristolochiaceae)" presentado por el Biol. Anna Paizanni Guillén, consideramos que reúne los requisitos suficientes para ser publicado y defendido en Examen de Grado de Maestra en Ciencias.

Sin otro particular por el momento, reiteramos a usted un cordial saludo.

A T E N T A M E N T E
Morelia, Michoacán, a 27 de enero de 2016

MIEMBROS DE LA COMISIÓN REVISORA

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RESUMEN

La subsección *Pentandrae* (de aquí en adelante abreviada como *Pentandrae*) está situada en la sección *Gymnolobus*, propuesta por Duchartre (1854, 1864) y es la que tiene mayor riqueza de especies de *Aristolochia* en México. El clado que comprende a *Pentandrae* está caracterizado por dos sinapomorfias: 5 estambres y 5 carpelos (ginostemo con 5 lóbulos estigmáticos), mientras que las otras especies mexicanas tienen 6 estambres y 3 carpelos. A pesar de que *Pentandrae* es uno de los grupos con mayor riqueza de especies en México, es el menos estudiado de todo el género *Aristolochia*. Se distribuye desde el sur de Estados Unidos a Colombia (Isla San Andrés), incluyendo Guatemala, Belice, Honduras (Islas Cisne) y las Antillas, pero es casi en su totalidad endémica de México. 43 especies conocidas hasta la fecha. Adicionalmente, y parte de los resultados de este trabajo, se publico un manuscrito con cuatro especies nuevas. Desde el tratamiento de Pfeifer (1970), pocos estudios se han generado para *Pentandrae*. Los estudios moleculares para la familia Aristolochiaceae se han enfocado a nivel de géneros y subgéneros y solo algunos de estos trabajos han incluido algunas especies de *Pentandrae*, para representar al grupo. Estudios filogenéticos moleculares indican monófila del grupo, aunque en ellos solo se han incluido algunas especies. Hasta ahora solo evidencias morfológicas existen como prueba de que este grupo podría ser monofilético. Sin embargo, no existe algún trabajo molecular filogenético en el que se haya incluido a más de quince especies de *Pentandrae*, además de que los tratamientos morfológicos que existen son limitados. Se presenta en este trabajo un estudio taxonómico de *Pentandrae* que incluye 18 especies, así como la circunscripción de las mismas. Además, se presenta la primera hipótesis filogenética de este clado en donde se incluyeron el 73 % de las especies. Se descubrieron seis especies nuevas de las cuales se publicaron recientemente. Se generó una clave dicotómica para toda la subsección, se sinonimizaron dos nombres (*Aristolochia socorroensis* con *A. watsonii* y *A. peninsularis* con *A. monticola*) y se presentan en total 18 descripciones que incluyen las cuatro especies nuevas por publicarse y dos especies nuevas inéditas. El resultado de los análisis de probabilidad posterior (Mr. Bayes) y Maxima Verosimilitud de las regiones de cloroplasto concatenadas de *trnK-matK-trnK-psbA*, *rpL16* intron fueron las que presentaron mayor soporte. Se confirma la monofilia de *Pentandrae* y dentro de esta subsección se soportan tres clados, los cuales tienen relación con caracteres morfológicos, distribución, hábitat y altitud.

Palabras clave: *Aristolochia*, subsección *Pentandrae*, Aristolochiaceae, Taxonomía, Filogenia

ABSTRACT

In Mexico *Aristolochia* is present with about 70 species belonging to two subgenera (i.e. *Siphisia* and *Aristolochia*). The subsection *Pentandrae* (hereafter shortened to *Pentandrae*) is traditionally placed in section *Gymnolobus* proposed by Duchartre (1854, 1864) and is part of *Aristolochia* subgenus *Aristolochia*, besides is the most species-rich *Aristolochia* lineage in Mexico. The clade is characterized by two morphological synapomorphies: five stamens and five carpels (a 5-merous gynostemium). The representatives of *Pentandrae* are further characterized by a geophytic and procumbent habit (occasionally climbing), as well as a perennial subterranean organ. Although *Pentandrae* is a comparatively species-rich lineage, it is the least studied *Aristolochia* lineage on worldwide scale. Its distribution area ranges from the southern United States to Colombia (San Andrés Island), including Guatemala, Belize, Honduras (Swan Islands) and the West Indies, but it is most diverse in Mexico with 43 species known today. Additionally, a manuscript with four species new to science has been published recently (Paizanni et al. 2016). Since the treatment of Pfeifer (1970) relatively little research has been carried out on *Pentandrae* as a whole. Here I present the basis for a monography of *Pentandrae* (updated species descriptions), including a full species list, distribution areas, and an identification key. Moreover, a phylogeny based on nucleotide sequences of the chloroplast regions: *trnK-matK-trnK-psbA*, *rpL16* intron, *rpL32-trnL*$^{(UAG)}$ spacer and *psbJ-petA* spacer has been generated for 39 species from *Pentandrae*, plus 23 accessions as outgroup taxa. In a first attempt I tested two nuclear single copy genes. However, only the regions providing high statistical support (*trnK-matK-trnK-psbA* and *rpL16* intron) were used in combination to reconstruct a phylogenetic hypothesis. The molecular phylogenetic results confirm the monophyly of *Aristolochia* subsection *Pentandrae* for the first time, using a broad sampling. Including about 73% of the species, three well-supported clades were recovered within *Pentandrae*. In accordance with the phylogenetic hypotheses, habitats, altitudinal ranks of occurrence, patterns in distribution and morphological characters are discussed.

GENERAL INTRODUCTION AND OBJECTIVES

INTRODUCTION

Aristolochiaceae is together with Asaraceae, Lactoridaceae, and Hydnoraceae part of a Piperales clade that is characterized by the presence of a perianth, in contrast to the perianthless families Piperaceae and Saururaceae (Naumann *et al.* 2013). Currently, Aristolochiaceae

comprise only two genera (e.g. Horner et al. 2015): *Aristolochia* L. with about 500 species and a nearly worldwide distribution, and *Thottea* Rottb., with 35 species distributed in Asia (Neinhuis et al. 2005; Wanke et al. 2006).

The highest species richness of *Aristolochia* is found in the tropics and subtropics (Kelly 2000; Neinhuis et al. 2005; Ohi-Toma et al. 2006; Wanke et al. 2006; González et al. 2010). It has been subdivided in three subgenera: subgenus *Siphisia* occurring in Asia, North and Central America (Wagner *et al.* 2014, González et al. 2014), subgenus *Pararistolochia* present in tropical Africa and Australasia (Buchwalder et al. 2014) and subgenus *Aristolochia* occurring in America, Europe, Africa and the Middle East. The latter subgenus is further subdivided into several monophyletic groups, but the relationships of these remain largely unknown and are currently treated as operational taxonomic units (OTUs). Six OTUs are currently recognized: "Howardia" p.p. (including the former genera *Euglypha* and *Holostylis*), *Aristolochia* s.str., *Thyrsicae*, the "*Aristolochia lindneri* group", subsection *Pentandrae* (former genus *Einomeia* according to Rafinesque (1828) and Huber (1985, 1993)), as well as the "*Aristolochia grandiflora* complex" (Neinhuis et al. 2005).

In Mexico *Aristolochia* is present with about 70 species belonging to two subgenera (i.e. *Siphisia* and *Aristolochia*) (Pfeifer 1966, 1970; González et al. 2014). The subsection *Pentandrae* (hereafter shortened to *Pentandrae*) is traditionally placed in section *Gymnolobus*, proposed by Duchartre (1854, 1864) and is the most species-rich *Aristolochia* lineage in Mexico. The clade is characterized by two morphological synapomorphies: five stamens and five carpels (a 5-merous gynostemium), whereas all other Mexican species have six stamens and three carpels (Pfeifer 1966, 1970). The representatives of *Pentandrae* are further characterized by a geophytic and procumbent habit (occasionally climbing), as well as a perennial subterranean organ. Although *Pentandrae* is a comparatively species-rich lineage, it is the least studied *Aristolochia* lineage on worldwide scale. Its distribution area ranges from the southern United States to Colombia (San Andrés Island), including Guatemala, Belize, Honduras (Swan Islands) and the West Indies, but it is most diverse in Mexico (Pfeifer 1970; González et al. 2010). Thirty-five species have been recognized by Pfeifer (1970), who has published the only available, broad scale taxonomic revision of the subsection. Subsequently, eight additional species have been described from Mexico by Ortega and Ortega (1995), Santana-Michel (1995, 2002, 2007), Santana-Michel and Lemus-Juárez (1996), Calzada et al. (1997), Santana-Michel and Solís-Magallanes (2007), and Santana-Michel and Guzmán-

Hernández (2014), resulting in a total of 43 species known today. Additionally, a manuscript with four species new to science has been published recently (Paizanni et al. 2016) (Table 1).

Since the treatment of Pfeifer (1970) relatively little research has been carried out on *Pentandrae* as a whole. Molecular studies within the family Aristolochiaceae have especially focused on genus and subgenus level and only few studies have addressed particular lineages in more detail (e.g. Neinhuis et al. 2005; Ohi-Toma et al. 2006; Wanke et al. 2006). Molecular phylogenetic studies indicated that it is a monophyletic group although only a few species of *Pentandrae* were included. The species that are most closely related to *Pentandrae* are an informal group consisting of five species, named the *Aristolochia lindneri* group (Wanke et al. 2006; Ohi-Toma et al. 2006; González et al. 2010). Until now only morphological evidence exists that the group is monophyletic (González et al. 2010).

Until today, limited data is available to distinguish the species of *Pentandrae* beside morphological studies and their natural relationships remain largely unknown. Therefore, it is here proposed to present a monography including the majority of the species belonging to *Pentandrae* as well as a first molecular phylogenetic study of this clade. Together, this information will serve as the basis for more extensive research, including the biogeographic history and adaptive radiation of this group. The comparatively wide distribution of some species and the endemism of others (including island endemics) are facts that might help to answer questions about the dispersal of *Pentandrae*. In addition, this project will provide knowledge of a poorly known Mexican plant group.

The main objectives of this thesis are:

- Present a full species list, as well as the morphological circumscription and the distribution area of *Aristolochia* subsection *Pentandrae*.
- Perform a first molecular phylogenetic study of *Pentandrae* that will not only provide the basis for a revision of the clade, but will also allow obtaining future insight in the evolutionary biology of the species including their diversification and biogeography.

The particular objectives are:

- Provide the basis for a monography of *Pentandrae*.
- Prepare an identification key and updated species descriptions.
- Update the general distribution maps of *Pentandrae* using a geographic information system.
- Obtain a first molecular phylogenetic hypothesis.

CHAPTER 1. TAXONOMY OF *ARISTOLOCHIA* SUBSECTION *PENTANDRAE*

1.1 INTRODUCTION

In Mexico *Aristolochia* is present with about 70 species belonging to two subgenera (i.e. *Siphisia* and *Aristolochia*) (Pfeifer 1966, 1970; González et al. 2014). The subsection *Pentandrae* (hereafter shortened to *Pentandrae*) is the most species-rich *Aristolochia* lineage in Mexico and is characterized by two morphological synapomorphies: five stamens and five carpels (a 5-merous gynostemium). Rafinesque (1828) was the first to propose this group based on the aforementioned synapomorphies, but he considered it as genus *Einomeia*. Later Duchartre (1854) provided a new classification where he considered the genera proposed by Rafinesque (1828) as different sections into *Aristolochia*, but the ranks into this work were unclear. It was not until Duchartre (1864) that these classifications were clarified. Duchartre (1864) placed *Pentandrae* as a subsection in section *Gymnolobus* and considered *Einomeia* as synonym. Afterwards, different authors followed this subsection as segregate (Kolsch, 1859; Schmidt, 1935; Huber, 1985, 1993). Recent phylogenetic analyses based on molecular and morphological data have shown *Pentandrae* species are nested within the genus Aristolochia (González and Stevenson, 2002; Neinhuis et al. 2005; Ohi-Toma et al. 2006; Wanke et al. 2006).

Pfeifer (1970), who has published the only available, broad scale taxonomic revision of the subsection, has recognized 35 *Pentandrae* species. Subsequently, eight additional species have been described from Mexico by Ortega and Ortega (1995), Santana-Michel (1995, 2002, 2007), Santana-Michel and Lemus-Juárez (1996), Calzada et al. (1997), Santana-Michel and Solís-Magallanes (2007), and Santana-Michel and Guzmán-Hernández (2014), resulting in a total of 43 species known today. Additionally, a manuscript with four species new to science has been published recently (Paizanni et al. 2016) (Table 1).

Since the treatment of Pfeifer (1970), relatively little research has been carried out on *Pentandrae* as a whole. Fourty-five years later, limited data are available to distinguish the species of *Pentandrae* beside morphological studies, and their natural relationships remain largely unknown. Consequently, it is here proposed to present the basis of a monography of this clade, including a full species list with identification key, updated species descriptions of the subsection *Pentandrae* and 18 species, with their respective illustrations, as well as an updated general distribution map of the clade. The comparatively wide distribution of some species and

the endemism of others (including island endemics) are facts that might help to answer questions about the dispersal of *Pentandrae*. In addition, this project will provide knowledge of a poorly known Mexican plant group.

The taxonomic objectives are:

- Present a full species list.
- Provide the basis for a monography of *Pentandrae*.
- Prepare an identification key and updated species descriptions.
- Update the general distribution map of *Pentandrae* using a geographic information system.

1.2 MATERIAL AND METHODS

Specimens of the pentandrous species were carefully studied in the following 21 herbaria in Mexico and Europe: Herbarium of the Universidad Autónoma Agraria Antonio Narro (ANSM), Coahuila; Herbarium of the Natural History Museum of Denmark (C), Copenhagen, Denmark; Herbarium of the Instituto Politécnico Nacional (CIIDIR), Durango; Technische Universität Dresden (DR) Dresden, Germany; Herbarium of the Escuela Nacional de Ciencias Biológicas (ENCB), Mexico City; Herbarium of George B. Hinton (GBH), Nuevo León; Herbarium of the Universidad Autónoma de Guadalajara (GUADA), Jalisco; Herbarium of the Instituto de la Universidad de Guadalajara (IBUG), Jalisco; Centro de Investigaciones Biológicas del Noreste, S.C. (HCIB), Baja California Sur; Herbaria of the Instituto de Ecología (IEB and XAL), Michoacán, Veracruz; Herbarium of the Estación de Biología Chamela (MEXU), Jalisco; National Herbarium of Mexico (MEXU), Universidad Nacional Autónoma de México; Herbarium of the Universidad Autónoma de Nuevo León (NL); Herbarium of the Universidad Autónoma de Querétaro (QMEX); Herbarium of the Universidad Autónoma de San Luis Potosí (SLPM) and the Herbarium of the Universidad de Guadalajara, Centro Universitario de la Costa Sur (ZEA). These herbaria contain the most important collections of species of *Aristolochia* subsection *Pentandrae* in Mexico.

Additionally, material was loaned from the following four herbaria in the United States of America: University of Arizona (ARIZ), Arizona, Tucson; Baylor University (BAYLU), Texas, Waco; University of Texas at Austin (TEX), Texas, Austin; University of California (UCR), Riverside, California.

All material studied was used as a basis for selection of the localities for the field trips,

updating the distribution maps, and often allowed the collection of DNA samples from species that could not yet be collected in the field by ourselves.

1.2.1. Databases, distribution maps and taxonomic treatment

This work includes a starting point for a monographic revision of *Pentandrae*, such as the update of species circumscriptions including proposing synonymy, and the treatment of species new to science. In addition, an identification key, photos, and/or drawings of the species are presented. Distributions maps were made with the software Quantum GIS version 2.8 Wien (QGIS Development Team 2015) with projection WGS84, using the Digital Elevation Model from the CGIAR-CSI SRTM 90m database (http://srtm.csi.cgiar.org) as base map. Morphological data and the specimen information from the herbaria and the field trip have been added to different databases. The specimen database includes all the information of the label of the herbarium specimens, as well as the information recovered during the field trip. The morphological database includes all the measurements of the principal characters of every species. This latter information will later also be used for principle component analyses.

1.2.2. Fieldwork

Collecting took place from mid June to early October 2014, and during 2015 in January (Yucatán) and in September (Baja California Sur). The fieldwork was supported by the Technical University of Dresden (Germany) through financing by the German Academic Exchange Service (DAAD), as well as funding by the Instituto de Ecología, A.C. The Centro Regional del Bajío of the Instituto de Ecología, A.C. and the Universidad Michoacana de San Nicolás de Hidalgo provided vehicles.

During the field trip the activities performed were: Collect samples for herbarium specimens and for DNA; Collect living plants for cultivation; Obtain geographical coordinates with a GPS; Collection of flowers in falcon tubes with 70% alcohol to investigate flower visitors and pollinators in the future as well as for morphological research; Collections of stem segments in falcon tubes with 70 % alcohol later anatomical investigation in the research group of the TU Dresden.

1.3 RESULTS

In the state of **Baja California Sur,** we collected the following species: *Aristolochia monticola* and *A. watsonii*; in **Colima**: *A. occidentalis* (type locality*), A. luzmariana* (type locality), *A. colimensis* (type locality), *A. aff. foetida, Aristolochi sp.* (unidentified sp. 1); in **Jalisco**: *Aristolochia foetida, A. pringlei, A. palmeri, A. tequilana* (type locality), *A. bracteosa, A. rzedowskiana, A. pacifica, A. tuitensis, A. nahua, Aristolochia sp*. (unidentified sp. 2), *Aristolochia sp*. (unidentified sp. 3), *A.* aff. *flexuosa*; in **Nayarit:** *A.* aff. *watsonii*, *A. durangensis, A. savannoidea*; in **Sinaloa**: *A. watsonii*; in **Durango:** A. *cordata* (type locality), A. wrightii, A. aff. *tequilana*, *A. watsonii, Aristolochia sp*. (unidentified sp. 3); in **Chihuahua:** *A. wrightii*; in **Estado de México:** *A. conversiae*; in **Puebla:** *A. pueblana* (type locality); in **Oaxaca:** *A. teretiflora, A. oaxacana*; in **Guanajuato:** *A. versabilifolia*; in **San Luis Potosí:** *A. versabilifolia, A. nana*; In **Coahuila:** *A. lassa* (type locality), *A. pentandra;* in **Nuevo León:** *A. secunda, A. pentandra, A. aff. watsonii*; in **Michoacán**: *Aristolocia flexuosa*; in **Guerrero**: *Aristolochia sp.* (unidentified sp. 4) (Fig. 1).

In total, **127 herbarium specimens** and **111 DNA samples** were collected during the field trips. During fieldwork six new species were discovered (see below) and 40 living plants were collected. A first manuscript has been published by Systematic Botany: "Four new species of *Aristolochia* subsection *Pentandrae* from western Mexico" (Paizanni Guillén et al. 2016). The remaining new species in the table 1 were previously collected and two of the new species are only known from herbarium material.

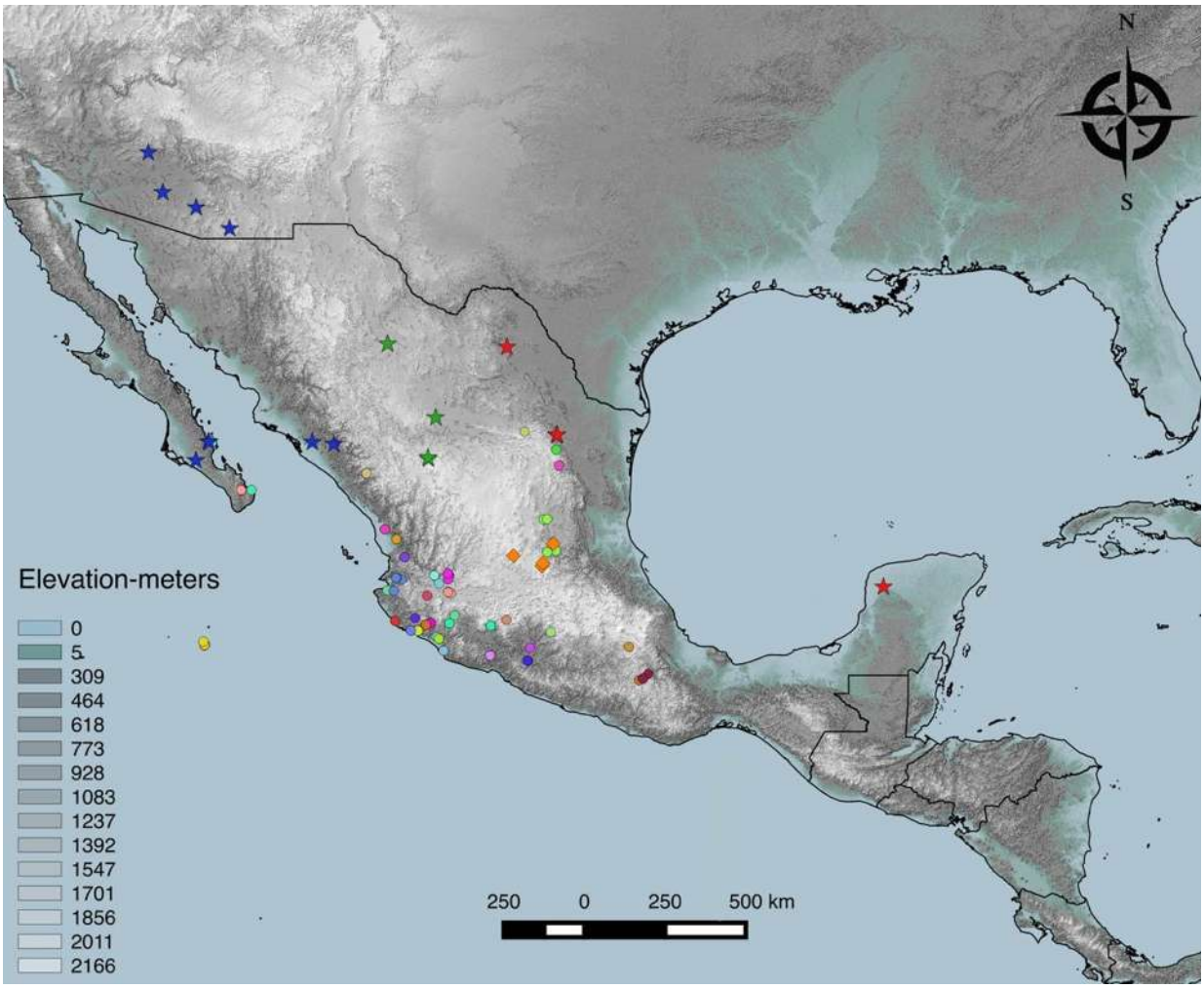

Figure 1 Distribution map showing our field collections from 2014 and 2015.

1.3.1 Taxonomic treatment

Note: The manuscript "Four New species of *Aristolochia* subsection *Pentandrae* from Western Mexico" has been published and is presented in Appendix 1.

Aristolochia* subsection *Pentandrae Duch., in DC. Famille des Aristolochiées. 4:1-17 1854; DC. Prod. 15-1:440 1864 –Lectotype: *Aristolochia pentandra* Jacq.

Geophytic herbs, usually procumbent, sometimes climbing or erect (e.g. *A. erecta*, *A.* sp. nov. inedit. (Huetamo, Michoacán)), with **perennial** subterranean organs; **stems** with 2-3 **subterranean internodes**; **aerial internodes** occasionally geniculate, sometimes purplish red, sparsely or dense covered with septate trichomes. **Petioles** terete, sometimes purplish red, sparsely covered with septate trichomes. **Leaf blades** hastate-deltate-trilobate (21 spp.), orbicular to widely ovate (cordiform) (18 spp.), lanceolate-ovate (8 spp.), lanceolate to ensiform (6spp.), or sagittate oblong with base auriculate (5 spp.)., the majority of the species

each having a similar leaf shape, although some can show different shapes, e.g. orbicular to hastate leaves, even in the same branch, discolorous, base commonly cordate, subcordate, auriculate, sagitate or hastate, rare obtuse or rounded (*A. emiliae*, *A.* sp. nov. inedit. (El Chavarín, Colima)), **lobes** commonly sinuous, rounded or some species with anchor-shaped (*A.* sp. nov. inedit. (El Chavarín, Colima)), apex acute, acuminate, mucronulate, long acuminate, obtuse or attenuate; palmatinerved, actinodromous, sometimes basal veins purple-pink, **margin** entire; **adaxial leaf** side green, margin sometimes purple-pink, with sparse to dense septate trichomes, with acroscopic orientation; **abaxial leaf** side pale green, reticulate (*A. buntingii, A. durangensis, A. monticola*?), veins pale yellow with dense septate trichomes, especially along the veins. **Flowers** axillary, solitary or placed in a racemose inflorescence, **flower pedicels or peduncle** with dense or sparce septate trichomes. **Bract** ovate, cordiform, lanceolate, lanceolate-ovate, deltate, widely triangular to widely ovate. **Perianth** geniculate, rectilinear or erect; when dry white-yellowish, pale yellow becoming purple toward the limb, veins brown-orange, dense to sparse septate trichomes along veins; **utricle** spheroid, subspheroid, obovoid, ellipsoid or obloid, internal surface yellow with purple dots at the base or toward the syrinx, covered with mucilaginous trichomes; **tube** straight or bent (10°-) 90º-120°(-170º), basally widened, straight, widened toward the limb, internal surface with black-purple, purplish red short strigulose, scabrous or villous trichomes, rare glabrous; **limb** usually erect, rarely bent (*A. rzedowskiana, A.* sp. nov. inedit. (Sierra Verde, Jalisco)), ovate to cordiform, obovate, widely ovate, orbicular, elliptic, oblong, lanceolate, lanceolate-ovate, trilobate (*A. cordata*), rhombic (*A. karwinskii*), short triangulate, **base** attenuate, cordate, rounded, slightly cordate, emarginated, cleft, **apex** acute, narrowly acute, apiculate, acuminate, long acuminate, ensiform, flexuous, attenuate, mucronulate, obtuse, slightly apiculate, retuse, or caudate emarginated, color yellow, yellow green, coral cream to purplish red, maroon, brown; smooth, with protuberances or trichomes, in lateral view flat or cup-shaped; **throat** reniform, ovoid, elliptic, widely elliptic, deltate, obovoid, cylindrical, narrowly cylindrical, pentagon-shaped, sometimes ringed, color yellow to dark purple, minutely granular, glabrous or covered by trichomes; **syrinx** infundibuliform, eccentric. **Gynostemium** coroniform, with **stipe,** rarely subsessile, **stigmatic lobes** 5, **anthers** 5. **Ovary inferior**, oblanceolate, lanceolate, linear or narrowly elliptic, with sparse to dense or lanate septate trichomes (Fig. 2). **Fruit** a capsule, spheroid, subglobose, obloid, ellipsoid, ovoid to obovoid, green to purplish red when young, brown

yellow when mature, with septicidal dehiscence at the apex (Fig. 3). **Seeds** deltoid, upper part black, lower part yellow-brown, with granular surface (Fig. 3).

Phenology— The majority of the species flowering in the second half of the rainy season or shortly after it, from July to September. In desert areas, where the rainy season ends around September-October, the flowers appear from October to May. The fruiting season is similar, from July to November and January to May, respectively

Distribution and Habitat— The majority of the pentandrous species are favored by disturbed areas, where the competition between plants is less, especially in open areas. The areas are rocky (andesitic, basalt, diorite, igneous rocks, limestone, among others) and the most common soil types are poorly drained, alluvial, clay, ferralsols, sand, gypsum, vertisol, etc.

The principal vegetation types are tropical deciduous forest, oak forest, oak-pine forest, grasslands, desert scrub, xeric grassland, volcanic areas, juniper grassland, savannas, and gypsum areas, among others. The distribution for few species is broad (*Aristolochia pentandrae*, *A. watsonii*, *A. wrightii* and *A. versabilifolia*), but in general the species are usually known from a limited amount of localities and few of them even from the type locality only. Dispersal studies are required to understand the distribution patterns of the species of this peculiar group within the genus *Aristolochia*. Altitudinal distribution ranges from zero to 2,500 (-3,200) m (Figs. 4 y 5).

Common Name and Uses— "Camote de Indio"; "Cigarrera" (Jalisco), "Orejita de ratón", "Chifladores" (Oaxaca), the latter referring to its use as a whistle, in this case, children cut the utricle and the tube, and use the short limb to blow as a whistle; "Camote de la vibora" (Veracruz), the local people use a piece of root to chew against the poison of snakes and some insects; "Hierba del Indio" "Yerba del Indio"(north and central Mexico) used in a tea against stomach pain, as well to reduce weight, cholesterol, diabetes, headache and also against snake bites, toasted and grounded powder to cure wounds of cattle; Wright Dutchman`s Pipevine (*Aristolochia wrightii*, Texas. USA), Swanflower (*A. erecta, USA*), Cory´s Pipevine (*A. coryi*).

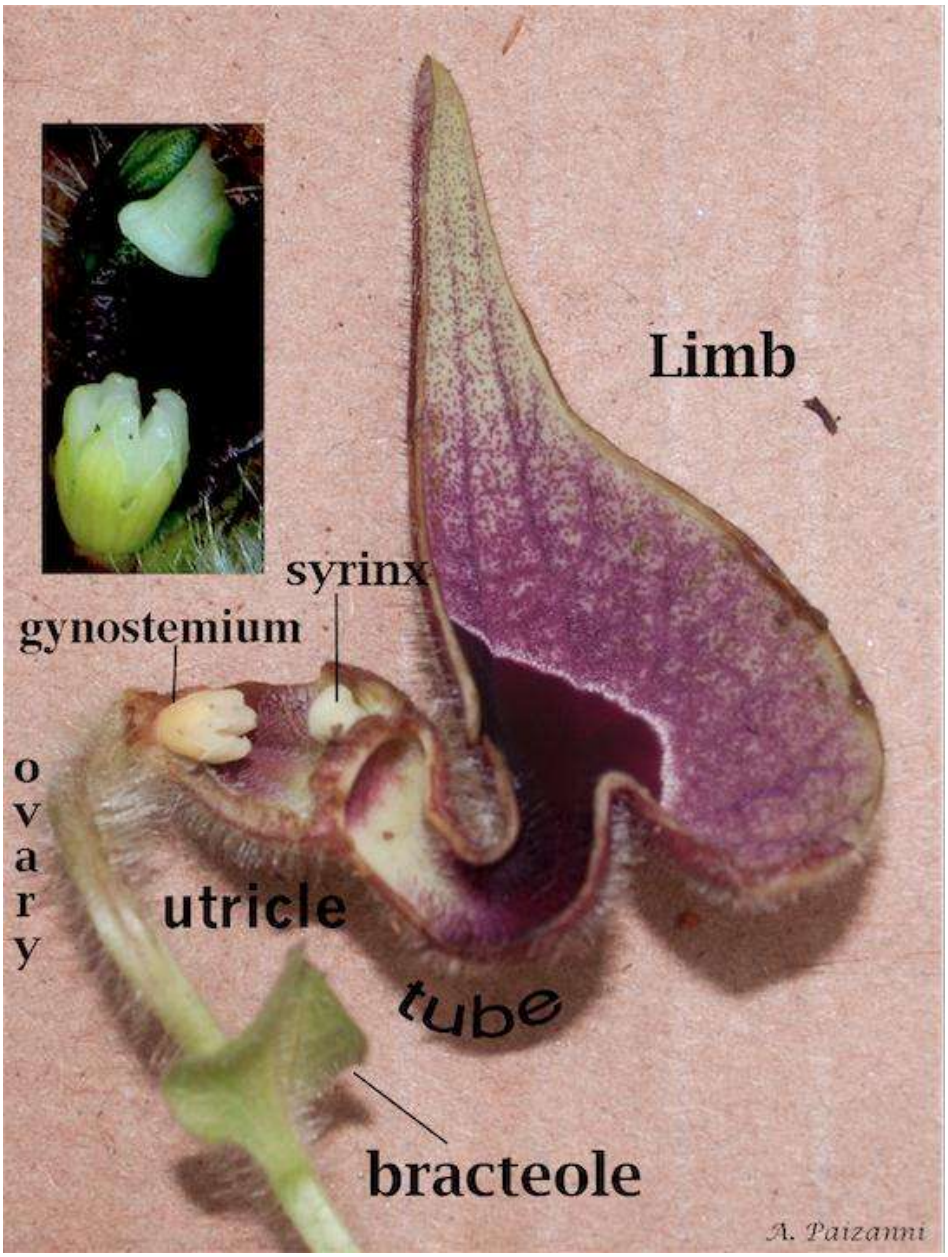

Fig. 2 Flower of *Aristolochia cardiantha* showing the structures of the perianth, ovary, and bracteole.

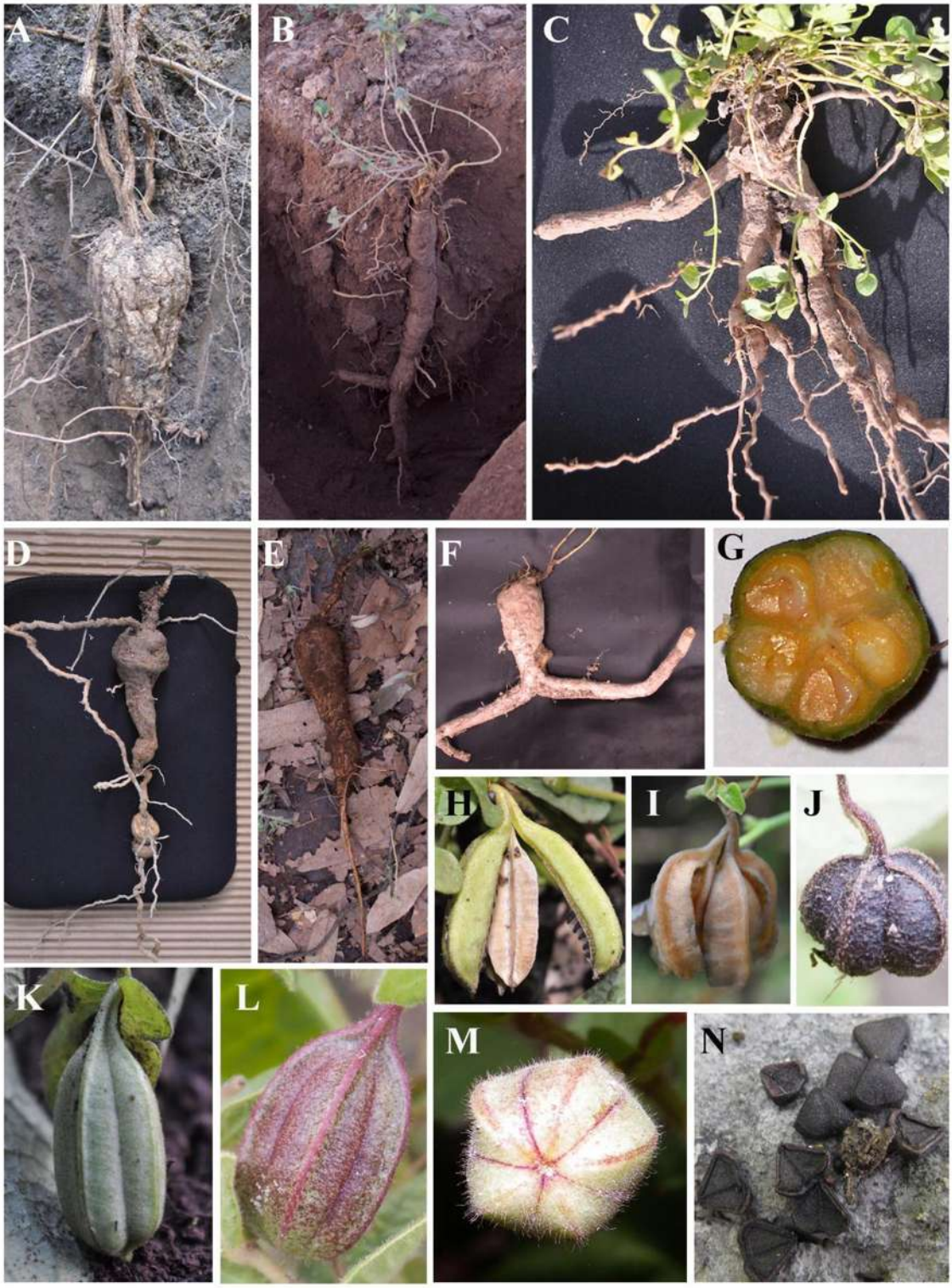

Fig. 3 Representation of the following structures in *Aristolochia* subsection *Pentandrae*: Perennial subterranean organs (A-F), Capsular fruits: G. transversal view. H-I, mature fruit. J-L, different shapes of the fruit. M. Apex view and seeds (N). (Photographs by J. Manuel Ramírez Amezcua: A-B, and by J.F. Santana Michel: G).

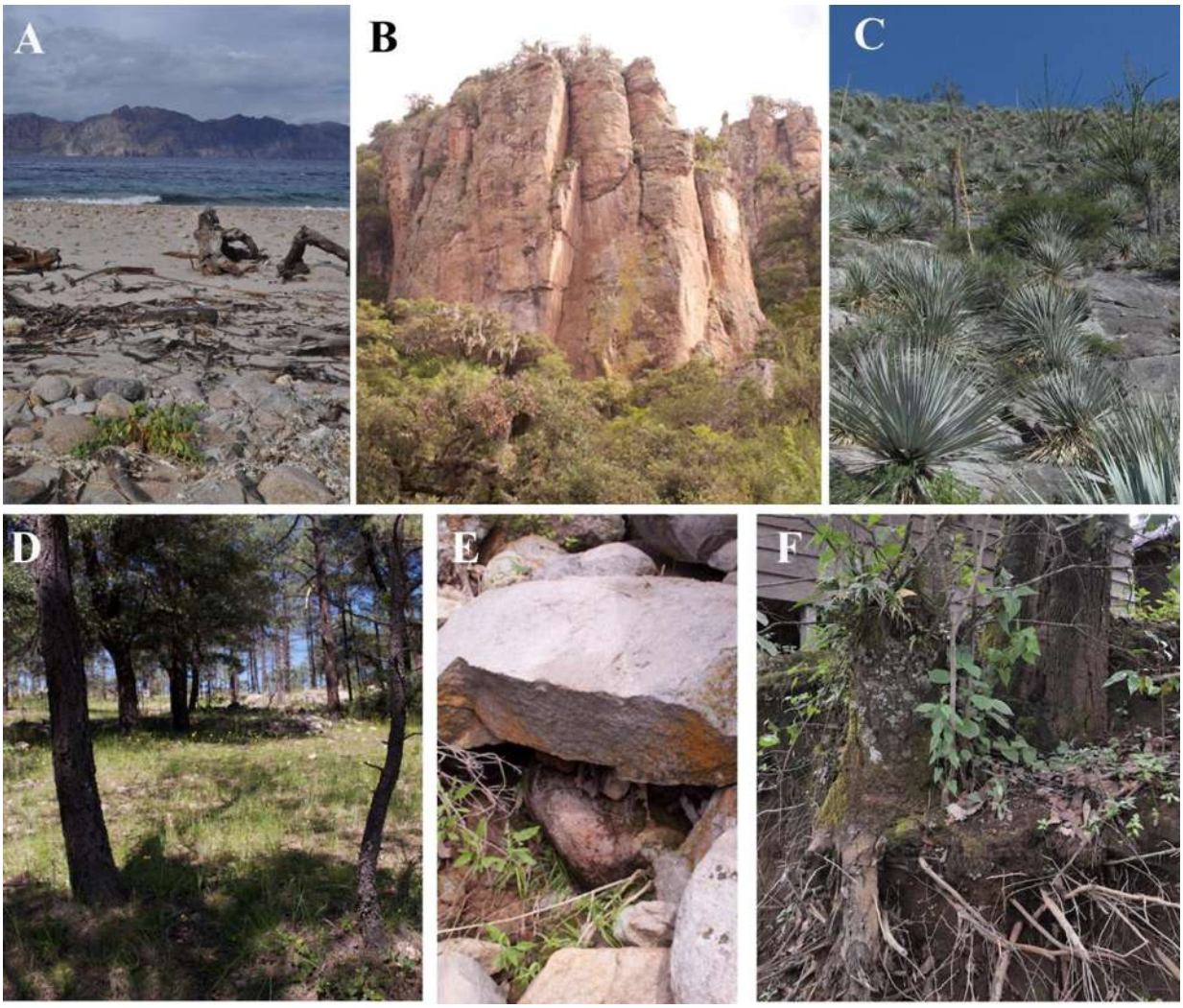

Fig. 4 Principal habitats from *Aristolochia* subsection *Pentandrae*. A. Dune vegetation. B. Tropical deciduous forest. C. Desert scrub. D. Pine forest. E. habitat of *A. palmeri* with oak forest. F. habitat of *A. luzmariana* with pine forest in transition with cloud forest.

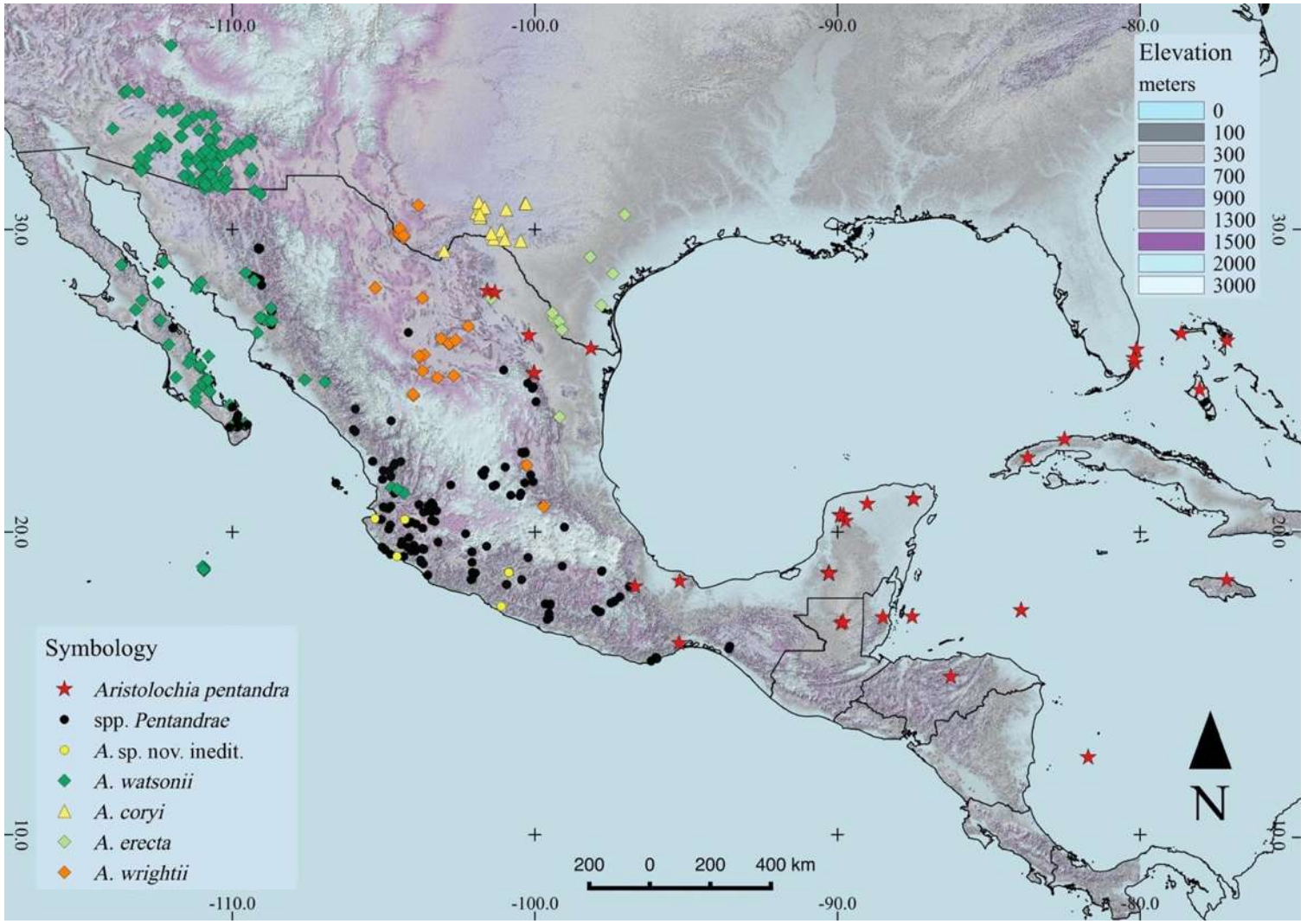

Fig. 5 Distribution map of *Aristolochia* subsection *Pentandrae*, showing with stars the widely distributed *Aristolochia pentandrae*, with circles the species growing in Mexico, with squares the species growing in Mexico and USA and with triangles *Aristolochia coryi*, the species that is known only from United States. Map generated by QGIS 2.8-Wien, projection WGS84, using the Digital Elevation Model from CGIAR-CSI SRTM 90m Database (http://srtm.csi.cgiar.org) as a base map.

1.3.1.1 Key to the species of *Aristolochia* subsection *Pentandrae*

The groups below refer to the normal pattern of leaf shapes. As well mostly all the leaves bases are cordate, subcoradate to auriculate. The species of *Aristolochia* subsection *Pentandrae* may show variation of leaf shapes in the plants, but some of them have remarkable plasticity (see group VI).

Leaves orbicular to widely ovate (cordiform) (Fig. 6)…….…….....I

Leaves lanceolate to ensiform (Fig. 7)…….……...……..……II

Leaves lanceolate-ovate (Fig. 8)…….. ……..……...…….III

Leaves hastate, deltate or trilobate (Fig. 9)……..……...……IV

Leaves sagittate or oblong with base auriculate (Fig. 10)…..…..……....V

Leaves with different shapes (Fig. 11)……...…….....VI

I-Leaves orbicular to widely ovate (cordiform)

1a Peduncle 8-15 cm long; ovary lanate……..…….……2

1b Peduncle (0.02-)0.5-1.5 (-3.5) cm long; ovary with dense septate trichomes.....3

2a Limb lanceolate-ovate, apex ensiform, pink purple area covered by verrucose protuberances, around throat ringed starting with a slim line white, becoming purplish red, throat yellow……..*Aristolochia foetida* (Fig. 12A, B)

2b Limb widely ovate, apex cuspidate, pink-purplish to purplish red area, around throat ringed starting with a slim white line, then purplish red, margin white……*A. cardiantha* (Fig. 12C, D)

3a Perianth 1-2 (-2.6) cm long……...……..……...4

3b Perianth (2-)2.5-7(-10) cm long…….……..…10

4a Abaxial leaves side reticulate; limb purple dark…….....….*A. buntingii* (Paizanni et al. 2016, Fig. 2)

4b Abaxial leaaves side not reticulate, limb purple pink to yellowish……...5

5a Utricle spheroid, obovoid to ellipsoid; tube basally widened……..….....6

5b Utricle ellipsoid to irregularly ellipsoid; tube basally straight or widened towards the limb…….……....9

6a Leaves orbicular; limb ovate to cordiform, yellowish area covered by purplish red papillose protuberances ……...*A. nahua* (Paizanni et al. 2016, Fig. 2)

6b Leaves cordiform to hastate; limb ovate to orbicular, white yellow to purplish red…….7

7a Throat ovate; leaves becoming abruptly small at end of stem; limb white-yellow, smooth ……..…….…….*A. conversiae* (Fig. 12G, H)

7b Throat reniform; leaves usually with same shape pattern; limb purplish red or yellowish covered by dark purple to purple protuberances…….…….…....8

8a Limb orbicular, purplish red area covered by dark purple protuberances……....*A. tuitensis* (Paizanni et al. 2016, Fig. 7)

8b Limb ovate, yellowish area, covered by purple protuberances…….…...*A. pacifica* (Paizanni et al. 2016, Fig. 2)

9a Limb widely ovate, surface with a short vermiform appendage, growing in Nayarit…….....*A. savannoidea* (Paizanni et al. 2016, Fig.7)

9b Limb elliptic, surface with pectinate-fimbriate annulus at the throat, margin smooth on the inner face, growing on Tres Marias Island……...….....*A. tresmariae* (Fig. 12K)

10a Utricle subspheroid to obovoid, 0.5-0.8 cm long……...…...11

10b Utricle ellipsoid to obovoid, (-0.5) 1-2 cm long……..……..…...12

11a Limb broadly heart-shaped, purplish red; throat yellow-green……....*A. mutabilis* (Fig. 12I, J)

11b Limb ovate, cream with purple dots; throat covered with dense pusplish red dots…….….*A. tequilana* (Fig. 12E, F)

12a Limb narrowly triangulate to lanceolate-ovate……..……...….13

12b Limb widely ovate, orbicular or elliptic …….……...……..….15

13a Flower rectilinear; tube straight; limb maroon to purplish red or pink red; throat yellow……..…14

13b Flower geniculate, tube bent 90º-110º; limb vinaceous; throat white……...…....*A. nelsonii* (Fig. 13F, J)

14a Tube 0.7-1.5 cm long; limb maroon to purplish red; throat yellow with purple dots……..……..……...……*A. pentandra* (Fig. 13J)

14b Tube 2.5-3 cm long; limb pink red; throat yellow……...*A. teretiflora* (Fig. 13A, B)

15a Leaves with velvety pubescence; limb coral-cream or purplish red with papillose protuberances…….....*A. monticola* (Fig. 13C-E)

15b Leaves with sparse to dense septate trichome; limb purplish red, purple dark or maroon…..…….……..….....16

16a Bract cordiform……..…….…...17

16b Bract elliptic to lanceolate-ovate……...……18

17a Tube widened toward the utricle; limb maroon, tuberculat…….....*A. durangensis* (Fig. 13H, I)

17b Tube slim toward the utricle, abruptly widened toward the limb; limb maroon to brown, velvety……..*A. variifolia* (Fig. 14F)

18a Leaves 5-9 cm wide; limb orbicular black, smooth…….…..…….*A.* sp. nov. inedit. (Tlalcoyunque) (Fig. 14D, E)

18b Leaves 2-4.5 (-5.5) cm wide; limb ovate to widely ovate with purplish red-bullate reticulate protuberances……...*A. rzedowskiana* (Fig. 14B, C)

II- leaves lanceolate to ensiform

1a Base of leaves sagittate (lobes up to 2 cm long)……..…....…2

1b Base of leaves attenuate or auriculate (lobes no more than 0.5 cm long)……...…..……3

2a Limb orbicular, apex obtuse ……...…….....*A. acontophylla* (Fig. 14G)

2b Limb ovate, apex acuminate …….……...*A. palmeri* (Fig. 14A)

3a Plants climbing, usually with different leaf shapes ……..……4

3b Plants erect, with lanceolate to ensiform leaves…….……...5

4a Limb broadly heart-shaped to deltate, dark violet, apex acute; throat as well as zone around it yellow green with white trichomes …….....*A. mutabilis* (Fig. 12I, J)

4b Limb lanceolate-ovate to ovate purplish red, apex long acuminate to ensiform, flexuous; throat white……..…..….....*A. nelsonii* (Fig. 13F, J)

5a Base of leaves attenuate, perianth 5-8 (-10) cm long, apex long acuminate ……...…….*A. erecta* (Fig. 15A, B)

5b Base of leaves auriculate, perianth 3-5 cm long, apex attenuate ……..*A. davilae* (Fig. 15E)

III- Leaves lanceolate-ovate

1a Leaf apex obtuse or obtuse-mucronulate……...2

1b Leaf apex acute or attenuate……..…….....3

2a Flowers geniculate, tube bent at least 90º; limb orbicular, apex obtuse …….....**.....***A. secunda* (Fig. 15H, I)

2b Flowers rectilinear, tube erect or subarcuate 10-30º; limb ovate, apex retuse ….....….....…….....*A. bracteosa* (Fig. 15C, D)

3a Leaves amplexicaul or semi-amplexicaul……..……...….4

3b Leaves not amplexicaul or semi-amplexicaul…….…...5

4a Perianth 3-4 cm long, limb oblong, bent 90º, with striate brown lines and toward throat marginally ringed yellow-green……*A. flexuosa* (Fig. 15F, G)

4b Perianth 2-2.5 (-3) cm long, limb ovate, not bent, dark red and toward throat yellow…..….....**.....**….....*A. oaxacana* (Fig. 16D, E)

5a Bract cordiform; limb narrowly oblong, dark violet toward apex……...*A. brevipes* (Fig. 16A, B)

5b Bract lanceolate-ovate; limb lanceolate to lanceolate-ovate, purplish red, dark red or yellow-green toward apex…….**.....**…..6

6a Flower rectilinear; tube erect; limb yellow-green……...*A. occidentalis* (Fig. 16C)

6b Flower geniculate; tube bent 35º to 110º……..….7

7a Leaf base auriculate (lobes 1-3 cm long); limb erect; throat white with purplish red dots…….……*A.* sp. nov. inedit. (Quila) (Fig. 16F, G)

7b Leaf base lobate (0.5-0.8 cm long); limb bent at least 60-90º; throat yellow with red dark dots…….……..*A.* sp. nov. inedit. (Sierra Verde) (Fig. 16H, I)

IV Leaves hastate, deltate or trilobate

1a Leaves deltate or triangular……...……...…..2

1b Leaves hastate to trilobate……...……...…6

2a Plant erect; leaves (5.5-) 9-13 (-20) cm long; throat yellow-green…..*A.* sp. nov. inedit. (Huetamo) (Fig. 17A, B)

2b Plant procumbent or climbing; leaves (0.5-) 1.5-4 (-5) cm long; throat yellow or purplish red…….....….....3

3a Tube slightly bent 20º-30º or erect…….……...4

3b Tube bent 70º-90º……..……...…..……..….5

4a Limb lanceolate-ovate; tube 2-4 cm long, straight……*A. teretiflora* (Fig. 13A, B)

4b Limb trilobate; tube 0.5-0.7 cm long, widened at the base……...*A. cordata* (Fig. 17C, D)

5a Perianth 1-2.5 (-3) cm; leaves becoming abruptly small at the end of the stem; limb ovate white-yellow; throat purplish red…….....*A. conversiae* (Fig. 12G, H)

5b Perianth 4.5-5.5 cm long; leaves more or less the same size; limb rhombic purplish red, throat deep purple ……..*A. karwinskii* (Fig. 17H, I)

6a Flower rectilinear or slightly curveted, limb lanceolate…….……...…..…7

6b Flower geniculate; limb orbicular, ovate to widely ovate or elliptic to widely elliptic…….....….....12

7a Perianth 2-3 (-4) cm long, tube 0.5-0.7 cm long……...……...8

7b Perianth 4.5-6 (-7) cm long, tube 1-2.4 cm long……..……9

8a Limb short triangular, rounded at the base; throat dark……...……..*A. whitei* (Fig. 18G)

8b Limb lanceolate-ovate, base subcordate; throat yellow with purple dots....*A. versabilifolia* (Fig. 17E, F)

9a Leaves (4-) 8-10 cm long……...……..……..…..10

9b Leaves 1-3.5 (-4) cm long……..……11

10a Throat white with purple dots; leaves with basal lobes rounded……...*A. luzmariana* (Fig. 18C, D)

10b Throat yellow with purple dots; leaves with basal lobes sinuous…....……..*A. pentandra* (Fig. 13J)

11a Limb black purple, papillose; throat as well as zone around it yellow with purple dots or dark purple, apex acuminate ……....….*A. wrightii* (Fig. 18E, F)

11b Limb purplish red, minutely tuberculate near the apex; throat as well as zone around it yellow with purple dots and covered with white trichomes……..*A. nana* (Fig. 18A, B)

12a Leaf base anchor-shaped……..……....…...13

12b Leaf base rounded or sinuous……..…….……14

13a Limb orbicular, purplish red; throat elliptic……..……...*A. emiliae* (Fig. 18G, H)

13b Limb ovate, dark violet; throat ovoid……....*A.* sp. nov. inedit. (El Chavarín) (Fig. 18I, J)

14a Limb (widely) elliptic, margin dark red, center white to pale yellow, covered with purple dots more dense in the throat……..…….....….*A. coryi* (Fig. 19F, G)

14b Limb heart-shaped to ovate, widely ovate or orbicular, purplish red, brown yellow to coral cream……...…….....15

15a Leaves basal lobes ½ high…….……16

15b Leaves basal lobes 1/3 to ¼ high……...…...17

16a Abaxial leaf side with markedly yellow veins; limb broadly heart-shaped to deltate, dark violet; throat as well as zone around it yellow green with white trichomes

….....……..….....……...…….*A. mutabilis* (Fig. 12I, J)

16b Abaxial leaf side velvety; limb ovate to elliptic, covered by purplish red or coral-cream papillose protuberances; throat purplish red covered by villous purple trichomes……....*A. monticola* (Fig. 13C-E)

17a Perianth (0.4-)0.5-1-2 (-2) cm long; tube basally widened…….……..18

17b Perianth (2-)2.5-7 (9-) cm long; tube basally straight……..……19

18a Throat elliptic to ovoid, yellow-white; limb purplish red glabrous at the lower part becoming yellow-orange toward the limb; tube basally widened…..*A. lassa* (Fig. 19D, E)

18b Throat reniform, dark purple; limb ovate, yellowish area, covered by purple protuberances…….....*A. pacifica*

19a Limb orbicular, yellow green, maroon in the center, apex rounded....*A. manantlanensis* (Fig. 19C)

19b Limb ovate to widely ovate, purplish red to brown, apex acute to long acuminate…....…….....20

20a Tube straight; limb apex long acuminate to ensiform, flexuous, base cordate; throat white…….....……...……..….*A. nelsonii* (Fig. 13F, G)

20b Tube slim toward the utricle, abruptly widened toward the limb; limb apex acute, base emarginated; throat pale…….…….…….....….*A. variifolia* (Fig. 14F)

V Leaves sagittate or oblong with base auriculate

1a Apex limb flexuous, long acuminate, yellow-greenish…….....*A. pringlei* (Fig. 19A, B)

1b Apex limb short, acute to attenuate, brown purple, purplish red or maroon……...…2

2a Tube with the widest part in the middle…….....*A. micrantha* (Fig. 20E, F)

2b Tube with same diameter over its full length…….…..……..3

3a Bract lanceolate-ovate 0.9-1 x 1.7 cm; limb bent 90º brown purple, around the throat maroon; throat yellow with purple dots…….....*A. colimensis* (Fig. 20A, B)

3b Bract triangulate or lanceolate 0.2-0.6 x 0.1-0.4 cm; limb straight purplish red; throat as well as zone around it yellow with purple dots……..…4

4a Leaves apex long acuminate (sometimes hastate); limb margin maroon, throat yellow-brown……..……...…….*A. watsonii* (Fig. 20C, D)

4b Leaves apex acute mucronulate (sometimes hastate); limb purplish red, throat yellow with purple dots……...…….*A. sinaloae* (Fig. 20G, H)

VII Leaves with different shapes

*This group should be used for specimens with branches showing abruptly different leaf shapes, e.g. trilobate, hastate, and cordate to linear leaves on one single branch.

1a Leaves base obtuse, rounded or subcordate, basal lobes anchor-shaped (rarely widely ovate to ovate), (group IV)……..……...*A.* sp. nov. inedit. (El Chavarín) (Fig. 18I, J)

1b Leaves base cordate or auriculate, basal lobes auriculate to sinuous…….…....2

2a Leaves trilobate with basal lobes ½ high……...……..……..3

2b Leaves trilobate with basal lobes 1/3 to 1/4 high……...……...…4

3a Abaxial leaf side with markedly yellow veins; limb broadly heart-shaped to deltate, dark violet; throat as well as zone around it yellow green, with white trichomes (groups I, II and IV)….....…….……..*A. mutabilis* (Fig. 12I, J)

3b Abaxial leaf side velvety; limb ovate to elliptic, covered by purplish red or coral-cream papillose protuberances; throat purplish red, covered by villous purple trichomes (groups I and IV)…….…….…….....….*A. monticola* (Fig. 13C-E)

4a Flower rectilinear, tube straight; limb maroon to purplish red; throat yellow with purple dots (groups I and IV)…….…….*A. pentandra* (Fig. 13J)

4b Flower geniculate, tube bent 90º-110º; limb purplish red; throat white or paler…….....…5

5a Limb widely ovate, apex acute, base emarginated; tube slim toward the utricle, abruptly widened toward the limb (groups I and IV)…...……..…….……...*A. variifolia* (Fig. 14F)

5b Limb lanceolate-ovate to ovate, apex long acuminate to ensiform, flexuous, base cordate; tube straight (groups II and IV)…….……*A. nelsonii* (Fig. 13F, J)

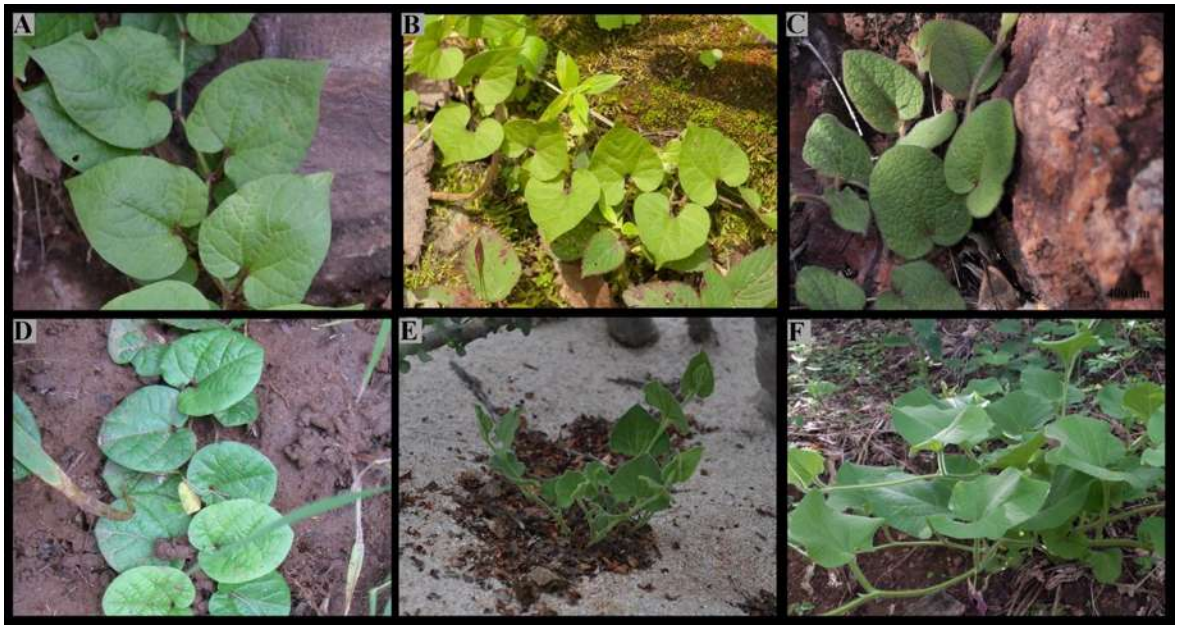

Fig. 6 Group I. Leaves orbicular to widely ovate (cordiform). A. *Aristolochia tequilana*. B. *A. conversiae*. C. *A. buntingii*. D. *A. nahua*. E. *A. monticola*. F. *A. cardiantha*.

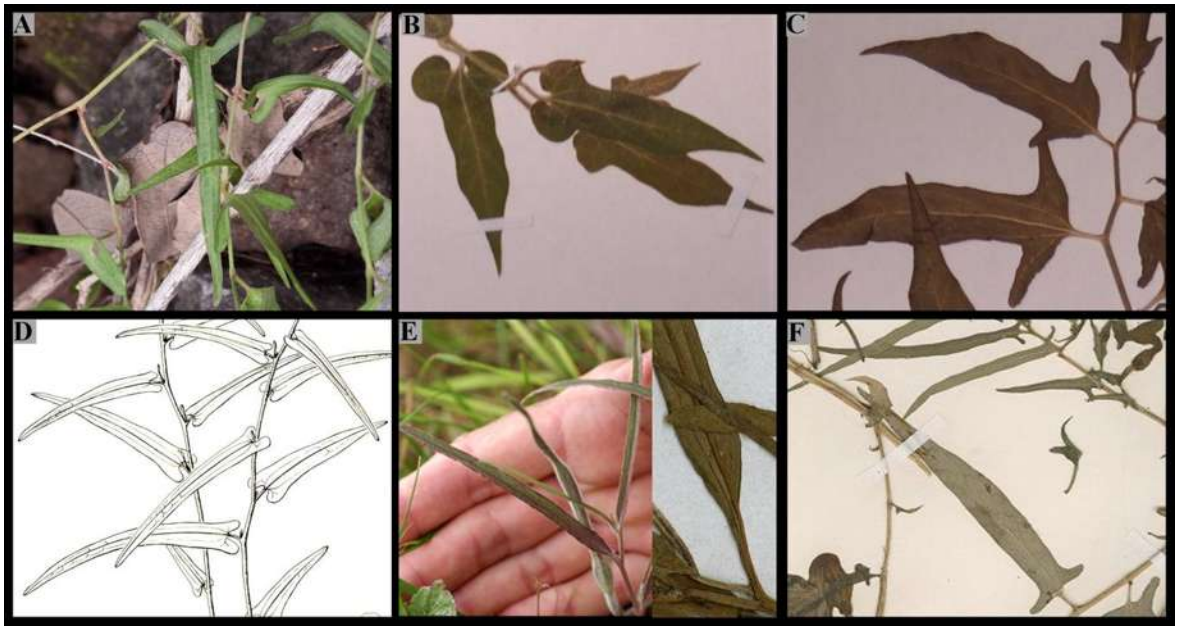

Fig. 7 Group II. Leaves lanceolate to ensiform-orbicular. A. *Aristolochia palmeri*. B. *A. nelsonii* C. *A. mutabilis*. D. *A. davilae*. E. *A. erecta*. F. *A. acontophylla* (B, C, pictures from herbarium MEXU; D. from Calzada et al. 1997; E. from herbarium TEX by Vascular Plant

Image Library (http://botany.csdl.tamu.edu/FLORA/imaxxars.htm 19/01/2016); F. from the US and MICH herbarium).

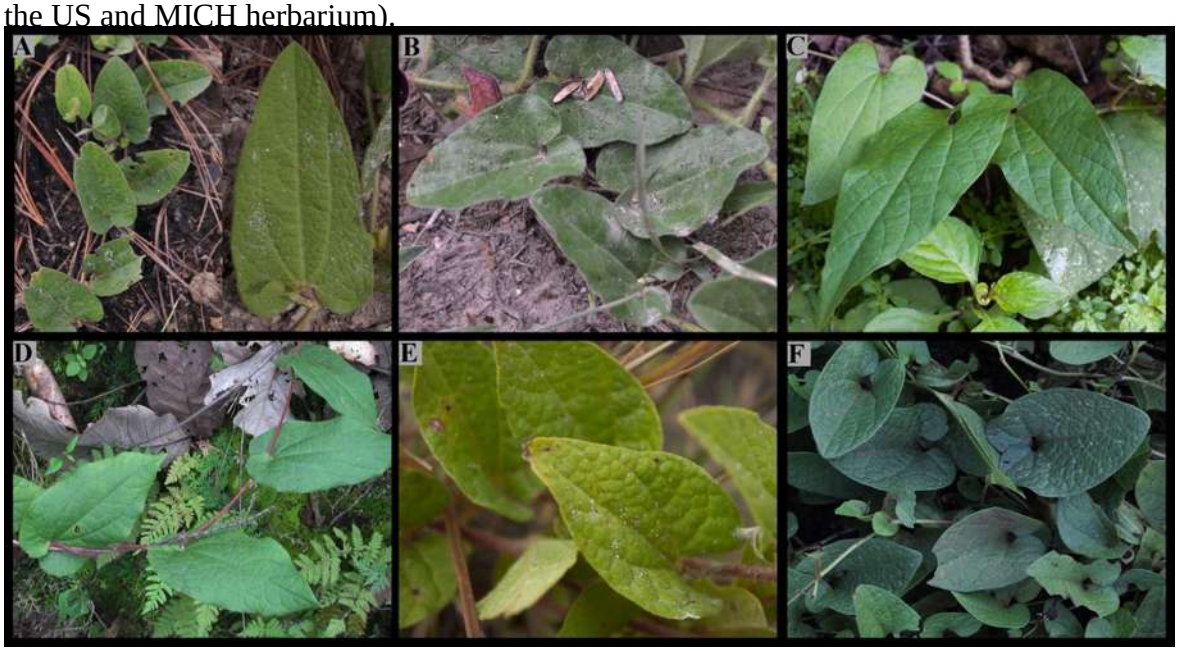

Fig. 8 Group III. Leaves lanceolate-ovate. A. *Aristolochia bracteosa*. B. *A. secunda*. C. *A. occidentalis*. D. *A. flexuosa*. E. *A. oaxacana*. F. *A. brevipes*.

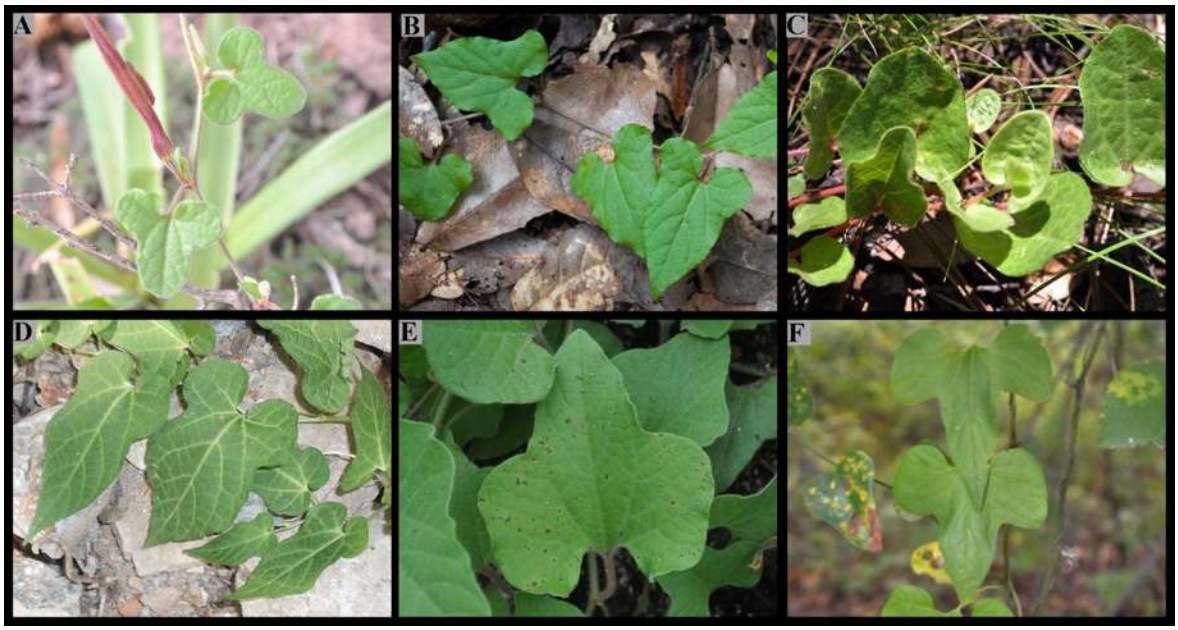

Fig. 9 Group IV. Leaves hastate, deltate or trilobate. A. *Aristolochia nana*. B. *A. luzamariana*. C. *A. cordata*. D. *A. nelsonii*. E. *A. monticola*. F. *A. pentandra* (D. picture by Marie-Stéphanie Samain).

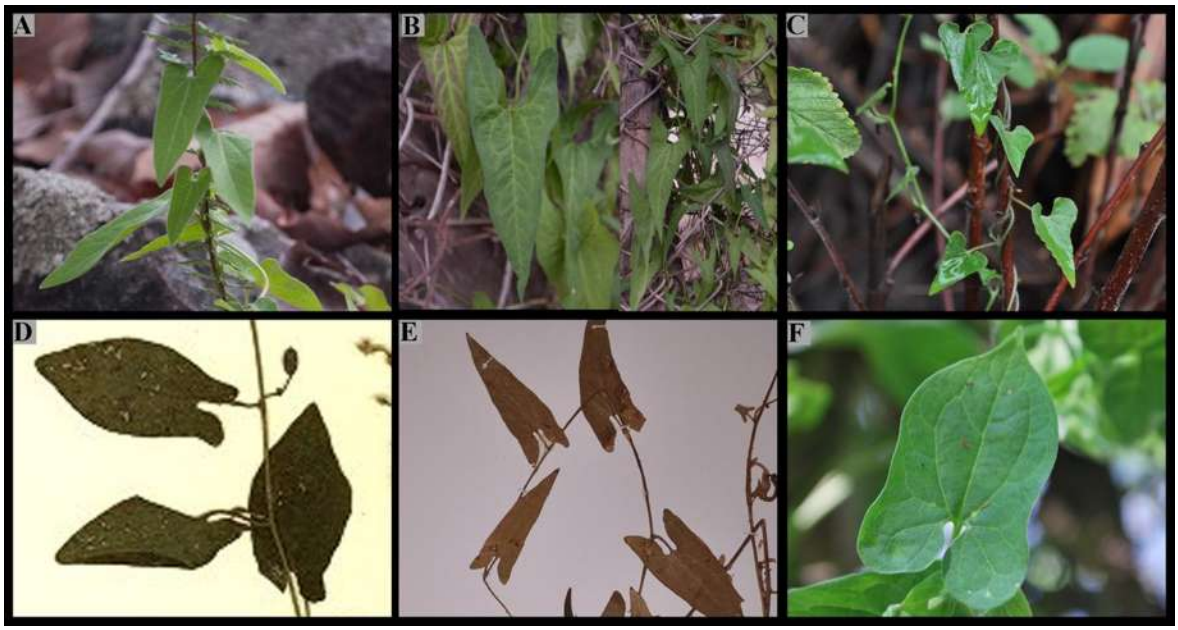

Fig. 10 Group V. Leaves sagittate or oblong with base auriculate. A. *Aristolochia pringlei*. B. *A. watsonii*. C. *A. watsonii*. D. *A. micrantha*. E. *A. sinaloae*. F. *A. colimensis* (D. from the US herbarium; E. from ARIZ herbarium).

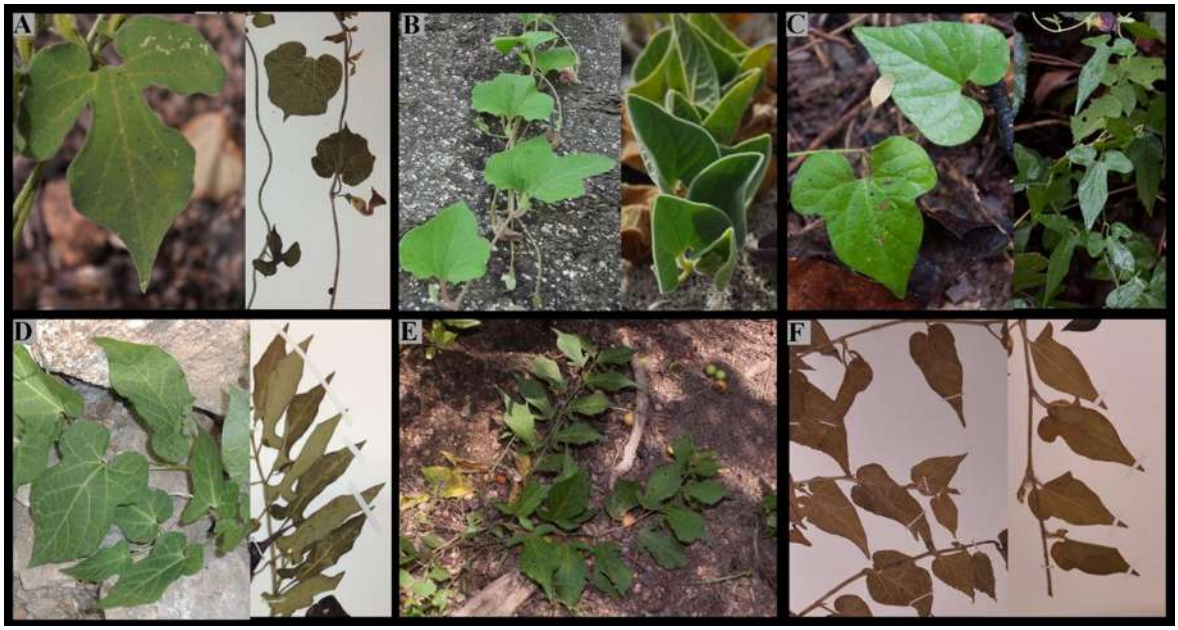

Fig. 11 Group VI. Leaves with different shapes. A. *Aristolochia mutabilis*. B. *A. monticola*. C. *A. pentandra*. D. *A. nelsonii*. E. *A.* sp. nov. inedit. (El Chavarín). F. *A. variifolia* (A, D and F. from MEXU herbarium; D. picture from Marie-Stéphanie Samain).

1.3.1.2 Descriptions

Aristolochia bracteosa Duch, Ann. Sci. Nat. Sér. 4.2:37. 1854. amended description — Type: (based upon *A. rotunda* in herb. Deless. Sessé & Mociño lecta, (holotype: G!; isotypes: F ex G!, MO ex G!).

= *Aristolochia guadalajarana* Watson, Proc. Amer. Acad. 22:448. 1887—Type: Palmer, Edward 27 (holotype: GH!; isotypes: MO, US (2), NY, MEXU!).

= *Aristolochia rotunda* Sessé et Mociño, Fl. Mexic. Ed. 2. 209. 1894, non L. (1753), non Georgi (1797), non Bové ex Duchr. (1864). (holotype: Sessé et Mociño s. n., G!; isotypes: MO ex G!, F ex G).

Geophytic herb, procumbent, **perennial subterranean organs** 50-70 x 0.4 -1 cm; **stems** with 2-3 subterranean internodes, swollen nodes, 10-20 mm long; **aerial internodes** (2.5-) 3-4.5(-5.6) cm long, sparsely covered with septate trichomes. **Petioles** terete (0.6-) 0.7-1 x 0.6-1.3 mm, sparsely covered with septate trichomes. **Leaf blades** elliptic to ovate, discolorous, base cordate, apex acuminate, (2.8-) 3.7-7.5 (-9.5) x (1-) 2-4.5 cm, palmatinerved, actinodromous, basal veins sometimes purple-pink, margin entire, sometimes purple-pink; **adaxial leaf** side green, with a dense septate trichomes, 0.05 mm long and with septate trichomes, 0.7-1 mm long, sparse and with acroscopic orientation, **abaxial leaf** side green pale, with dense septate trichomes, escpecially along the veins. **Flowers axillary,** solitary, growing in the apical part, **peduncle** 0.6-0.7 x 0.3-0.5 mm. **Bract** lanceolate, 1-1.2 x 0.04-0.1 cm. **Perianth** erect, 5-6 cm long, green-yellowish, becoming purple toward the limb, veins yellow, dense septate trichomes along the veins; **utricle** spheroid gibbous, 0.9-1.2 x 0.35-0.6 cm, internal surface yellow with purple dots, covered with moniliform mucilaginous trichomes; **tube** bent 10°-30°, basally straight, 10-16 x 2-3.5 mm, internal surface with black-purple short setose trichomes, 1 mm long, and white setose trichomes toward the syrinx, 0.05 mm long; **limb** ovate, cordate at the base, retuse at the apex, 3-3.6 x 0.8-1 (-1.6) cm; purplish-red verrucose, in lateral view flat; **throat** obovoid, dark-maroon, minutely granular and glabrous; **syrinx** infundibuliform, eccentric, 2.5 mm long, 2.5-3 mm wide at base and 2 mm wide at apex. **Gynostemium** coroniform, stipitate, 2.5-3 x 1.3 mm wide; **stipe** 0.5-1 mm long, **stigmatic lobes** 5, 0.5-1.5 mm long, **anthers** 5, 1 mm long. **Ovary inferior,** lanceolate 5 x (0.8-) 0.9 mm, with sparse to dense septate trichomes. **Fruit** a capsule, obloid to ellipsoid, 1.5 x 0.7-0.9 cm wide, with septicidal dehiscence at the apex. **Seeds** deltoid, 3.5-4 x 3-4.5 mm, upper part black, lower part yellow-brown, with granular surface. Figure 15C, D.

Principal characteristics—This species is easily distinguished by its elliptic to ovate leaves straight; the ovate limb, which is cordate at the base, retuse at the apex, and maroon to purplish red and verrucose and the throat which is obovoid, dark-maroon.

Phenology— This species is reported to flower from June to early October and has been

collected in fruit from April and July to August.

Distribution and Habitat— *Aristolochia bracteosa* is distributed in the states of Jalisco and Nayarit and grows in open secondary vegetation near oak forest, on volcanic highland, from 1,000 to 1,800 m altitude.

Additional Specimens Examined—MEXICO. Jalisco: Mun. Guadalajara, Barranca de, 3 Jul 1991, *Luis Fdo. Castellanos Renteria et al. 18* (IBUG). Mun. Ixtlahuacán del Río, ejido Los Pitayitos 30 km al N de Ixtlahuacán, 1400 m, 23 Mar 1988, *A. Flores Macías 828* (IBUG). Mun. Tala, rumbo a Villa Felicidad a 1 km saliendo del poblado 20°39´28.8″N, 103°40´59″ W, 1379 m, 7 Aug 2014, *A. Paizanni Guillén et al. 238* (IEB); La Primavera, bosque escuela distrito 9°, 1600 m, 26 Jun 1986, *Aarón Rodríguez C. 293* (IBUG); Agua Dulce, hacienda de Huaxtla, río Salado, 30km al W de Guadalajara, 1400 m, 13 Aug 1975, *L.M. Villarreal de Puga* 15605 (IBUG). Mun. Zapopan, La Primavera, 10 Jul 1998, *H. White & W.C. Holmes 554* (BAYLU); Cerro del Col, a 3 km al W de Santa Lucía, 13 Oct 1992, *M. Cházaro B. & R. Acevedo R. 6982* (IEB); Bosque del Nixticuil, 20°45´10″ N, 103°24´34″ W, 1625 m, 30 Jun 2006, *F.Mercado-Muñoz et al. 137, 139* (IBUG); Arroyo del Atlicolte, rancho La Mesa, San Esteban, 1460 m, 10 Jun 1973, *L.M. Villarreal de Puga 4830* (IBUG); La Venta, 20 km al W de Guadalajara, 20°41´53″ N, 103°33´34″ W, 145 m, 23 Jun 1967, *L.M. Villarreal de Puga 4072* (IBUG); Sierra de La Venta, carr. Ameca-Pto. Tortugas, 1450 m, 30 Sep 1968, *L.M. Villarreal de Puga 2682* (IBUG); río Blanco, Jun 1886, *Edward Palmer 27* (MEXU); Baños del Padre 33 km por la carretera Guadalajara-Ameca, 1600 m, 30 Jul 1967, *L.M. Villarreal de Puga 852* (IBUG). Nayarit: Mun. Tepic, 25 miles southeast of Tepic along highway to Guadalajara, 1067-1219 m, 16 Jul 1951, H. S. Gentry 10873 (ARIZ, MEXU). Mun. Zapotamitas, Mex. Hwy 15, E of Zapotamitas, 21.28312 N, -104.63214 W, 1350 m, 29 Jul 1998, *W.C. Holmes & H.L. White 9671* (BAYLU).

Aristolochia brevipes Benth. Pl. Hartweg. 15. 1839, amended description.—TYPE: MEXICO. Aguascalientes, *Hartweg 85* (holotype: K!; isotypes: BM!, E!, F ex K, GH, K!, LD!, NY, P!,).

≡ *Eionomeia brevipes* (Benth.) Klotzsch, Monatsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin 1859: 606. 1859. (post 18 Aug 1859) (Monatsber. Königl. Preuss. Akad. Wiss. Berlin).

= *Aristolochia subclausa* Watson, Proc. Amer. Acad. 20:372.1885. (TYPE: MEXICO. Guanajuato, Tarandacuao, *Dúges, s.n.*, Aug. 1884, (holotype: GH!)).

Geophytic herb, procumbent, **perennial subterranean organs** 26-30 x 2.5 cm; **stems** with 2-3 subterranean internodes, swollen nodes, 6-8 mm long; **aerial internodes** (3.2-) 4-5.5(-6.5) cm long, sparsely covered with septate trichomes. **Petioles** terete (1.5-) 2-3 (-4) x 0.09-0.6 cm, sparsely covered with septate trichomes. **Leaf blades** ovate, discolorous, base cordate to lobate, apex acute, (1.5-) 2.5-5 x (1.3-) 2-3.6 (4.8) cm, palmatinerved, actinodromous, basal veins sometimes purplish red, margin entire; **adaxial leaf** side green, with a dense septate trichomes, (0.05) mm long and with septate trichomes, (0.7-1) mm

long, sparse and with acroscopic orientation, **abaxial leaf** side green pale, with dense septate trichomes, especially along the veins. **Flowers axillary,** solitary or placed in a racemose inflorescence, **flower pedicels** (peduncle) 5-13-20 x 0.1-0.2 mm with septate trichomes. **Bract** cordiform, 0.7-1.2 x 0.2-0.6 cm. **Perianth** geniculate, (3-)4-5 cm long, pale yellow becoming purple-brown toward the limb, with veins brown-orange, sparce septate trichomes along the veins; **utricle** ellipsoid, gibbous, 0.6-0.9 x (0.4-)0.6-0.8 cm, internal surface yellow with purple dots (at the base) covered with moniliform mucilaginous trichomes; **tube** bent 35°-40°, straight, widened toward the limb, (0.7) 10-16 x 0.3-0.4 (10) mm, internal surface with white uniseriate trichomes with strigulose trichomes; **limb** narrowly oblong, cordate at the base, emarginate at the apex, (1.6-)2-3 x (0.5-) 0.7-1 cm, dark-violet toward the apex covered by dark violet verrucose protuberances, around the throat pale yellow with purplish red dots and with a dark violet margin, in lateral view flat; **throat** narrowly cylindrical, pale-yellow with purplish red dots, covered by white trichomes; **syrinx** infundibuliform, slightly eccentric, 1-1.5 mm long, 3 mm wide at base and 1.5-2 mm wide at apex. **Gynostemium** coroniform, stipitate, 1-2 x 1 mm; **stipe,** 0.07-0.1 mm long, **stigmatic lobes** 5, 0.6-1 mm long, **anthers** 5, 1.5 mm long. **Ovary inferior**, linear, (5-)7-10 x 1-2 mm, with sparse to dense septate trichomes. **Fruit** a capsule, ellipsoid, (1.3-)2-3 x (1.2-)1.5-2 cm, green when young, brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 4-5 x (3-)4-5 mm, upper part black, lower part yellow-brown, with granular surface.

Principal characteristics—This species is easy to distinguish because usually grows in volcanic soils, the limb is narrowly oblong, cordate at the base, emarginate at the apex, (1.6-)2-3 x (0.5-) 0.7-1 cm, dark-violet toward the apex covered by dark violet verrucose protuberances, around the throat pale yellow with purplish red dots and with a dark violet margin; the bract is cordiform and large, 0.7-1.2 x 0.2-0.6 cm. Moreover, it is distinguished by growing in volcanic soils. Figure 16A, B.

Phenology— This species is known to flower from July to September and has been collected in fruit in August to September.

Distribution and Habitat— *Aristolochia brevipes* is known from the states of Aguascalientes, Guanajuato, Hidalgo, Mexico and Michoacán, it grows in secondary vegetation, open areas, near pine-oak forest, in volcanic soils from 1,000 to 2,400 m altitude.

Additional Specimens Examined—MEXICO. Edo. Mexico: Mun. Temamatla, 3 km al SE de San Pablo Atlazalpan sobre corriente de roca basáltica, 25 Sept. 1983, *M. González G., J. Rzedowski R. 38319* (IEB);

Mun. Acolman, cerro Chiconautla vertiente S, 2,400 m, 18 Jul 1973, *Rzedowski 30933* (IBUG). Michoacán: Mun. Pátzcuaro, Ladera NE del Cerro Blanco, 14 Jul 1996, *S. Zamudio R. 9823* (IEB). Mun. Tiripetío, por el camino de terrecería que esta atrás de la parada de autobuses viniendo de Morelia hacia Pátzcuaro. En una zona lotificada, 19.57310º N, -101.35415º W, 2186 m, 27 Sep 2015, *A. Paizanni Guillén & J.M. Ramírez Amezcua 318* (IEB).

Aristolochia cardiantha Pfeifer Taxon. Re. Pentand. Aristolochia, 2. 1970. amended description.—TYPE: MEXICO. Guerrero: Mun. Placeres del Oro, Puerto de Oro, 500 m, 15 Jul 1936, *Hinton 9096* (Holotype: US!; isotypes: GH!, K!, MO!, NA).

Geophytic herb, procumbent, **perennial subterranean organs** not seen; **stems** with 1-2 **subterranean internodes**, swollen nodes, 1.2-45 mm long; **aerial internodes** 9-14 cm long, sparsely covered with septate trichomes. **Petioles** terete, (3-)4-8.5 x 0.1-0.3cm, green, sometimes purple-pink, pilose. **Leaf blades** cordiform, discolorous, base cordate and slightly bended, apex abruptly acute to acuminate, (6.0-)10-21 x (6-)9-22 cm; palmatinerved, actinodromous, basal veins purple-pink, margin entire; **adaxial leaf** side green, with orange septate trichomes, dense and with acroscopic orientation; **abaxial leaf** side pale green, veins pale yellow with dense orange septate trichomes, especially along the veins. **Flowers** axillary, solitary, or placed in a racemose inflorescence, growing at the base of the plant, **flower pedicels** (peduncle) 11-15 cm x 0.1-0.4 mm, with dense septate trichomes. **Bract** ovate, 0.9-1.5 x 0.7-1 cm. **Perianth** geniculate, 6.5-11.5 cm long, brown-yellowish, becoming purplish red toward the limb, veins brown-orange, dense septate trichomes along the veins; **utricle** obovoid, (1-)1.5-2 x (0.5-)1-1.5 cm, internal surface yellow with purple dots at the base, covered with mucilaginous trichomes, yellow, purplish red toward the syrinx; **tube** bent 150°-170°, basally widened, 1.5-2.5(-3) x (3-)4-10 mm, internal surface with purplish-dark villous trichomes, 0.3-0.4 mm long; **limb** widely ovate, cordate at the base, cuspidate at the apex, (3-)5-8 x (3.3-)5-8.5 cm, pink-purplish to purplish red area, around the throat ringed starting with a slim line white, then purplish red, margin white, with anastomosing and reticulate venation, covered by verrucose protuberances, in lateral view flat; **throat** pentagon shape, maroon or occasionally yellow, minutely granular and glabrous, covered by purplish red septate trichomes; **syrinx** long tubular, eccentric, 3-3.5 mm long, 3-3.5 mm wide at base and 1.5-2 mm wide at apex. **Gynostemium** coroniform, stipitate, 2-3.5 x 2 mm; **stipe**, 0.1-0.1 mm long, **stigmatic lobes** 5, 1 mm long, **anthers** 5, 2-2.5 mm long. **Ovary inferior**, linear, 1 x 1.5 mm, with lanate septate trichomes. **Fruit** a capsule, ellipsoid, 3-4 x (1-)2-3 cm, brown yellow when

mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 5-7 x 5-6 mm, upper part black, lower part yellow-brown, with granular surface. Figure 12C, D.

Principal characteristics—*Aristolochia cardiantha* is vegetatively similar to *A. foetida* because of the cordiform leaves, straight; the lanate ovary, and the long peduncle or pedicel ((8-)9-12.5 x 0.01 mm), but the principal confusion between this species is when the flowers are inmature, because the flower of *A. cardiantha* is not widened at the base of the limb like at its maturity. However, *Aristolochia cardiantha* can be easily distinguished by its widely ovate limb, cordate at the base, pink-purplish to purplish red area, around the throat ringed starting with a slim line white, then maroon, occasionally the throat, yellow and with a margin white. The surface is anastomosing, and with a reticulate venation, covered by verrucose protuberances. Since 1970 this species was only known from its type location. In the present study, we have registered additional locations for this species and we noticed the coloration variation on the limb.

Phenology—This species flowers from July to September and has been collected in fruit in August to October.

Distribution and Habitat—*Aristolochia cardiantha* is distributed in the states Michoacán and Guerrero. This species grows in open secondary vegetation in tropical deciduous forest, tropical subdeciduous forest, on flat areas or in canyons, from 500 to 1,600 m altitude.

Additional Specimens Examined—MEXICO. Guerrero: Mina. Puerto de Oro, 500 m, 15 Jul 1936, *Hinton 9096* (BD); Adelante de Quechultenango, 16 Aug 1978, *Blanco C., C. Toledo M., E. F. Cabrera C. 495 A* (IEB); Mun. Tixtla de Guerrero, cerro a la orilla de la laguna de Tixtla 17° 37´ 30″ N, 99° 29´ 30″ W, 1600 m, 24 Aug 1998, *B. Ludlow, N. Diego P. 208* (IEB, MEXU); Tlalchapa 18° 25´ 31″ N, 100° 26´ 21 ″ W, 530 m, 2 Aug 2002, *P. Carrillo Reyes y E. Carranza 3272* (IEB, MEXU); Mun. Coyuca de Catalán, pasando Placeres del Oro rumbo a Puerto del Oro (Topotipo), 18°16´03.8″ N, 100°55´39.7″ W, 441 m, 21 Jun 2014, *A. Paizanni Guillén et al. 191* (IEB); A 50m antes de llegar a Puerto del Oro (Topotipo) 18°16´18.6″ N, 100°56´32.0″ W, 536 m, 21 Jun 2014, *A. Paizanni Guillén et al. 192* (IEB). Michoacán: Mun. Arteaga, 10 km de Arteaga, carr. a Cuatro, 24 Oct 2001, *E. Carranza G. 6287* (IEB); 10 km de Arteaga, carr. a Cuatro Caminos, 24 Oct 2001, *E. Carranza G. 6287* (IEB, MEXU). Mun. La Huacana, Sierra las Cruces, Cañada Las Cruces, ca. 6 km (by air) SW of Los Ranchos 18°40´20″ N, 102°3´40″ W, 600 m, 14 Aug 2004, *V. W. Steinmann 4526* (IEB). Mun. Nocupétaro, 9 km al N de Nocupétaro, 28 Sep 1982, *J. C. Soto N. 4625* (IEB). Tiquicheo de Nicolás Romero, en El Guayabo Saucón, 4 Sep 1982, *J. C. Soto N. 4290* (IEB).

Aristolochia coryi I.M. Johnst. J. Arnold. Arbor. 21:256. 1940, amended description.—TYPE: UNITED STATES. Texas: Mun. Arteaga, Substation no. 14, Edwards Co. 8 June 1934, *V. L. Cory 8491* (holotype: GH!)

Geophytic herb, procumbent, **perennial subterranean organs** 15-24 x 0.5-1.7 cm; **stems** slightly ribbed with 2-3 **subterranean internodes**, not seen, 3-15 mm long; **aerial internodes** slightly geniculate, (1.0-)2-5.0 cm long, sparsely covered with septate trichomes. **Petioles** terete, (0.5-)1.0-2.5 x 0.03-0.05 cm, green, sometimes purple-pink, pilose with septate trichomes. **Leaf blades** hastate to deltate, discolorous, base auriculate, apex acute mucronulate, (0.7-)1.5-3(-5) x (0.6-)1.5-2.6(-6) cm; palmatinerved, actinodromous, margin entire; **adaxial leaf** side green, margin sometimes purple-pink, with septate trichomes, dense and with acroscopic orientation; **abaxial leaf** side pale green, veins pale yellow with dense septate trichomes, especially along the veins. **Flowers axillary**, solitary, **peduncles**, (1-)2-5(-8) x 0.01 mm with sparce septate trichomes). **Bract** ovate, (0.3-)0.4-1.8 x (0.1-)0.2-0.5 cm. **Perianth** geniculate, (1.3-)2.3-4 cm long, brown-yellow, becoming purplish red toward the limb, veins brown-orange, with dense septate trichomes along the veins; **utricle** obovoid gibbous, 0.5-0.7 x 0.3-0.5 cm, internal surface yellow with purple dots at the base, covered with mucilaginous trichomes; **tube** bent 60°-90°, basally straight, widened toward the limb, (0.7-)1-1.7 x 0.2-0.3 mm, with white villose trichomes; **limb** elliptic to widely elliptic, emarginated to cordate at the base, obtuse to acute at the apex, (1.0-)1.5-2.0 x (0.3-) 0.5-1 cm, dark red marginal area, center pale yellow covered with purple dots more dense in the throat, in lateral view flat; **throat** elliptic, pale yellow, minutely granular, covered by white trichomes; **syrinx** infundibuliform, eccentric, 2-2.5 mm long, 2 mm wide at base and 1 mm wide at apex. **Gynostemium** coroniform, stipitate, 2.5 x 1.7 mm; **stipe,** 0.05 mm long, **stigmatic lobes** 5, 1 mm long, **anthers** 5, 1.5 mm long. **Ovary inferior**, oblanceolate, 0.6-12 x 0.15 mm, with dense septate trichomes. **Fruit** a capsule, spheroid to elliptic 0.9-2.0 x (0.7-)1-1.6 cm, green when young, brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 5-6 x 5 mm, upper part black, lower part yellow-brown, with granular surface. Figure 19F, G.

Principal characteristics—*Aristolochia coryi* is easy to recognize by its elliptic to widely elliptic limb, with the margin dark red, the center pale yellow covered with purple dots, becoming dense toward the throat and the long straight tube. The leaf shape of *A. coryi* is

similar to the leaves from *A. wrightii* but the latter has an elongate narrow limb, which is dark purple with verrucose protuberances.

Phenology—This species is reported to flower from April to early November and has been collected in fruit from April-May to June-August.

Distribution and Habitat—*Aristolochia coryi* is known from Texas in the United States of America. This species grows along limestone hillside, calcareous soils, clay soils, stream sides with *Celtis*, *Sophora secundiflora*, *Karwinskia*, and *Rhus*. It is common in shade of shrubby motts, from 800 to 1,200 m altitude.

Common Name and Uses—"Cory Dutchman´s pipe".

Additional Specimens Examined—UNITED STATES. Texas: Co. Kerr, Kerr Wildlife management area, about 30 miles northwest of Kerrville, 13 Jul 1958, *Frank W. Gould 8261* (BAYLU); Kerr Wildlife management area, in East bobcat pasture, 0.5 miles northwest of headquartes building, near the dump site UTM 4511, 33266, 22 Jul 2005, *L.L. Sanchez 3935* (BAYLU); Kerr Wildlife management area, across highway 1340 from main entrance UTM 4515, 33257, 28 May 2005, *L.L. Sanchez 3854* (BAYLU). Co. Kimble, At Walter Buck Wildlife Management Area, in Hunt Area 1, near House Blind 1, 30 May 2009, *L. L. Hansen 6579* (TEX, BAYLU). Co. Kinney, Kickapoo Caverns State Park. In large creek which empties into E Fork Sycamore Creek from the west at crossing of park jeep road near northwestern park boundary, 29.624167 N, -100.468056 W, 1715 ft, 20 Jun 1993, *Mark H. Mayfield & Burford Westlund 1733* (TEX). Co. Menard, Westernmost part of county where highway 190 enters into Schleicher Co. 30.866667 N, -100.3 W, 8 Jun 2001, *B. L. Turner 21-776* (TEX). Co. Pecos Just southwest of Sheffield, on and below rimrock of Mesa, 21 Jun 1963, *D. S. Correll & D. C. Wasshausen 27976* (LL); Along highway, 40-45 miles southeast of Stockton toward Sanderson, 3200 ft, 1 Jul 1955, *Barton H. Warnock 13464* (LL); Limestone hills 20 to 35 miles south of Ft. Stockton along Sanderson highway, 3100 ft, 1 Jul 1955, *Barton H. Warnock 13276* (LL); Limestone soil along highway 40 to 45 miles southeast of Ft. Stockton toward Sanderson, 3200 ft, 1 Jul 1955, *Barton H. Warnock 13396* (LL); Limestone Mesa hillside six miles north of Fort Stockton, 3450 ft, 4 Nov 1946, *Barton H. Warnock 46751* (TEX); Along highway one mile east of Fort Stockton, 19 Apr 1946, *Barton H. Warnock 46146* (TEX); Pecos Crevice in caprock 10 feet below top, 27 Jul 1943, B. C. Tharp 43-535 (TEX); Hillside 6 miles north of Forth Stockton, 3450 ft, 4 Nov 1946, *Barton H. Warnock 46751* (TEX). Co. Presidio, Limestone north-facing cliffs, 15 miles southeast of Redford, 14 May 1959, *D. S. Correll & I. M. Johnston 21916* (LL); 13.8 miles east of Redford. Eagle Creek Canyon, 7 Nov 1964, *D. S. Correll & Helen B. Correll 30521* (LL); Eagle Crack, 13.8 miles E. of Redford, 16 Apr 1965, *D. S. Correll & Helen B. Correll 30869* (LL). Co. Real 12.2 miles north of US 83/FM 336, west side of US 83 in highway right-of-way. Growing just south of Haby Ranch Rd/US 83, 584 m, 11 May 2000, *K. H. Clary et al. 410* (TEX). Co. Schleicher Westernmost part of county where highway 190 enters Menard Co, 30.866667 N, -100.316667 W, 8 Jun 2001, *B. L. Turner 21-813* (TEX). Co. Sutton Easternmost part of county, 2.2 mi. north on Harrell Rd. exit from IH 10, 12 Oct 1998, *B. L. Turner 98-470* (TEX). Co. Terrell Fifteen miles north of Sanderson, 3800 feet, 10 Aug 1955, *Barton H. Warnock 14000* (BAYLU); Along highway; fifteen miles north of Sanderson; low limestone hills, 3800 ft, 10 Aug 1955, *Barton H. Warnock 14000* (LL); Low hills in Big Canyon; about

thirty miles north of Sanderson toward Sheffield, 2800 ft, 1 Jun 1957, *Barton H. Warnock & W. D. McBryde 14745* (LL, MEXU); Terrell Near lip of canyon ca. 0.95 airmiles S of well near house on Canon Ranch. or ca. 3.8 airmiles S to SSE of St. Rt. 349 bridge over Independence Creek. Oasis Ranch Quadrangle, 30.405617 N, -101.813883 W, 2400-2420 ft, 1 May 2002, *W.R. Carr & Robert McCurdy 20853* (TEX); 0.5 miles west of intersection of highways 2400 and 1217 along highway 2400 where it drops off the escarpment, northeastern most part of the county, 30.566667 N, -101.933333 W, 17 Jul 2000, *B. L. Turner 20-429* (TEX); SW fork of short E-W canyon entering North Canyon from W just S of N fenceline of Oasis Ranch Preserve (TNC). Sheffield SE Quadrangle, 30.5 N, -101.816667 W, 2400 ft, 24 Apr 2001, *W. R. Carr 19592* (TEX); Low hills in big Canyon; about thirty miles north of Sanderson towar Sheffield, 2800 ft, 1 Jun 1957, *Barton H. Warnock 14745* (BAYLU, MEXU). Co. Val Verde, On open slope along route #183, 13 miles north of Comstock, 19 Jul 1957, *D. S. Correll & I. M. Johnston 18245* (LL); Rock crevices in Eagle Cave Canyon just east of Langtry, 21 Apr 1966, *D. S. Correll & R. C. Rollins 32604* (LL); S side of a NE-SW stretch of Phillips Canyon, 300-1000 ft SW of a road crossing, ca. 0.5-0.6 airmiles S o SSW of confluence of Phillips and Huffstedter canyons, 1.6 airmiles NNW of mouth of Bluff Canyon at Devils River, on A.O. Baker Ranch. Telephone Canyon Quadrangle, 29.966667 N, -101.107778 W, 1520 ft, 4 Apr 2000, *W.R. Carr et al 18809* (TEX); Amistad National Recreation Area: Pecos River picnic area and boat launch. Sample plot no. 95. Seminole Canyon Quad (1:24,000), 24 May 2002, *Jackie M. Poole 4577* (TEX); Mouth of Pecos River along delta which clogs up the once beautiful Pecos River. 29.7 N, -101.366667 W, 1 May 1999, B. L. Turner 99-217 (TEX); 5 mi. e. of Shumla, 29 Aug 1953, *B. L. Turner & B. C. Tharp 53-481* (TEX); Outcrops of Devils River Limestone on lower walls of nameless canyon, ca. 1.1-1.2 mi N of concrete stock tank at old windmill, ca. 3.9-4.0 air miles N to NNW of mouth of Painted Canyon at Pecos River, roughly 8 mi ENE of Langtry. Pecos River Ranch. Shumla Quadrangle, 29.875 N, -101.445278 W, 1400 ft, 10 Jun 1999, *W. R. Carr 18313* (TEX); N side of road to camp / houses, E side of Devils River ca. 1.8 airmiles SSE of mouth of Little Satan Canyon. Satan Canyon Quadrangle. Devils River State Natural Area Southern Unit, 29.675517 N, -100.993467 W, 1250-1300 ft, 16 Oct 2012, *W. R. Carr 32070* (TEX).

Aristolochia emiliae Santana-Michel & Solís-Magallanes Acta Bot. Mex. 82: 7-13. 2007. amended description.—Type: MEXICO. Jalisco: Mun. La Huerta, Estación de Investigación, Experimentación y Difusión Chamela, UNAM, en la vereda El Búho antes del arroyo La Chachalaca, 180 m. 11 September 1998, *F. J. Santana–Michel 9238* (holotype: ZEA!; isotypes: ENCB!, IBUG!, IEB!, MEXU!).

Geophytic herb, procumbent or erects, **perennial subterranean organs** (not seen); **stems** with **subterranean internodes** (not seen); **aerial internodes** geniculate, (1.5-)2-3 cm long, sparsely covered with septate trichomes. **Petioles** terete, (0.3-)0.4-0.9(-1) x 0.4-0.5 cm, green, sometimes purple-pink, sparsely covered with septate trichomes. **Leaf blades** trilobate, like anchor shape discolorous, base hastate, apex caudate, (2.5-)5-8.5(-10) x (1-)1.5-3.5(-4.5) cm; palmatinerved, actinodromous, margin entire; **adaxial leaf** side

green, with septate trichomes, 0.7-1 mm long, sparse and with acroscopic orientation; **abaxial leaf** side pale green, (veins pale yellow) with dense septate trichomes, especially along the veins. **Flowers** axillary, solitary or placed in a racemose inflorescence growing at the base, **flower pedicels** 5-8 x 1 mm. **Bract** lanceolate, 0.5-0.8 x 0.3-0.5 cm. **Perianth** geniculate, 2-2.8 cm long, white-yellowish, becoming purple toward the limb, veins brown-orange, sparce septate trichomes along the veins; **utricle** obloid, 0.9-0.11 x 0.08-0.09 cm, internal surface not seen; **tube** bent 130°-180° basally straight, widened toward the limb, 1.3-2.1 x 4-5 mm, internal surface (not seen); **limb** orbicular, subcordate at the base, apiculate at the apex, 2-3.5 x 2-2.2 cm, deep purple red area covered by white uncinate trichomes 0.3-0.4 mm long, in lateral view cup-shaped; **throat** elliptic, dark-purple, glabrous; **syrinx** infundibuliform, eccentric, 3-3.5 mm long, 2-2.5 mm wide at base and 1 mm wide at apex. **Gynostemium** coroniform, stipitate, 2-3 x 1-2 mm; **stipe,** 0.5-1 mm long, **stigmatic lobes** 5, 1-1.5 mm long, **anthers** 5, 1.5-2 mm long. **Ovary inferior**, lanceolate-ovate, 6 x 9 mm, with sparse septate trichomes. **Fruit** a capsule, ovoid, 2-3 x 1.7-2.5 cm, purple when young, brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 5-6 x 4.5-5.5 mm, upper part black, lower part yellow-brown, with granular surface. Figure 18G, H.

Principal characteristics—This species can be distinguished because the of the anchor-shaped leaves, the orbicular limb, subcordate at the base, apiculate at the apex, deep purple red area, covered by white uncinate trichomes and the throat oblate principally. This species is similar to *Aristolochia* sp. nov. inedit. (El Chavarín) because of the trilobate leaf shape and differs in the limb which has an ovate shape, and is purple pink covered by whitish to hyaline trichomes and the throat, which is ovoid dark-purple.

Phenology—According to our data this species flowers from September to November, and has been collected in fruit in November.

Distribution and Habitat—*Aristolochia emiliae* is known only from the type locality in tropical deciduous forest with *Lysiloma microphyllum, Bursera instabilis, B. heteresthes, Jatropha malacophylla, J. standleyi, Croton pseudoniveus, Cordia alliodora, Heliocarpus pallidus, Trichilia trifolia subsp. Palmeri, Lonchocarpus eriocarinalis, Guettarda elliptica, Spondias purpurea, Caesalpinia coriaria, Ceiba aesculifolia, Thouinia paucidentata, Ficus cotinifolia, Bernardia spongiosa, Crescentia alata.* It grows from 100 to 200 m altitude.

Additional Specimens Examined—MEXICO. Jalisco: Mun. La Huerta, Estación de Investigación, Experimentación y Difiusión Chamela, UNAM, 150 m. 29 September 1982, *J. A. Solís-Magallanes 3849, Emily J. Lott ibid 1532;1699* (MEXU).

Aristolochia flexuosa Duchr. Ann. Sci. Nat. sér. 4 2:36. 1854. amended description.—

TYPE: MEXICO. Michoacán: Mun. Apatzingán, 1841, *Ghiesbreght 216* (holotype: P!).

Geophytic herb, climbing to procumbent, **perennial subterranean organs** 15-20 x 0.6-0.8 cm, **stems** with 1-2 **subterranean internodes**, swollen nodes, 5-15 mm long; **aerial internodes** (4-) 6.5-10 (-11.5) cm long, sparsely covered with septate trichomes, purple-pink. **Petioles** terete (0.2-) 0.6-1.2 x 0.2-0.4 mm, pink-purple, sparsely covered with septate trichomes. **Leaf blades** elliptic to ovate, discolorous, base cordate, apex acuminate, (2.0-) 4.5-9 (-13) x (1-) 3-4.5(-5.5) cm, palmatinerved actinodromous, basal veins pink-purple, margin entire; **adaxial leaf** side green with septate trichomes, 0.7-1 mm long, sparse and with acroscopic orientation; **abaxial leaf** side pale green. **Flowers** axillary, solitary or placed in a racemose inflorescence, growing in the apical part, **flower pedicels** 0.5-1 x 0.3-0.4 mm. **Bract** lanceolate, 1-1.5 x 0.2-0.3 cm. **Perianth** geniculate, 3-4 cm long, green-yellowish veins brown-orange, with dense septate trichomes along the veins; **utricle,** elliptic gibbous (0.5-) 6 x 0.4-0.5 cm, inside surface yellow with purple dots, covered with moniliform mucilaginous trichomes; **tube** slightly bent 40°, basally straight, 7 x 2-4 mm, internal surface with short setose trichomes; **limb** oblong, cleft at the base, retuse at the apex, 2- 4 x 0.6-0.7 cm; bent 90° respect the tube and utricle, base yellow-green with striate brown lines toward the apex and a marginally ringed yellow green, in lateral view flat; **throat** elliptic, dark-purple, covered by white septate trichomes around; **syrinx** infundibuliform, eccentric, 1.5 mm long, 4 mm wide at base and 1.5 mm wide at apex. **Gynostemium** coroniform, stipitate, 2.5 x 2.5 mm wide; **stipe** 0.5-1 mm long, **stigmatic lobes** 5, 0.5-1 mm long, **anthers** 5, 1-1.5 mm long. **Ovary inferior**, lanceolate 7-10 x 1.6-2.5 mm, with sparse to dense septate trichomes. **Fruit** a capsule, obloid to ellipsoid, 1.5 x 0.7-0.9 cm wide, green when young, brown yellow when mature, with septicidal dehiscence at the apex. Seeds deltoid, 3.5-4 x 3-4.5 mm wide, upper part black, lower part yellow-brown, with granular surface. Figure 15F, G.

Principal characteristics—*Aristolochia flexuosa* is easily to recognize by his limb oblong, cleft at the base, retuse at the apex; bent 90° respect the tube and utricle, base yellow-green with striate brown lines toward the apex and a marginally ringed yellow green and the throat elliptic, dark-purple.

Phenology—This species has been found flowering in October. The fruiting season remains unknown.

Distribution and Habitat—*Aristolochia flexuosa* is known from the municipalities of Apatzingán, Uruapan and Ziracuaretiro in the state of Michoacán, it grows in canyons of disturbed pine-oak forest as well as forest with tropical elements in humid canyons with *Pinus, Bursera Acacia, Quercus, Boconia, Ficus cotinifolia.* From 1,300 to 1,500 m altitude.

Additional Specimens Examined—MEXICO. Michoacán: Mun. Uruapan, del poblado de Jucutacato pasando la cascada de la Tzaráracua por el camino que va hacia la Tzararacuita, 19°20´58.08″ N, 102°4´48.16″ W, 1,435 m, 31 August 2013, *A. Paizanni Guillén & J. M. Ramírez-Amezcua 118* (IEB); Desde el poblado Jucutacato, rumbo a la cascada de la Tzararácua 19°21´01.9″ N, 102°04´43.9″ W, 1,451 m, 5 July 2014, *A. Paizanni Guillén et al. 194* (IEB), Desde el poblado Jucuctacato, rumbo a la cascada de la Tzararácuita (Topotipo), 19°21´01.7″ N, 102°04´48.31″ W, 441 m, 5 October 2014, *A. Paizanni Guillén & J.M. Ramírez Amezcua 297* (IEB).

Aristolochia foetida Kunth Nov. Gen. et Sp. Pl. 2:147, t.114. 1817 (*ex* ic.) amended description.—TYPE: Valladolid (Mexico), *Bonpland A.J.A. and F.W.H.A. von Humboldt s.n.* (holotype: P!).

= *Aristolochia velutina* Duch, Ann. Sci, Nat. sér. 4, 2:39. 1854. (Type: Sessé et Mociño lecta, s.n., ex herb. Pavon proveniens, FI!)

≡ *Howardia foetida* (H.B.K.) Klotzsch, Monatsb. Acad. Berlin. 1859:619. 1859.

= *Aristolochia anguicida* Pav. ex Duch. In Prodromus Systematis Naturalis Regni Vegetabilis 15-1:443. 1864, non Jacq. (1762), nec Sieber ex Duchr. (1864). (Type: Sessé et Mociño lecta, s.n., ex herb. Pavon proveniens, (holotype: FI))

= *Aristolochia valentina* Duchr. ex Jacks. Index Kew. 190. 1893, sphalm. = Aristolochia velutina Duchr.

= Aristolochia longipes Watson, Proc. Amer. Acad. 22:447. 1887. (Type: Palmer, Edward 139, (holotype: GH; isotypes: NY, US))

Geophytic herb, procumbent, **perennial subterranean organs** not seen; **subterranean internodes**, not seen; **aerial internodes** slightly geniculate, 5-7(-10) cm long, sparsely covered with septate trichomes. **Petioles** terete, (3-)4-6(-9) x 0.9-1.2 cm, green, sometimes purple-pink, sparsely covered with septate trichomes. **Leaf blades** cordiform, rare reniform, discolorous, erect, base cordate and slightly bended, apex abruptly acute to acuminate, (4.5-)8-12.3(-27) x (3.5-)7-10(-20) cm; palmatinerved, actinodromous, in some leaves basal veins purple-pink margin entire; **adaxial leaf** side green, with septate trichomes, sparse and with acroscopic orientation; **abaxial leaf** side pale green, (veins pale

yellow) with dense septate trichomes, especially along the veins. **Flowers** axillary, solitary or placed in a racemose inflorescence, **flower pedicels** (peduncle) (8-)9-12.5 x 0.01 mm. **Bract** lanceolate, 0.4-1 x 0.5 cm. **Perianth** geniculate, 7-9(-11) cm long, white-yellowish, becoming purple toward the tube, veins brown-orange, sparce septate trichomes along the veins; **utricle** obovoid 1-1.7 x (0.5-)0.7-1 cm, internal surface yellow with purple dots at the base, covered with moniliform mucilaginous trichomes; **tube** bent 20°-40°, basally widened, 10-20 x 7-10 mm, internal surface with black-purple trichomes, cover with short setose trichomes; **limb** lanceolate-ovate, cordate to rounded at the base, ensiform at the apex, 6-8 x 0.9-1.5(-2) cm, pink-purple area covered by verrrucose protuberance, around the throat ringed starting with a slim line white, then purple red, in lateral view flat; **throat** widely elliptic, yellow, glabrous covered by small white and purple trichomes; **syrinx** long tubular, eccentric, 3-5 mm long, 3-6 mm wide at base and 2-3 mm wide at apex. **Gynostemium** coroniform, stipitate, 3-3.5 x 2 mm; **stipe,** 0.1 mm long, **stigmatic lobes** 5, 1 mm long, **anthers** 5, 2 mm long. **Ovary inferior**, oblanceolate, 10-15 x 2-5 mm, with lanate septate trichomes. **Fruit** a capsule, elliptic to ovoid, 2-4 x 1-1.6 cm, purple to dark-purple when young, brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 3.5-4 x 3-4.5 mm, upper part black, lower part yellow-brown, with granular surface. Figure 12A, B.

Principal characteristics—*Aristolochia foetida* is similar to *A. cardiantha* by the cordifom leaves, erects; ovary lanate; long peduncle or pedicel ((8-)9-12.5 x 0.01 mm), but the principal confusion between this species is when the flowers are immature, because the flower of *A. cardiantha* is not widened at the baseof the limb as when is mature. However, *Aristolochia foetida* it can be easily distinguishing by its limb lanceolate-ovate, cordate to rounded at the base, ensiform at the apex, with a purple area covered by verrucose protuberance, around the throat ringed starting with a slim line white, then purple red and the throat yellow.

Phenology—This species flowers from May to October and has been collected in fruit in August to October.

Distribution and Habitat—*Aristolochia foetida* is distributed in the center and west of Mexico in the states of Jalisco, Colima, Michoacán and Zacatecas. This species grows in open secondary vegetation in tropical deciduous forest, tropical subdeciduous forest, on flat areas or in canyons, from 85 to 1,500 m altitude.

Common Name and Uses——"Cigarrera" (Cerro Viejo, Jalisco), referring to the shape of

the perianth.

Additional Specimens Examined— MEXICO. **Colima**: Mun. Comala, 19-20 km al NW de Colima, 1-2 km al E de Campo Cuatro, 13 Aug 1991, *L. Guzmán H., et al. 1345* (IEB); Rancho El Jabalí. 22 km NNW of the city of Colima at the Jalisco state line. La Atarjea Canyon, 1.5 km SE of Hacienda San Antonio on the road to Comala, SW foothills of the Volcán de Colima, 19º26´18″ N, 103º42´30″ W, 1300m, 19 Sep 1991, *A.C. Sanders, I. Garcia, T. Ross, B. Rothschild, L. Vazquez 11481* (UCR); Rancho El Jabali, 22 km NNW of the city of Colima at the Jalisco state line near Hacienda San Antonio. c. 1.5 km E of the ranch headquarters & just SW of Lago El Jabali, SW foothills of the Volcán de Colima, 19º26´50″ N, 103º42´ W, 1275m, 23 Aug 1988, *A.C. Sanders, I. Garcia R. et al. 8270* (UCR); Rancho El Jabali, 22 km (airline) NNW of Colima; the Colima/Jalisco line cuts through the ranch. Near Lago Epazote, SW foothills of the Volcán de Colima, 19º26.3´ N, 103º41.2´ W, 1400m, 18 May 1991, *A.C. Sanders et al. 11021* (UCR); 19-20 km al NW de Colima 1-2 km al E de Campo Cuatro, 1500 m, 13 Aug 1991, *L.Guzmán H. et al. 1345* (IBUG). Mun. Minatitlán 2 km al SE de Platanarillo, 10-11 km al E de Minatitlán, carr. Colima- Minatitlán, C. Grande, 19º23´3 N, 103º56´49″ W, 950m, 3 Sep 1990, *L. Guzmán H. & R. Cuevas G. 1077* (IEB). **Guerrero**: Mun. Chilpancingo, Alrededores de Chilpancingo, 17º34´46″ N, 99º30´50″ W, 1290m, 2 Aug 2007, *G. Ibarra Manríquez et al. 5453* (IEB); Alrededores de Chilpancingo, 17º34´46″ N, 99º30´50″ W, 1290m, 2 Aug 2007, *G. Ibarra Manríquez et al. 5453* (MEXU). Mun. Leonardo Bravo, Atlixtac, 0.5 km al W rumbo a Chichihualco, 17º37´51″ N, 99º39´31″ W, 1360m, 18 Sep 1997, *B. González H. 1182* (IEB, MEXU). Mun. Eduardo Neri, 2 km al E de Xochipala, 13 Oct 1981, *J. L. Redondo s.n.* (IEB). Mun. Tlapa, aprox. 5 km al W (WSW) de Tlapa, por la carr. Chilpancingo-Tlapa, 1350m, 23 May 1997, *E. Carranza y R.M. García 5279*(IEB). Mun. Zumpango del río, 6 km al SO de Xochipala carr. a Filo de Caballo, 1350 m.m, 15 Aug 1985, *J. C. Soto Núñez & Simón Román G. 9854* (IBUG, MEXU). Mun. Ahualulco, 100m. al SE de el Carmen al SE de Ahualulco, 30 Nov 1979, *F.Sandoval Vázquez 54* (IBUG). **Jalisco**: Mun. Ameca, ejido El Cuis, 15 May 1978, *Pedro C. Ríos s.n.* (IBUG); 1180m, 11 Jun 1986, *González Jiho M.G. 39* (IBUG); 2 km al sur de Ameca por la plaza de Toros, 1180 m, 12 Jun 1986, *M. A. Guerrero Nuñez 35* (IBUG); Hda. El Cabezón, 28 May 1976, *J.L. Cuenca Lopéz s.n.* (IBUG). Mun. Atengo, 20º16´42″ N, 104º12´52″ W, 1550 m, 22 Oct 1982, *J.B. López & M. Mariscal s.n.* (IBUG). Mun. Atoyac, laguna de Sayula, Isla Chica parte norte, 1300 m, 22 Jul 1993, *E.Villegas F. Y R. Ramírez D. 370* (IBUG). Mun. Chapala, Ca. 1 km E of San Juan Cosala, N side of Lago de Chapala, 1550-1600 m, 18 Jul 1997, *W.C. Holmes 9029* (BAYLU); ca. 1 km E of San Juan Cosala, N side of Lago de Chapala, 1550-1600 m, 18 Jul 1997, *W.C. Holmes 9029* (BAYLU); San Julián, rivera del lag de Chapala, 1560 m, 18 May 1979, *J.Cortés Moreno 25* (IBUG). Mun. Cocula, Santa Rosa de Lima a 4km al, 1350 m, 9 May 1972, *L.M. Villarreal de Puga 3904* (IBUG). Mun. El Limón, rancho El Recodo 2km al E de San Miguel Hidalgo, 2 km al E de San Miguel Hidalgo, 850 m, 9 Sep 1987, *F.J. Santana Michel 2963* (IBUG); Cerro de La Cruz 500 m. al SW de El Limón, 19º49´27″ N, 104º9´24″ W, 1000m.m, 5 Aug 1995, *F.J. Santana Michel 7274* (IBUG). Mun. Guadalajara, Barranca de Huentitán, abajo del Zoológico Guadalajara, 29 Jul 1990, *J. J. Guerrero N. 870* (IEB); Barranca de Huentitán, abajo del Zoológico Guadalajara, 29 Jul 1990, *J. J. Guerrero N. 870* (IEB); Barranca de Huentitán, microcuenca del Cerro del Diablo, 4 km al NE de Tonalá, 8 Aug 1990, *A. Flores M. 2504* (IEB); Barranca de Huentitán, microcuenca del Cerro del Diablo, 4 km al NE de Tonalá, 8 Aug 1990, *A. Flores M. 2504* (IEB); Ladera SW de la barranca del Río Santiago (Ibarra), entrando en Privada Panorámica y Volcán Sajama, al S del Parque

Independencia, 10 Aug 1996, *M. Harker S. 1741* (IEB); barranca de Oblatos 2.5 km. antes del balneario, 20º42´25″ N, 103º15´14″ W, 1210 m, 25 Jun 2000, *J.Acosta-Velázquez 89* (IBUG); Puente Guadalupe 800m. al poniente y 20 m. del río Santiago, 1400 m, 8 Jul 1986, *Castrejón A. & Hernández A. 009* (IBUG); Barranca de Oblatos del río Grande Santiago camino hasta el balneario Oblatos; disposición de ladera hacia el este o noreste, 1505-1300m.m, 10 Oct 1995, *M. Harker et al. 505* (IBUG); Barranca del río Santiago lado de Huentitán aprox 2km al N del funicular del CFE, 20º44.5´ N, 103º18.2´ W, 1080m.m, 7 Jun 1997, *P.Carrillo M. Ayón & M. Harker 213* (IBUG); Ladera SW de la barranca del Río Santiago (Ibarra) entrando en Privada Panorámica y Volcán Sajama al S del parque Independencia, 1465m.m, 10 Aug 1996, *M. Harker et al. 1741* (IBUG); Barranca de Huentitán el bajo abajo del zoológico Guadalajara, 1400 m, 13 Nov 1997, *J. J. Guerrero Nuño et al. 1367* (IBUG); Barranca de Huentitán el bajo abajo del zoológico Guadalajara, 1500 m, 29 Jul 1990, *J. Jesús Guerrero 870* (IBUG). Mun. Ixtlahuacán del Río, rancho Mezquitán carr. Colotlán, 1400m.m, 25 May 1979, *F. Miramontes Nava s.n.* (IBUG). Mun. Jocotepec, camino de ascenso al ¨Cerro Viejo¨por la poblado de Zapotitlán de Hgo., 1800 m, 20 Aug 1986, *Aaron Rodríguez C. 561* (IBUG). Mun. Juchitlán, El Llano, 1250 m, 12 Sep 1980, *J. F. Cobián Olmedo s.n.* (IBUG). Mun. La Huerta, Estación Chamela UNAM, camino Eje Central rumbo a Caliandra, 19º29´54.1″ N, 105º2´39.5″ W, 81m, 27 Sept 2014, *A. Paizanni Guillén et al. 294* (IEB). Mun. Ocotlán, rivera N de la laguna de Chapala carr. Ocotlán La Barca km 9, º´ N, º´ W, 1490m.m, 18 Mar 1989, *H.Arreola Nava & S. González C. 1101* (IBUG). Mun. Poncitlán, a 1km al S de Casa Blanca, Arroyo del Tigre, 1480 m, 8 Aug 1976, *L.M. Villarreal de Puga 9198* (IBUG). Mun. San Cristobal de la Barranca, Mezquitán 58 al N., 1500 m, 13 May 1979, *R. Lopéz Jauregui s.n.* (IBUG). Mun. San Martín Hidalgo, Sierra de Quila, río Grande, 20º19´42″ N, 104º4´13″ W, 1400 m, 2 Sep 1990, *J. Jesús Guerrero Nuño 903* (IBUG). Mun. Tala, San Isidro Mazatepec sur-oeste como a 2 km de un arroyo, 1500 m, 17 Oct 1982, *M. Buenrostro Lopéz 1ºA* (IBUG). Mun. Techaluta, 2 km E del poblado orilla de la vía del tren, 1300 m, 9 Jul 1993, *E.Villegas F. & R. Ramírez D. 319* (IBUG). Mun. Tlajomulco de Zuñiga, San Miguel Cuyutlán, camino hacia el Cerro Viejo, pasando el primer arroyo, 20º23´36.9″ N, 103º23´52.6″ W, 1292m, 18 Jul 2014, *A. Paizanni Guillén et al 205* (IEB); San Miguel Cuyutlán, camino hacia el Cerro Viejo, 4 m antes de llegar al primer arroyo, 20º23´51.6″ N, 103º24´3.1″ W, 1786m, 18 Jul 2014, *A. Paizanni Guillén et al 207* (IEB); El Aguaje, por el camino al arroyo Hondo cerca de La Laguna, Cajititlán, 11 Oct 1997, *C. Cortés R. & E. Ortiz C. 170* (IEB, IBUG). Mun. Tolimán, camino a Toxín, Puerto Toxín, 27 May 1990, *A. Rodríguez C., et al. 2040* (IEB, MEXU). Mun. Tonalá, Barranca de Colimilla, 1 Aug 1996, *R. Acevedo R. & M. Hernández-Galaviz 1620* (IEB); Barranca de Colimilla, 1 Aug 1996, *R. Acevedo R. & M. Hernández-Galaviz 1620* (IEB). Barranca de Colimilla cerca del camino, 1400 m, 1 Aug 1996, *R. Acevedo R. & M.Hernández-Galaviz 1620 (IBUG)* (IBUG); 2 km al N del rancho de La Cruz en cañada, 1600 m, 7. Jul 1990, *M.L. Lomelí 24* (IBUG). Mun. Tuxcacuesco, Cerro del Palacio, 5-6 km al WSW de Tuxcacuesco, 1100m, 8 Sep 1994, *F. J. Santana M. et al. 6815* (IEB); 9-10 km al SWW de Tolimán 4-5 km al SW de San Pedro Toxin , 19º33´53″ N, 103º59´46″ W, 1400-1700m, *R.Cuevas & L.Guzmán 3743* (IBUG). Mun. Zapopan, Las Animas márgenes Río Santiago Pto. de Guadalupe, 960 m, 15 Jul 1973, *L.M. Villarreal 4947* (IBUG). Mun. Zapotitlan de Vadillo, rancho El Jabali, 22 km NNW of the city of Colima at the Jalisco state line near hacienda San Antonio. Arroyo and cafetal below hacienda San Antonio, 19º26.6´ N, 103º40.5´ W, 1400 m, 20 Sep 1991, *A.C. Sanders, I. García R., T. Ross, B. Rothschild & L. Vazquez 11553* (UCR); La María, 22 km airline NNW of Colima [near Rancho El Jabali], SW foothills of the Volcán de Colima,

19º27.5´ N, 103º42.5´ W, 1250 m, 21 May 1991, *A.C. Sanders et al. 11111* (UCR); Rancho El Jabalí, 22 km (airline) NNW of Colima; Colima/Jalisco line passes through ranch. La Joya area, north of the airstrip and south of arroyo Santa Cruz, east of Cerro El Campanerio, SW foothills of the Volcán de Colima, 19º27´ N, 103º41´ W, 1420m, 22 Sep 1991, *A.C. Sanders et al. 11681* (UCR). Mun. Casimiro Castillo, carretera Barra de Navidad-Guadalajara, al NE de El Zapotillo, 340 m, 27 Sep 1992, *R. Ramírez Delgadillo 2931* (IBUG). **Michoacán:** Mun. Villamar, Cerro de Cotijarán, 1550m, 9 Jul 1986, *Baudelio Ceja 365* (UCR). Mun. Río Duero near dam, 1520 m, 11 Jul 1998, *W.C. Holmes, H.L. White, R. Joyner & J. Buis 316* (BAYLU); near dam; ca, 1520 m, 11 Jul 1998, *W.C.Ho1mes et al.* (BAYLU). Mun. Tiquicheo, en el Guayabo Saucón, 650 m, 4 Sep 1982, *J. C. Soto Núñez 4290* (IBUG). Mun. Jacona, Cerro Curutarán, 19º56´33.8″ N, 102º17´33.4″ W, 1850 m, 10 Aug 2007, *P. Reyes M., et al. 61* (IEB). Mun. Los Reyes, Camino a Palillos, 19 Aug 2004, *I. García R., A. Linares 6711* (IEB). **Zacatecas:** Mun. Juchipila, cerro El Piñón, Sierra de Morones, 1250-2130 m, 2 Aug 1996, *L.M. Villarreal de Puga 17121* (IBUG); Moyahua, los Otates, 1050 m, 20 Aug 1992, *E. D. Enríquez E. 106* (IBUG). Mun. Moyahua de Estrada, 3 km al oeste de Las Palmas, Palo Blanco, 3 Sep 1992, *E. D.Enríquez E. 195* (IBUG).

Aristolochia karwinskii Duch in DC. Prod. 15-1:442. 1864. amended description.—TYPE: MEXICO. *Karwinsky W.F. von 712* (holotype: LE!; isotypes: M!, MPU!).

Geophytic herb, procumbent, **perennial subterranean organs** not seen; **stems** with 2-3 **subterranean internodes**, not seen; **aerial internodes**, 1.2-2 cm long, sparsely covered with septate trichomes. **Petioles** terete, 0.4-0.7 x 0.02 cm, green, sometimes purplish red, sparsely covered with septate trichomes. **Leaf blades** deltoid, discolorous, base subcordate, apex acute, (0.7-)1.7-3.0 x (0.4-)1.2-3 cm; palmatinerved, actinodromous, margin entire; **adaxial leaf** side green, with septate trichomes with acroscopic orientation; **abaxial leaf** side pale green, with dense septate trichomes, especially along the veins. **Flowers** axillary, solitary or placed in a racemose inflorescence, **flower pedicels** (peduncle) 4-7 x 0.01-0.02 mm, with dense septate trichomes). **Bract** ovate, 1-2 x 0.7 cm. **Perianth** geniculate, 4-6(-8) cm long, brown-orange, becoming maroon toward the limb, veins brown-orange, with dense septate trichomes along the veins; **utricle** obovoid to ellipsoid, 0.7-0.8 x 0.5-0.7 cm, internal surface, not seen; **tube** bent 90°, basally straight, widened toward the limb, 1.5-1.7 x 4-6 mm, internal surface, not seen; **limb** rhombic, obtuse at the base, acute at the apex, 2.5-3 x 1-2 cm, **in** lateral view flat; **throat** elliptic; **syrinx**, not seen. **Gynostemium, stipe** not seen**, stigmatic lobes** 5, **anthers** 5. **Ovary inferior**, oblanceolate, 10 x 2 mm, with dense septate trichomes. **Fruit** not seen. Figure 17H, I.

Principal characteristics—*Aristolochia karwinskii* can be easily distinguished by its deltoid leaves, the rhombic limb, the elliptic throat, and the perianth that is up to 8 cm long.

***Notes*—**This species is one of the less represented ones in herbaria collections.

Phenology—This species is reported to flower from May. The fruiting season remains unknown.

Distribution and Habitat—This species is currently only known from Tamaulipas and its unclear type locality region which might be from Veracruz. It grows in rosette scrub, xerophytic scrub vegetation and is associated to limestone, from 2000 m altitude.

Additional Specimens Examined—MEXICO. Tamaulipas: Mun. Bustamante, 3 km al N de La Joya de Herrera, 2000 m, 24 May 1976, *F. González-Medrano 9111* (MEXU); 4 km al N de La Joya de Herrera, 2000m, 24 May 1976, *F. González-Medrano 9122*(MEXU).

Aristolochia lassa I.M. Johnst Arnold Arb. 21:255. 1940. amended description.—TYPE: MEXICO. Coahuila: Mun. Saltillo, common in bottom lands, May 1898, *Palmer 187* (holotype: GH!; isotypes: BM!, K!, MO! UC!).

= *Aristolochia watsonii sensu* Pfeifer Taxon. Re. Pentand. Aristolochia, 53. 1970.

Geophytic herb, procumbent and climbing, **perennial subterranean organs** 20-40; **stems** with 1-2 **subterranean internodes**, swollen nodes, 10-12 mm long; **aerial internodes**, 1.5-2 cm long, sparsely covered with septate trichomes. **Petioles** terete, (0.1-)0.8-1.3 x 0.05-0.2 cm, green, sometimes purple-pink, sparsely covered with septate trichomes. **Leaf blades** hastate to trilobed, discolorous, base auriculiform, apex acute, (0.5-)1.3-5.3 x (0.5-)1.3-4.0 cm; palmatinerved, actinodromous, basal veins purplish red, margin entire; **adaxial leaf** side green, glabrescent with some uncinate septate trichomes; **abaxial leaf** side pale green, veins pale yellow with sparce septate trichomes, especially along the veins. **Flowers axillary,** solitary or placed in a racemose inflorescence, **flower pedicels** (peduncle) 0.4-0.7 x 0.01-0.02 mm, with sparse septate trichomes. **Bract** deltate to widely ovate, 0.4-0.7 x 0.2-0.5 cm. **Perianth** geniculate, 1.5-2 cm long, yellow, becoming maroon toward the limb, veins brown-orange, with dense septate trichomes along the veins; **utricle** obovoid to elliptic, 0.4-0.5 x (0.2-)0.3-0.5 cm, internal surface yellow with purplish red dots at the base covered with mucilaginous trichomes; **tube** bent 70°-90° basally widened, 0.7-0.8 x 0.2-0.3 mm, internal surface with purplish red villose trichomes; **limb** widely ovate, cordate at the base, obtuse to acuminate at the apex, (0.4-)0.5-1.2 x (0.3-)0.5-0.7 cm, maroon glabrous at the lower part becoming yellow-orange toward the limb with purplish red villous trichomes, **in lateral view** flat; **throat** elliptic to ovoid yellow-white, minutely granular and glabrous, covered pusplish red villous

trichomes; **syrinx** infundibuliform, eccentric, 1.5 mm long, 1.5 mm wide at base and 1 mm wide at apex. **Gynostemium** coroniform, stipitate 1.5-2 x 1.5-2 mm; **stipe,** 0.1 mm long, **stigmatic lobes** 5, 0.05-1 mm long, **anthers** 5, 1 mm long. **Ovary inferior**, oblanceolate, 0.5-0.6 x 0.1-0.2 mm, with dense septate trichomes. **Fruit** obovoid to spheroid, 1.5 x 1.6 cm, young fruit not seen, brown-orange when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 4 x 3.5 mm, upper part black, lower part yellow-brown, with granular surface. Figure 19 A1,2.

Principal characteristics—*Aristolochia lassa* is characterized by its widely ovate limb, maroon glabrous at the lower part, becoming yellow-orange toward the limb with purplish red villous trichomes and the elliptic throat which isyellow with villous purplish red trichomes. The leaf shape is similar to *A. versabilifolia*, but the latter one occurs in Querétaro, Guanajuato and San Luis Potosí; the limb is lanceolate, maroon and around the throat yellow with purplish red dots. Furthermore, it is closely related to *A. karwinskii* but this species has larger deltoid leaves and the perianth is longer, reaching up to 8 cm.

Phenology—This species has been collected flowering from September and has been found in fruit in May.

Distribution and Habitat—*Aristolochia lassa* is distributed in the state of Coahuila in the municipalities of Coahuila and Nuevo León. It grows in grass lands or in open secondary vegetation, from 1769 to 2026 m altitude.

Notes—Pfeifer (1970) considered *Aristolochia lassa* as a synonym to *A. watsonii*. The description in Pfeifer´s publication is referring to *A. lassa.* However, few years later Pfeifer (1976) rectified this misunderstanding because the synonym of *A. watsonii* is *A. porphyrophylla*. As a consequence, we here recognize *Aristolochia lassa* as distinct from the two aforementioned species.

Additional Specimens Examined—MEXICO. **Coahuila:** Mun. Saltillo, In the Botanical Garden of "Universidad Agraria Antonio Narro", 25°21´24.7″ N, 101° 01´56.7″ W, 1769 m, 2 Sept 2014, *A. Paizanni Guillén et al. 261* (ANSM, IBUG, IEB, DR, MEXU). **Nuevo León:** Mun. Arramberri, Ascención->Sandia, 1900 m, 9 May 1992, *G.S. Hinton 21939* (GHB); 14 km aprox. del poblado La Sandía rumbo a La Ascención, 24°18´52.5″ N, 99°57´46.3″ W, 2026 m, 9 Sept 2014, *A. Paizanni Guillén et al. 267* (ANSM, IBUG, IEB, DR, MEXU).

Aristolochia monticola Brandegee Univ. Calif. Publ. Bot. 6:357. 1916. amended description.—TYPE: MEXICO. Baja California Sur: Mun. Los Cabos, on Sierra de la Laguna, 21 Jan 1899, *T.S. Brandegee 508 (116656* herbarium number*)* (holotype: UC!).

= *Aristolochia peninsularis* Brandegee amended description Univ. Calif. Publ. Bot. 6:357. 1916. —TYPE: MEXICO. Baja California Sur: Mun. Los Cabos, near San José del Cabo, 29 Sept 1890, *T.S. Brandegee 507 (116657 herbarium number)* (holotype: UC!).

Geophytic herb, procumbent, **perennial subterranean organs** 20-50 long; **stems** with 2-3 **subterranean internodes**, swollen nodes, 4-6 mm long; **aerial internodes** slightly geniculate, 3-6(-8.5) cm long, dense covered with septate trichomes. **Petioles** terete, (0.3-)1-3(-3.5) x 0.01-0.1 cm, green, sometimes purplish red, pubescent. **Leaf blades** tripartite, hastate or ovate, discolorous, base auriculate to hastate, apex mucronate, acute, (1.0-)3.3-7(-14) x (1.0-)2.7-7(-10) cm; palmatinerved, actinodromous, basal veins purplish red, margin entire; **adaxial leaf** side green, with velvety septate trichomes and with acroscopic orientation; **abaxial leaf** side pale green, veins white-yellow with velvety septate trichomes, especially along the veins. **Flowers** axillary, solitary or placed in a racemose inflorescence, **flower pedicels** (peduncle) (2.8-)3-10 x 0.1-0.3(-0.4) cm with dense septate trichomes). **Bract** ovate to hastate, (0.5-)0.6-1.5 x 0.3-0.7 cm. **Perianth** geniculate, 4.5-6(-8.5) cm long, pale green-yellowish, becoming purplish red toward the limb, veins white-yellow, with dense septate trichomes along the veins; **utricle** obovoid to ellipsoid, 0.6-1.2 x (0.4-)0.6-1 cm, internal surface yellow with purple dots at the base covered with moniliform mucilaginous trichomes; **tube** bent 45°-50°, basally straight, (8-)10-17 x (4-)5-10 mm, internal surface with purplish red villose trichomes; **limb** ovate to elliptic, cordate at the base, mucronulate at the apex, (1.2-)2-3 x 1-2 (-3) cm, purplish red or coral-cream marginal area, around the throat purplish red or coral-cream with purple dots, covered by purplish red to coral-cream papillose protuberances, in lateral view flat; **throat** elliptic, purplish red, minutely granular and covered by villous purple trichomes; **syrinx** infundibuliform, eccentric, 2 mm long, 1.5 mm wide at base and 1 mm wide at apex. **Gynostemium** coroniform, stipitate, 2.5 x 2 mm; **stipe,** 0.05-0.1(-0.6) mm long, **stigmatic lobes** 5, 1 mm long, **anthers** 5, 1.5 mm long. **Ovary inferior**, oblanceolate, 5 x 1.3 mm, veins purplish red with lanate septate trichomes. **Fruit** a capsule, ovoid to ellipsoid, 1-2-(4) x (0.7-)1-2.5 cm, gray-green when young, with veins purplish red, brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 5-6 x 5-6 mm, upper part black, lower part yellow-brown, with granular surface. Figure 13C-E.

Principal characteristics—*Aristolochia peninsularis* is here considered as a synonym of *A. monticola*, similar to Pfeifer (1970). The main differences considered by Brandegee (1916) to separate these two species were the following: *A. peninsularis* grows in sandy

soils close to the beach and its the leaves are usually ovate to saggittate, whereas *A. monticola* grows in tropical, subtropical forest and has auriculate leaves. However, during fieldwork we compared the flowers of both species which were exactly the same. The limb usually is coral-cream, sometimes purplish red with papillose protuberances. Additionally, we also noticed the variability in leaf shape and and plant size, depending on the habitat. Hence we adopted the name *A. monticola*, following Pfeifer (1970).

Phenology—*Aristolochia monticola* has been found flowering from January-May to September-November and has been collected in fruit in September.

Distribution and Habitat—This species is currently only known from Baja California Sur and some islands in the Sea of Cortés. It grows in granite, sand soils in tropical deciduous forest on granite boulders, close to creeks, in arid scrub vegetation, coastal dune as well as on beaches, from 0 to 600 m altitude.

Common Name and Uses—"Hierba del indio". The perennial subterranean organs are used to reduced weight, cholesterol, diabetes headache and against snakebites. It is prepared by boiling the subterranean organs and the infusion is drunk, and the local people keep a glass jar with slices in their house (½ slice in ½ liter boiled water and drunk 3 times/day).

Additional Specimens Examined—MEXICO. Baja California Sur: Mun. La Paz, playas Ostiones, isla San José, 26º45´ N, 111º58´ W, 3m, 8 Mar 2006, *M. Domínguez León 3903* (HCIB); Isla San José, Los Ostiones, 25º1.943´ N, 110º42.82´ W, 3 m, 20 Sep 2015, *A. Paizanni Guillén & S. Müller 302* (IEB); Isla San José, Los Ostiones, 25º1.943´ N, 110º42.82´ W, 3 m, 20 Sep 2015, *A. Paizanni Guillén & S. Müller 304* (IEB); La Burrera, a 27 km al E de Todos Santos, 550m, 14 Oct 1985, *P. Tenorio L. et al. 10488* (MEXU); La Burrera, a 27 km al E de Todos Santos, 550 m, 14 Oct 1985, *P. Tenorio L. Et al. 10488* (MEXU); San Bartolo, 100 m N of the road MEXICO-1 from La Paz to Los Barriles, 23º44´23″ N, 109º51´36.7″ W, 432m, 24 Sep 2015, *M. S. Samain & S. Wanke 2015-014* (IEB); San Isidro, Arroyo Boca de Alamos, 3 km SW of the road Los Barriles-Santa Teresa, 23º52´1.74″ N, 109º49´2.7″ W, 150m, 24 Sep 2015, *M. S. Samain & S. Wanke 2015-015* (IEB); After the town El Sargento, follow the principal road, 9m, 18 Sep 2015, *A. Paizanni Guillén y S. Müller 298* (IEB). Mun. Los Cabos, La Ribera, carr. entre el hotel Punta Colorada y el rancho Las Lagunas, 23º30´ N, 109º35´ W, 15 m, 3 May 2001, *R. Domínguez Cadena 2365* (HCIB); Sol de Mayo NW de Santiago, 23º28´ N, 109º50´ W, 350m, 2 Nov 1986, *J.L. León 2239* (MEXU, HCIB); Sierra de la Laguna, rancho El Mesquitillo, about 14 km E of Todos Santos, 23º27´35.2″ N, 110º5´26.7″ W, 344m, 19 Sep 2015, *M. S. Samain & S. Wanke 2015-008* (IEB); Sierra de la Laguna, rancho El Mesquitillo, about 14 km E of Todos Santos, 23º27´35.2″ N, 110º5´26.7″ W, 344m, 19 Sep 2015, *M. S. Samain & S. Wanke 2015-009*(IEB); 10 km after the town Santiago (Sol de Mayo), on the direction to the San Dionisio canyon, 23.53496 N, -109.7836 W, 339 m, 23 Sep 2015, *A. Paizanni Guillén y S. Müller 312* (IEB); San Dionisio canyon, in the ranch El Refugio, 23.55198 N, -109.81461 W, 340m, 23 Sep 2015, *A. Paizanni Guillén y S. Müller 313* (IEB); San Dionisio canyon, in the ranch El Refugio, 23.55149 N, -109.81638 W, 343m, 23 Sep

2015, *A. Paizanni Guillén y S. Müller 315* (IEB); Going to Cabo Pulmo, between the hotel Punta Colorada and rancho Las Laguna, in the rocky mountain area, 23.55327 N, -109.49857 W, 18m, 25 Sep 2015, *A. Paizanni Guillén y S. Müller 317* (IEB); Sol de Mayo NW Santiago, 23º28´ N, 109º50´ W, 350m, 2 Nov 1986, *J.L. León 2239* (MEXU, HCIB). Mun. Mulegé, sierra San Francisco: North of San Ignacio, cañón San Gregorio, northeast of the ranch San Francisco de la Sierra, 27º40´ N, 112º59´ W, 500-800m, 18 Oct 1997, *J. Rebman and J. Delgadillo 4437* (HCIB, UCR). Mun. Todos Santos, Sierra de la Laguna: Northeast of Todos Santos; cañón La Burrera vicinity of rancho Corral Grande (topotipo), 23º30´20" N, 110º1´27" W, 580 m, 27 Oct 1998, *J. Rabman, M. Domínguez, J. Barry and S. Wolf 5720* (UCR). Costa del Golfo de California, 3 m, 8 Mar 2006, *M. Dominguez Leon 3903* (ARIZ).

Aristolochia rzedowskiana Santana-Michel & Guzmán Acta. Bot. Mex. 106:1-7 2014, amended description.—TYPE: MEXICO. Jalisco: Mun. Puerto Vallarta, Potrero de Abajo, entre La Estancia y Las Palmas de Arriba, por la carr. Mascota-Puerto Vallarta, 360 m, 9 Dec 2006, *F. J. Santana-Michel & L. Guzmán-Hernández 12366* (holotype: ZEA!; isotypes: ENCB, IBUG!, IEB and MEXU!).

Geophytic herb, procumbent, **perennial subterranean organs** 15-25 x 0.8-1 cm; **stems** with 1-2 **subterranean internodes**, swollen nodes, 0.8-25 mm long; **aerial internodes** geniculate, (1.5-)4-6.5 cm long, sparsely covered with septate trichomes. **Petioles** terete, (0.8-)1-2.5(-4.3) x 0.01-0.4 cm, green, sometimes purplish red, dense covered with septate trichomes. **Leaf blades** ovate to hastate, discolorous, base auriculate, apex acuminate to attenuate, (2.5-)3.5-7(-11) x (1.5-)3-4(-5) cm; palmatinerved, actinodromous, basal veins purplish red, margin entire; **adaxial leaf** side green, with septate trichomes sparse and with acroscopic orientation; **abaxial leaf** side pale green, veins pale yellow, with sparse septate trichomes, especially along the veins. **Flowers** axillary, solitary or placed in a racemose inflorescence, **flower pedicels** (peduncle) (4-)5-8.5(12) x 1-1.5 mm, with sparse septate trichomes. **Bract** lanceolate, (0.4-)0.5-0.7(-1.1) x 0.2-0.35(-5) cm. **Perianth** geniculate, 3.5-5 cm long, white-yellowish, becoming purplish red toward the limb, veins yellowish-brown, sparse septate trichomes along the veins; **utricle** subglobose to obovoid, 0.5-0.8 x 0.4-0.6 cm, internal surface yellow with purple dots at the base, covered with moniliform mucilaginous trichomes; **tube** bent 80°-90°, basally widened, (1.0-)1-1.5(-2) x 2.5-3.5 mm, internal surface glabrous with purplish red dots starting at the throat and toward the syrinx pale-yellow; **limb** widely ovate to ovate, cordate at the base, attenuate or obtuse at the apex, 2.5-3.5 x 1-1.5 cm, yellowish marginal area covered by violet to purplish red-bullate reticulate protuberances, in lateral view flat; thro**at** ovaid, pale yellow with purplish red dots or violet, minutely granular and glabrous; **syrinx** tubular, eccentric, 2-3.3 mm long,

1.5-1.8 mm wide at base and 1 mm wide at apex. **Gynostemium** coroniform, 1.5-3.5 x 1.5-2 mm; **stipe,** 0.6-1.5 mm long, **stigmatic lobes** 5, 1 mm long, **anthers** 5, 1-1.4 long. **Ovary inferior**, oblanceolate, 5-7(-8) x 1.5-2 mm, with sparse to dense septate trichomes. **Fruit** a capsule, subglobose, 1.8-2.5 x 1.8-2 cm, brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 4-5 x 4-6 mm, 1 mm height, upper part black, lower part yellow-brown, with granular surface. Figure 14B, C.

Principal characteristics—*Aristolochia rzedowskiana* is similar to *A. variifolia*, *A. karwinskii* and *A. manantlanensis* because of its geniculate flowers which are more than 3 cm long, its short peduncle or pedicels and the tube which is bent more than 90°. However, *A. rzedowskiana* is easy to distinguish by its widely ovate to ovate limb, with a yellowish marginal area covered by violet to purplish, red-bullate reticulate protuberances.

Phenology—This species is reported to flower from August, November to December and has been collected in fruit from November.

Distribution and Habitat—*Aristolochia rzedowskiana* is known from the municipalities of Cabo Corrientes, Puerto Vallarta, San Sebastián del Oeste, and Talpa de Allende in the state of Jalisco. This species grows in tropical subdeciduous forest with *Brosimum alicastrum, Astronium graveolens, Ficus insipida, Cecropia obtusifolia, Couepia polyandra. Acacia polyphylla*, *Cymbopetalum hintonii, Enterolobium cyclocarpum, Hura polyandra, Sapium macrocarpum, Randia armata*, *Bursera simaruba, Luehea candida;* tropical deciduous forest with *Psidium sartorianum, Cnidoscolus, Annona, Pedilanthus, Stemmadenia, Opuntia* and pine-oak forest with *Quercus magnoliifolia, Q. resinosa, Q. castanea, Q. gentry, Q. elliptica, Clethra* and *Lysiloma acapulcense.*

Additional Specimens Examined—MEXICO. JALISCO: Mun. Cuale, bridge El Zapote, carr. Cuale-Puerto Vallarta, 7 km of Cuale to the bridge Los Lobos, 20° 25′ 30.6″N, 104° 4′ 45.3″W, 1130 m, 29 Nov 2009, *A. Paizanni Guillén* et al. 71 (IBUG). Mun. Puerto Vallarta, between Las Palmas and Mascota, km 56, 20° 49′ 57.2″N, 105° 00′ 35.8″W, 357m, 15 Sept 2014, *A. Paizanni Guillén* et al.274 (IEB, MEXU). Mun. San Sebastián del Oeste, road San Sebastián to Los Reyes, 20° 46′50.5″N, 104°50′ 25.4″W, 1489 m, bosque *Pinus-Quercus*, con *Clethra*, 16 Sept 2014, *A. Paizanni Guillén* et al. 275 (IEB, MEXU); Potrero El Bajo antes de llegar a La Palma, Carretera libre a Pto. Vallarta después de La Estancia, 13Q, 500048N, 2303259W, 431 m, 22 Nov. 2009 A. Paizanni Guillén et al. 62 (IBUG); Los Arrayanes antes de llegar a Las Palmas, Carr. libre a Pto. Vallarta después de La Estancia, 13Q 498804N, 2303611W, 271 m, 22 Nov 2009, *A. Paizanni Guillén et al. 63* (IBUG); km 86 brecha Mascota-San Felipe de Hijar. 20° 50′ 91″N, 104° 45′ 57″W, 1300-1330 m, 15 Aug 1998, *R. Ramírez Delgadillo et al. 5601* (IBUG); 9 km (en linea recta) y 14.9 km (por la brecha a La Palma) al NW de La Estancia, 20° 48′ 57″N, 104° 58′ 48″W, 500-600 m, 28 Jul 2004, *P. Carrillo-Reyes & F. Nicolalde 4296* (IBUG). Mun. Talpa de Allende, 16-17 km al SSE de Talpa por el camino a La Cuesta, 20° 14′ 15″N, 104° 46′ 40″W, 1370 m, 22 Aug 2004, *P. Carrillo-Reyes & A. Kennedy*

4391 (ZEA).

Aristolochia watsonii Wooton & Standl. Contr. U.S. Natl. Herb. 16: 117. 1913 (based upon *A. brevipes* Benth. var. *acuminata* Watson), amended description.

= *Aristolochia brevipes* var. *acuminta* Watson, Proc. Amer. Acad. Arts 18: 148. 1883, non *A. acuminata* Lam. 1783. (Lectotype: N. Mex, 1851-52, *Wright 1701*, (syntypes: GH!, MO, NY (2), UC!, US).

= *Aristolochia porphyrophylla* Pfeifer, Taxon. Rev. Pentandr. Species Aristolochia 94. 1970. (TYPE: *Gooding 1279*, (holotype: US; isotype: NY).

= *Aristolochia socorroensis* Pfeifer, Taxon. Rev. Pentandr. Species Aristolochia 82. 1970. TYPE: MEXICO. Archipiélago Revillagigedo: Mun. Socorro Island, vicinity of Academy Bay, 18º 50 ¾´ N, 110º 56 ¾ ´ W, 15 m, 15 March 1957, *Moran 5910* (holotype: CAS; isotypes: MEXU!, RSA!, SD, UC).

Geophytic herb, procumbent, **perennial subterranean organs** 18-28 x (0.4-)0.94-1.75 cm long; **stems** slightly ribbed with 1-2 **subterranean internodes**, swollen nodes, (0.5-)13-28 mm long; **aerial internodes**, (2.0-)2-7(-12) cm long, sparsely covered with septate trichomes. **Petioles** terete, (0.7-)2-3 x 0.01-0.1 cm, green, sometimes purple-pink, sparsely covered with septate trichomes. discolorous, base auriculate, apex narrowly acute, (1.2-)3.5-10(-14.5) x (0.7-)1.5-5(-8) cm; palmatinerved, actinodromous, margin entire; **adaxial leaf** side green and purple red when is expose to the sun, veins pale yellow, with septate trichomes dense and with acroscopic orientation; **abaxial leaf** side pale green, with sparse septate trichomes, especially along the veins. **Flowers** axillary, solitary, or placed in a racemose inflorescence, **flower pedicels** (peduncle) 1-4 x 0.5-0.8 mm, with dense septate trichomes. **Bract** widely triangular to widely ovate, 0.3-0.7 x (0.1-)0.2-0.35 cm. **Perianth** geniculate, (1.2-)3.4-5(-6.2) cm long, pale yellow becoming purple in the tube and toward the limb pale yellow, veins brown-purple, dense septate trichomes along the veins; **utricle** obloid gibbous, **(**0.5-)0.6-1.0 x (0.2-)0.4-0.7 cm, internal surface yellow with purple dots covered with moniliform mucilaginous trichomes; **tube** bent 90°-120°, straight, (0.6)1-2 x (0.1-)0.2-0.4 mm, internal surface with short strigulose trichomes white at the base purple at the apex; **limb** lanceolate-ovate, cordate at the base, acute to narrowly acute at the apex, (0.9-)2-4(-5.2) x (0.2-)0.4-0.6 (1.0) cm, purplish red to maroon marginal area minutely granular and glabrous, covered by a white to dark-purple villose trichomes around the troath, in lateral view flat; **throat** obovoid, yellow with deep purple red dots, covered by villose white trichomes; **syrinx** infundibuliform, eccentric, 1.3 mm long, 2-4 mm wide at

base and 1 mm wide at apex. **Gynostemium** coroniform, stipitate, 1-2.5 x 2-2.5 mm; **stipe,** 1-2.5 mm long, **stigmatic lobes** 5, 1.0 mm long, **anthers** 5, 1.2-1.5 mm long. **Ovary inferior**, narrowly elliptic, (5-)8-10 x 1-2(-5) mm, with sparse to dense septate trichomes. **Fruit** a capsule, obloid to ellipsoid, (1.3-)1.5-2(-2.7) x (0.7-)1-1.6 cm, green when young brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 4 x 3-4 mm, upper part black, lower part yellow-brown, with granular surface. Figure 20C, D.

Principal characteristics—This species is easy to recognize by its sagittate to hastate leaves. The limb lanceolate-ovate, cordate at the base, acute to narrowly acute at the apex, with a purplish red to maroon marginal area minutely granular and glabrous, covered by white to dark-purple villose trichomes around, the obovoid throat, yellow with deep purple red dots, covered by villose white trichomes.

Notes—*Aristolochia watsonii* is one of the species with a wide distribution in*Aristolochia* subsection *Pentandrae* and has previously been confused with *A. porphyrophylla* described by Pfeifer and corrected by the same author in 1976 (see notes below *A. lassa*). During the fieldwork and comparing herbarium specimens, we realized *Aristolochia socorroensis* is one of the morphological variations of *A. watsonii*. The principal differences are in the limb coloration maroon and the rounded limb apex. These differences are not visible in dry material, thus the specimens from *A. watsonii* and *A. socorroensis* look the same. They are here sinonymized and the variation is considered to be a consequence of geographic isolation.

Phenology—This species is reported to flower from January to April and from August to December. It has been collected in fruit from January to March and August to September.

Distribution and Habitat—*Aristolochia watsonii* is known from the south of the United States of America (Arizona and New Mexico) and from northwestern Mexico; growing principally in desert vegetation with *Larrea*, *Prosopis*, *Acacia constricta* and *Opuntia fulgida*, in riparian desert canyons with *Artemisia ludoviciana*, *Brickellia desertorum*, *Cercidium microphyllum*, *Carnegiaea gigantea*, *Fouquieria splendens*, alluvial soils, sandy soils, semidesert grassland, tropical deciduous forest, usually in secondary vegetation from 25 to 1,500 m altitude.

Common Name and Uses—"Hierba del Indio" (herb of the indian).

Additional Specimens Examined—**Baja California:** Mun. Ensenada, canyon La Borreguera, Sierra La Libertad, 9 miles east of Misión San Borja, 28º50´ N, 113º40´ W, 2400 m, 29 Mar 1978, *Kevin C. Nixon, Clark P. Cowan and Mary L. Sauls 1173* (ARIZ, MEXU); Cañón La Borreguera, sierra La Libertad, 9 miles east of Misión San Borja, 28º50´ N, 113º40´ W, 2400 m, 29 Mar 1978, *Kevin C. Nixon, et al. 1173* (ARIZ,

MEXU); Cañón La Borreguera, sierra La Libertad- 9 miles east of Mision San Borja, 28.833333 N, -113.666667 W, 732 m, 29 Mar 1978, *Kevin C. Nixon et al. 1173* (ARIZ, MEXU). **Baja California Sur:** Mun. Comondú, Benito Juárez, arroyo Las Bramonas, 20.6 km NE of Ciudad Constitución, 25º6´N, 111º52´8.9 W, 28 m, 23 Sep 2015, *M. S. Samain & S. Wanke 2015-012* (IEB); La Fortuna, 22 km along the road E of Ciento Ventiocho, in arroyo, 24º39´29.5″ N, 111º12´22.5″ W, 137 m, 23 Sep 2015, *M. S. Samain & S. Wanke 2015-013* (IEB); Arroyo ejido Los Naranjos, La Purisima, 26º12´ N, 112º6´ W, 250 m, 16 Feb 1991, *M. Domínguez L. 201* (HCIB); Arroyo, carr. Matancitas, Pto San Carlos, 28 m, 14 May 2010, *M. Domínguez León 4649* (HCIB). Mun. La Paz, bahía Magdalena, rancho Agua de los Coyotes, 24º17´ N, 111º13´ W, 30 m, 21 Nov 1996, *J. León de la Luz 8099* (HCIB); Ojo de Agua, cañón de La Burrera, NE Todos Santos, 23º30´ N, 110º2´ W, 600 m, 11 Dec 1990, *M. Domínguez L. 53* (HCIB, MEXU); Rancho El Potrero, Punta Coyote, 14 km al NW de rancho El Potrero, 24º38´ N, 110º45´ W, 140 m, 19 Oct 1996, *M. Domínguez L. 1729* (IEB);Arroyo Las Cruces, 500 m to the SSW of the end of the airstrip of the rancho Las Cruces Baja resort, 24º11´53.5″ N, 110º5´15.1″ W, 21 m, 18 Sep 2015, *M. S. Samain & S. Wanke 2015-007* (IEB); Arroyo about 1 km NNW of La Ribera, 23º35´59.5″ N, 109º35´47.9″ W, 30 m, 25 Sep 2015, *M. S. Samain & S. Wanke 2015-016* (IEB); Los Burros, 1 km away to the harbor, 25.04573 N, -110.83360 W, 13 m, 20 Sep 2015, *A. Paizanni Guillén y S. Müller 305* (IEB); Bay close to La Higuera, 25.03090 N, -110.80692 W, 0 m, 20 Sep 2015, *A. Paizanni Guillén y S. Müller 306* (IEB); Growing on the back yard of a house close to the town El Cien (originaly from San Hilario), 24.465561 N, -111.216761 W, 68 m, 22 Sep 2015, *A. Paizanni Guillén y S. Müller 308* (IEB); Arroyo del Ojo de Agua, ca de las ruinas de la Exmisión de Los Dolores, 15 km al N del poblado de San Evaristo, 25º3´20.8″ N, 110º53´28.1″ W, 80 m, 15 Mar 2003, *R. Domínguez Cadena 2795* (HCIB); N de Sierra de Las Cacachilas, arroyo Rancho Las Cruces, 25 km al W de La Paz, 690 m, 12 Nov 2013, *R. Domínguez Cadena 4158* (HCIB); La Paz, Sierra de la Giganta, Arroyo del rancho La Banderita, 24º59´38.64″ N, 110º57´23.7″ W, 345 m, 4 Mar 2005, *M. Domínguez León 3888* (HCIB); Rancho El Potrero, Punta Coyote, 24º38´99″ N, 110º45´ W, 140 m, 19 Oct 1996, *M. Domínguez L. 1729* (IEB); S. Giganta above pt. Econdido, 2000-3000 m, 22 Apr 1938, (MEXU); Arroyo Quisapol, E of la Presa along trail to laguna Caquihui, Sierra de la Giganta, 24º53´ N, 110º58´ W, m, 18 Nov 1959, *I. L. Wiggins 15543* (MEXU); 3.4 km W of Los Burros on the road to Santa María Toris, 25º2´40″ N, 110º51´13″ W, 150 m, 1 Nov 1997, *V.W. Steinmann & T. LaDoux 1254* (MEXU); Cañada de el agua southwest of Puerto Escondido, rocky arroyo bottom between first and second falls, 25º47´ 26´ N, 111º 21´ W, 200-300 m, 30 Nov 1961, *Annetta Carter 4349* (MEXU); Cañón de San Dionisio, a orilla del arroyo, 8 Sep 1985, *F. De Osio 5* (MEXU); 3.4 km W of Los Burros on the road to Santa María Toris, 25º2´40″ N, 110º51´13 W, 150 m, 1 Nov 1997, *V.W. Steinmann & T. LaDoux 1254* (MEXU); S. Giganta above pt. Econdido, 610-914 m, 22 Apr 1938, *H. Scott Gentry 3767* (ARIZ, MEXU); Sierra de la Giganta, El Salto, arroyo del Cañón del Tabor, 12 km al S de Loreto, 25º47´51.36″ N, 110º20´33.12″ W, 600 m, 7 Nov 2001, *R. Domínguez Cadena 2545* (HCIB); Rancho Agua Verde, Sierra de La Giganta, 25º26´ N, 111º3´ W, 14 Nov 2001, *José León de la Luz 10014* (HCIB); Southwest of Agua Verde along trail to Santa Maria in large box canyon, 25º29´2″ N, 111º3´30″ W, 100 m, 19 Oct 2001, *Jon P. Rebman 7582* (HCIB). Mun. Loreto, Rancho Agua Verde, 1300 m S of the village, 25º29´52.5″ N, 111º4´10.2″ W, 62 m, 21 Sep 2015, *M. S. Samain & S. Wanke 2015-010* (IEB); Rancho Los Corrales Viejos, 8 km N of the Transpeninsular Highway Ciudad Constitución-Loreto, 25º35´22.3″ N, 111º26´57.2″ W, 262 m, 22 Sep 2015, *M. S. Samain & S. Wanke 2015-011* (IEB); Mun. Los

Cabos, Miraflores, 27 Apr 1940, *Charles F. Harbison, 27262* (ARIZ); Cañón de San Dionisio, a orilla del arroyo, 8 Sep 1985, *F. De Osio 5* (MEXU); Mun. Mulegé, Sierra Guadalupe: west of Mulegé, west side of the mountain range, vicinity of rancho El Tule, east San Martin and La Vinorama, 26º81´ N, 112º72´ W, 260 m, 26 Apr 1998, *J. Rebman 5151* (HCIB, UCR); Rancho San Sebastián, arroyo de los Güeribos, al W del rancho San Sebastián, Sierra de Guadalupe, 27º0´ N, 112º24´ W, 600 m, 25 Oct 1997, *J.J. Pérez N. 1055* (HCIB); Arroyo del rancho de San Javier, Sierra de Guadalupe, 27º0´ N, 112º23´ W, 700 m, 26 Oct 1997, *J. L.León de la Luz 9003* (HCIB); Sierra Guadalupe: west of Mulegé, west side of the mountain range, vicinity of rancho El Tule, east San Martin and La Vinorama, 26º81´ N, 112º72´ W, 260 m, 26 Apr 1998, *J. Rebman 5151* (HCIB, UCR); Rancho San Sebastián Arroyo, Sierra de Guadalupe, 27º0´ N, 112º24´ W, 568 m, 25 Oct 1997, *M. Domínguez L. 2329* (HCIB, IEB); 3.4 km W of Los Burros on the road to Santa María Toris, 25º2´40″ N, 110º51´13 W, 150 m, 1 Nov 1997, *V. W. Steinmann & T. LaDoux 1254* (IEB, MEXU); Arroyo El Coyote ca. 3km west of aguaje de San Antonio. Note-headwaters of Arroyo del Coyote, narrow, steep-sided canyon draining westerly from the crest of the Sierra, 25º49´ N, 110º47´ W, 525 m, 1 Nov 1971, *R. Moran 18849* (MEXU); Arroyo bottom, rancho San Gregorio, cerro San Francisco, ca. 30 miles north of San Ignacio, 23 Dec 1987, *C.T. Mason, 3874* (ARIZ); Canyon del Higuera on the S end of the bay with rancho Los Burros, 25º1´44″ N, 110º48´26″ W, 35 m, 13 Nov 2003, *J. P. Rebman, J. Emming, J. Delgadillo & M. White 9754* (HCIB); Sierra San Francisco: North of San Ignacio, cañón San Gregorio, northeast of the ranch San Francisco de la Sierra, 27º40´ N, 112º59´ W, 500-800 m, 18 Oct 1997, *J. Rebman & J. Delgadillo 4437* (HCIB, UCR); Arroyo El Coyote ca. 3km west of Aguaje de San Antonio. Note-headwaters of Arroyo del Coyote, narrow, steep-sided canyon draining westerly from the crest of the Sierra, 25º49´ N, 110º47´ W, 525 m, 1 Nov 1971, *Reid Moran 18849* (MEXU); Rancho San Gregorio, Cerro San Francisco, ca. 30 miles north of San Ignacio, 23 Dec 1987, *C.T. Mason, Jr. 3874* (ARIZ, MEXU); Rancho San Gregorio, Cerro San Frnacisco, ca. 30 miles north of San Ignacio, 23 Dec 1987, *C.T. Mason, Jr. 3874* (ARIZ, MEXU). **Durango:** Mun. Tamazula, El Castillo, antes del Rancho El Carrizal, afuera de la tienda que está por el camino, 24º58´09.4″ N, 106º56´32.4″ W, 258 m, 6 Aug 2014, *A. Paizanni Guillén, et al. 237* (IEB). **Nayarit:** Mun. Tepic, ladera E del volcán Sangaguey, 21º25´ N, 104º33´ W, 1130 m, 30 Nov 1989, *P. Tenorio L. et al. 16877* (MEXU). **Sinaloa:** Mun. Culiacán, en el poblado El Capule, creciendo en la esquina de una casa del pueblo, 25º02´03.1″ N, 107º36´26.4 W, 94 m, 25 Jul 2014, *A. Paizanni Guillén et al. 222* (IEB). Mun. El Fuerte, San Blas, alrededores de San Blas, º´ N, º´ W, m, 28 Dec 1995, *A. García Mendoza et al. 6137* (MEXU). Mun. Mazatlán, El Tacote, 1000 m, *Ing. Jesus González Ortega 5728* (MEXU); Isla Tachechilte, 10-30 ft. 20 Jan 1945, *Howard Scott Gentry, 7125* (ARIZ). Mun. Agua Prieta , Southerly extension of Peloncillo Mtns, canyonland east of upper Bato Pito River, Peloncillo Mtns., 31º10´ N, 109º5´ W, 1372 m, 1 Sep 1979, *O. F. Clarke & C. Reddit s.n.* (UCR); Los Ojitos area, western tributary of arroyo San Bernardino, rancho San Bernardino (east of Agua Prieta on MEX 2), 31.300556 N, -109.261111 º´ W, 11232 m, 14 Aug 2007, *A. L. Reina-G, T. R. Van Devender 2007-734* (ARIZ); Peloncillo Mtns., Southerly extension of Peloncillo Mtns, canyonland east of upper Bato Pito River, 31º10´ N, 109º5´ W, 1300 to 1372 m, 1 Sep 1985, *Oscar F. Clarke s.n.* (UCR). **Sonora:** Mun. Alamos, río Mayo region, abandoned fields north of El Caracol campground, 10 mi. west of Alamos on the road to Navojoa, 27º5´ N, 109º5´ W, 459 m, 28 Aug 1980, *A.C. Sanders et al. 1888* (UCR); Abandoned fields north of El Caracol campground, 10 mi. west of Alamos on the road to Navojoa, río Mayo, 27º4´50″ N, 109º4´28″ W, 459 m, 28 Aug 1980, *A.C. Sanders, O.F. Clarke & S.*

Beckstrom-Sternberg 1888 (UCR); Alamos, Río Fuerte, 8 Feb 1937, *Howard Scott Gentry 2999* (ARIZ); El Guayabo crossing of río Cuchujaqui, 1.1 miles northeast-southeast Alamos, 27 N, -108.795833 W, 340 m, 5 Feb 1992, *T. R. VanDevender et al. 92-264* (ARIZ); Arroyo el Huirotal, rancho Uvalama, southeast slopes of the Sierra de Alamos, 11 km west-southwest Alamos, 600 m, 30 Jan 1992, *T. R. Van Devender et al. 92-62* (ARIZ); Arroyo El Huirotal, rancho Uvalama, southeast slopes of the Sierra de Alamos, 11 km west-southwest Alamos, 600 m, 30 Jan 1992, *T. R. Van Devender et al. 92-62* (ARIZ); Alamos, in back yard of Motel Somar, 27.025 N, -108.933333 W, 400 m, 29 Dec 1990, *T. R. & R. K. Van Devender, 90-578* (ARIZ); Alamos, 27.025 N, -108.933333 W, 390 m, 19 Mar 1993, *Mark Fishbein et al. 1093* (ARIZ); El Caracol trailer park, Sierra de Alamos, 350 m, 26 Dec 1994, *Mark Fishbein et al. 1972* (ARIZ); Arroyo El Cobre, at Choquincahui, about 7 mi north of Guirocoba, 26.983333 N, -108.683333 W, 580 m, 17 Mar 1995, *Mark Fishbein et al. 2205* (ARIZ); Arroyo Gochico, ca. 12 km west of San Bernardo and 1 km east of Gochico Viejo, 27.4 N, -108.725 W, 500 m, 13 Mar 1995, *V.W. Steinmann et al. 598A* (ARIZ); Alamos, río Fuerte, 8 Feb 1937, *Howard Scott Gentry, 2999* (ARIZ, MEXU); El Caracol trailer park, Sierra de Alamos, 350 m, 26 Dec 1994, *Mark Fishbein et al. 1972* (ARIZ, MEXU, UCR). Mun. Guaymas, Sonoran Desert, 58 mi south of Hermosillo, 27 mi north of Guaymas on Hwy 15, 28º15´ N, 111º2´ W, 17 Dec 1967, *O. F. Clarke et al. 953-3* (UCR); 58 mi south of Hermosillo, 27 mi north of Guaymas on Hwy 15, Sonoran Desert, 28º15´ N, 111º2´ W, 17 Dec 1967, *O. F. Clarke et al. 0953-03* (UCR); La Pintada Canyon, about 48 miles north of Guaymas and 4 miles east of Mexico Highway 15., 14 Mar 1984, *G. Joseph s.n.* (ARIZ). Mun. Hermosillo, Canyon las Barajitas, ca. 3 km inland, 28.058333 N, -111.180556, 15 Mar 1996, *R. S. Felger et al. 96-117* (ARIZ). Mun. Huatabampo, canyon Nacapules, about 6 km north of bahia San Carlos., 25 Feb 1985, *R. S. Felger & R.S. Devine 85-239* (ARIZ); Cañón de Nacapule, ca 4 km north of bahia San Carlos, 11 Aug 1985, *R. S. Felger & Mark A. Dimmitt 85-838* (ARIZ); Camahuiroa between Agiabampo and Las Bocas on the Gulf of California, 15 Mar 1993, *T. R. VanDevender et al. 93-292* (ARIZ); Diez de Abril vicinity 8 km north Diez de Abril at Arroyo Muerto and powerline road; 5.25 km north Melchor Ocampo. 2941.600km N, 682.450km E., 26.586111 N, -109.170833 W, 55 m, 20 Oct 1994, *S.L. Friedman & K.J. Johnson 457-94* (ARIZ); Canyon Nacapules, about 6 km north of bahia San Carlos, 25 Feb 1985, *R. S. Felger & R.S. Devine 85-239* (ARIZ, MEXU); Cañón de Nacapulie ca 4 km north of bahia San Carlos, 11 Aug 1985, *R. S. Felger & M. A. Dimmitt 85-838* (ARIZ, MEXU). Mun. Isla Tiburón, isla Tiburón. Hast Mozax, large basaltic hill in the vicinity of Pazj Hax waterhole, 28.92561 N, -112.27058 W, m, 15 Sep 2007, *B. T. Wilder & A. Swanson 07-380* (ARIZ). Isla Tiburón, SW part of central valley, ca. 13 miles south of Tecomate, 28.25 N, -112.45 W, 183 m, 20 Feb 1968, *R.S.Felger & Alexander Russell 17296* (ARIZ). Mun. Sahuaripa, rancho La Ventana, 32 km (by air) NNE of elevation, W, 31 Mar 2011, *A. L. Reina G et al 2011-84* (ARIZ). Mun. Teachive de Masiaca, Teachive de Masiaca, 75 m, 22 Sep 1993, *T. R. Van Devender et al. 93-976* (ARIZ). Mun. Yécora, Curea, 28.311667 N, -109.278333 W, 490 m, 12 Jan 2001, *T. R. Van Devender et al. 2001-99* (ARIZ). Arroyo Las Tinajas below ruins of Toledo smelter, near Loma Maderista, 3.5 km south (by air, across river) of Tonichi, west side of the río Yaqui, 28.5675 N, -109.556944 W, 220 m, 16 Mar 2005, *T. R. Van Devender & A.L. Reina G. 2005-270* (ARIZ). **United States. Arizona:** County Cochise, 2015, *C. M. Roll* (ARIZ); Huachuca flats, Huachuca Mountains, 31.636 N, -110.3583 W, 2 Aug 1909, *L. N. Goodding 287* (ARIZ); Five miles south of corner near Fry on Highway 92, 31.55667 N, -110.29444 W, 1432 m, 8 Sep 1944, *R. A. Darrow, F. W. Gould, W. S. Phillips, L. M. Pultz 1358* (ARIZ); East of highway about 15 miles south of Buena school,

31.337286 N, -110.25639 W, 10 Jul 1958, *L. N. Goodding 211-58* (ARIZ); AVA Ranch near Rock Tank, Chiricahua Mountains, 31.8887 N, -109.4206 W, 1463 m, 23 Jun 1968, *R. J. Barr 68-312* (ARIZ); Along Babocamari River, San Ignacio del Babocamari ranch, 3 miles west of Huachuca City, 31.62777 N, -110.384468 W, 1341 m, 30 May 1974, *T. R. Van Devender A. M. Phillips, III s.n.* (ARIZ); 5 miles east of junction of State Highways 90 and 82 on Highway 82, north of Sierra Vista, 31.554412 N, -110.217897 W, 1402 m, 18 Apr 1981, *Belinda Casto s.n.* (ARIZ); Along highway 80, south of Saint David., 31.90417 N, -110.21361 W, 16 Sep 1983, *C. T. Mason & Jr. R. W. Hoshaw 3667* (ARIZ); Outside Gleeson on the road to Pearce, 33.7723 N, -110.6887 W, m, 2 Apr 1990, *Steven J. Dentali, s.n.* (ARIZ); Chiricahua National Monument; Flats around lower Bonito creek., 32.00856 N, -109.35822 W, 5200 m, 28 Sep 2002, *Meg Quinn, 256* (ARIZ); Upper San Pedro River floodplain, 4-5 miles south of Hwy 82, ~1 mile east of San Pedro, most northern rocky outcrop of Charleston Hills, west side, 31.65125 N, -110.1679 W, 4029 m, 3 Aug 2002, *E. Makings, & C. Paradzik 1152* (ARIZ); Along highway 80, south of Saint David, 16 Sep 1983, *C. T. Mason, Jr & R. W. Hoshaw 3667* (ARIZ, MEXU, UCR). County. Coconino, Grand Canyon of the Colorado river; Clear Creek, 86.7 miles below Lees Ferry, 36.0819 N, -112.036 W, 825 m, 21 Aug 1970, *R. M. Turner & M. A. Turner 70-34* (ARIZ). East of mouth of Clear Creek. 84 miles below Lees Ferry on the Colorado River., º´ 36.0816 N, -112.0366 º´ W, m, 8 May 1971, *A. H. Holmgren, Noel H. Holmgren, Patricia K. Holmgren, Doris E. Holmgren, & Aaron B. Ross. 15605* (ARIZ). County. Gila, Natural Drainage Area, Parker Creek canyon, sierra Ancha Mountains, 33.7967 N, -110.9698 W, 1371 m, 25 Aug 1946, *F. W. Gould 3726* (ARIZ); Tonto N.F., Rock Cr., 3 Bar study area., 33.7189 N, -111.2946 W, 1067 m, 29 Aug 1960, *C. P. Pase 1136* (ARIZ); South slope below state highway #77, about 3 miles north of Dripping Springs turn-off; Mescal Mountains, 33.3489 N, -110.5357 W, 1219 m, 11 Apr 1965, *W. E. Niles 571* (ARIZ); County. Graham, vicinity Hawks Hollow plots, 32.8345 N, -109.8195 W, 1100 m, Dec , *M. , Moeller & R., Moeller 10949* (ARIZ); 18 mi. E. Safford., 32.833506 N, -109.396074 W, 1097 m, 12 Apr 1935, *M. Moelle & R. Moeller 10704* (ARIZ); Sycamore Canyon at Jackson Cabin, road near Coronado National Forest Boundary,. Galiuro Mountains, 32.4622 N, -110.2933 W, 1341 m, 4 Aug 1981, *T. R. Van Devender & F. W. Reichenbacher (731) s.n.* (ARIZ); In Spring Canyon, upstream from confluence with Gila River, 32.9107 N, -109.5012 W, m, 27 Mar 1978, *L. A. McGill, Minkley, et al. 2083* (ARIZ); Pinaleno Mountains; Marijilda Canyon road, 0.9 miles north of Hwy 366., º´ 32.694 N, -109.7574 º´ W, 3840 m, 7 Sep 1988, *Steven P. McLaughlin & R. S. Felger 5099* (ARIZ); About 1/2 mile east of Hooker Cienega, 32.564 N, -110.0477 W, m, 17 Sep 1979, *C. Jenkins & G. Yatskievych (79-712) J. Cicero, P. Fischer & C. Revak 2430* (ARIZ); County. Greenlee, Boyles, San Francisco River, 32.975911 N, -109.371959 W, m, 5 Aug 1912, *L. N. Goodding 1279* (ARIZ); County. Maricopa, Tonto National Forest, Butcher Jones campground, Saguaro Lake, 33.576944 N, -111.510278 W, 17 Sep 1998, *A. E. Brant, J. Stone 4070* (ARIZ); Cottonwood Creek near Lake Pleasant, º´ 33.891372 N, -111.952487 º´ W, m, 11 Jun 1965, *E. Lehto 5184* (ARIZ); Usery Mountain Regional Park, 33.4687 N, -111.6093 W, 25 Nov 1966, *D. Keil, 794* (ARIZ); Sonoran Desert National Monument; Vekol Valley bosque, 32.709125 N, -112.230953 W, 1995 m, 15 Aug 2001, *R. S. Felger, 01-428* (ARIZ); Sonoran Desert National Monument; Vekol Valley Road, 32.7605 N, -112.2459 W, 1940 m, 8 Aug 1999, *K. Mauz, 99-74* (ARIZ); Sonoran Desert National Monument, 5.8 miles on Hwy 238 (Maricopa Road) west of east boundary of the Monument, then 0.8 mile on dirt road north of Hwy 238, 33.0275 N, -112.409264 W, 1490 m, 2 Oct 2002, *R. S. Felger & G. Maskarinec 02-354* (ARIZ); West of the

base of Hat Mountains, Sauceda Mountains. B. M. Goldwater Gunnery Range, 32.6445 N, -112.743 W, m, 23 Nov 2001, *S. Rutman, s.n.* (ARIZ); Sand Tank Mountains, Sonoran Desert National Monument. Sand Tank Wash, on northwest side of Javelina Mountain, 32.75353 N, -112.4635 W, 2200 m, 16 Mar 2003, *S. Rutman, 2003-118* (ARIZ); Sand Tank Mountains, Sonoran Desert National Monument, 32.757222 N, -112.485278 W, 2000 m, 27 Nov 2003, *S. Rutman, 20031127-7* (ARIZ). County. Mohave, Sonoran desert, southeast Aquarius Mountains, upper Kaiser Spring Wash, 34º35´12″ N, 113º28´56″ W, 671 m, 28 Apr 2009, *M. A. Elvin 6287* (ARIZ); 1 mile west of Burro Creek, 34.5372 N, -113.5722 W, m, 20 Apr 1938, *Crooks & Darrow s.n.* (ARIZ). Cnty. Pima, Tortolita Mountains, Tortolita Mountain park, Rail X Ranch Rd. access, 32.5098 N, -111.0312 W, 3989 m, 31 Aug 2014, *R. Lindley, 624* (ARIZ); Cienega Creek Natural Reserve, Agua Verde Creek, 32.0177 N, -110.6318 W, 3350 m, 24 May 2014, *J. Fonseca & D. Backer 2014-321* (ARIZ); Molino Canyon Overlook parking lot, on right at trail entrance, 32.32677 N, -110.70049 W, 4138 m, 20 Sep 2014, *D. Bird & J. Tedford 59* (ARIZ); Molino Canyon Overlook parking lot, on right at trail entrance, 32.32677 N, -110.70049 W, 1261 m, 20 Sep 2014, *D. Bird & J. Tedford 59* (ARIZ); Sonoran desert, San Xavier indian reservation, south of Tucson, 1 mile SE of Black Mountain, 32º4´18″ N, 111º1´29″ W, 808 m, 24 Aug 1984, *S.D. Boyd & J. Hirshberg, et al. s.n.* (UCR); San Xavier indian reservation, south of Tucson, 1 mile SE of Black Mountain elevation, Sonoran Desert, 32º4´18″ N, 111º1´29″ W, 808 m, 24 Aug 1984, *S. D. Boyd & J. Hirshberg, et al. s.n.* (UCR); Wilmont, 32.1223 N, -110.8445 W, 853 m, 20 Aug 1903, *Thornber s.n.* (ARIZ); County, Wilmont, 32.1223 N, -110.8445 W, 731 m, 8 Sep 1903, *Thornber s.n.* (ARIZ); Tucson, 32.2217 N, -110.9265 W, m, 1 Aug 1891, *J. W. Toumey s.n.* (ARIZ); Santa Catalina Mountains, Pima Canyon, 32.3542 N, -110.9387 W, 27 Apr 1913, *Thornber & Brown s.n.* (ARIZ); Wilmont, 32.1223 N, -110.8445 W, 853 m, 20 Aug 1903, *Thornber s.n.* (ARIZ); Range Reserve, Wilmot, 32.1223 N, -110.8445 W, 29 Aug 1902, *Thornber s.n.* (ARIZ); Organ Pipe Cactus National Monument; Cipriano Well, 32.001624 N, -113.026454 W, 388 m, 27 Apr 1939, *A. A. Nichol s.n.* (ARIZ); Small range reserve, Wilmot, 32.12222 N, -110.84389 W, m, 16 Aug 1901, *J. J. Thornber s.n.* (ARIZ); Santa Rita Mountains, Stone Cabin Canyon, 31.7679 N, -110.8484 W, *Thornber Griffiths 201* (ARIZ); Upper Sabino canyon 1/2 miles above Ranger Station; Santa Catalina Mountains, 32.415 N, -110.7166 W, 914 m, 12 Oct 1944, *F. W. Gould 2860* (ARIZ); 2 miles southeast of Walls Well, near northern end of Ajo Mountains, 32.351144 N, -112.8357 W, m, 30 Aug 1945, *F. W. Gould & H. S. Haskell 3218* (ARIZ); Southern slopes of Santa Catalina Mountains, near Mount Lemmon road, 32.4647 N, -110.7397 W, 1371 m, 25 Oct 1945, *F. W. Gould 3459* (ARIZ); Near Tucson, 32.22167 N, -110.92583 W, 1 Nov 1925, *R. H. Peebles 617* (ARIZ); Saguaro National Park, Rincon Mountain District; 1.2 miles north of visitors center; Saguaro National Monument, East., 32.1513 N, -110.6086 W, m, 23 Apr 1966, *W. F. Steenbergh s.n.* (ARIZ); Barrel Canyon, north end of Santa Rita Mountains, 31.8687 N, -110.677 W, 1371 m, 13 Aug 1976, *S. P. McLaughlin W. Van Asdall and L. Haynes 1348* (ARIZ); Bingham Swamp, ca. 2 miles north of Redinton, San Pedro Valley, 31.45 N, -110.483333 W, 853 m, 21 Jul 1979, *R. M. Turner P. M. Bergthold 79-185* (ARIZ); Organ Pipe Cactus National Monument; Dos Lomitas, 32.04167 N, -112.87472 W, 426 m, 17 Nov 1974, *P. L. Warren s.n.* (ARIZ); Southwest of Tumamoc Hill, Tucson, 32.2 N, -111 W, 5 Aug 1983, *J. E. Bowers, 2698* (ARIZ); Espiritu Canyon, Rincon Mountains, 32.3148 N, -110.4767 W, 27 Aug 1983, *J. E. Bowers & S. P. McLaughlin R-1281* (ARIZ); Saguaro National Park, Rincon Mountain District; Along the Manning Camp trail, Rincon Mountains, 32.2095 N, -110.554 W, 4570 m, 22 Jul 1982, *J. E. Bowers & S. P. McLaughlin R-321* (ARIZ); Picture Rock

Canyon above Wade Rd., SW of Ina Road, 762 m, 8 Apr 1986, *D. Scheidemantel 22* (ARIZ); Organ Pipe Cactus National Monument; Arroyo of Aguajita Spring, 1 mile by road east of Quitobaquito; ca. 50 m north of Mexican border, 31.940833 N, -113.013333 W, m, 13 Sep 1986, *R. S. Felger & L. Leigh 86-275* (ARIZ); Waterman Mountains, 32.3414 N, -111.449 W, 2550 m, 27 Sep 1988, *T. R. Van Devender & P. Jenkins 88-744* (ARIZ); Waterman Mountains, 32.3414 N, -111.449 W, 2550 m, 30 Nov 1988, *T. R. Van Devender & M. K. O'Rourke 88-830* (ARIZ); Ragged Top Peak area; near Silverbell Road and Gas Pipeline Road, 32.4495 N, -111.4901 W, m, 31 Aug 1989, *J. F. Wiens, 89RT-32* (ARIZ); Saguaro National Park, Tucson Mountain District; Cam-Boh Picnic Area on Picture Rocks Road, 32.2787 N, -111.1826 W, 2490 m, 10 Nov 1989, *T. R. Van Devender, 89-338* (ARIZ); At the corner of Anklam and Speedway, 32.2283 N, -111.0544 W, 2620 m, 30 Apr 1989, *P. D. Jenkins, 89-40* (ARIZ); US For. Serv. Road 505, 14.2 miles SE of Sahuarita, 31.8247 N, -110.8158 W, 4400 m, 12 Aug 1986, *S. P. McLaughlin, 3779* (ARIZ); Organ Pipe Cactus National Monument; Aguajita Spring and vicinity, 31.940833 N, -113.013333 W, 1100 m, 6 Apr 1988, *R. S. Felger & K. A. Dahl 88-267* (ARIZ); Along Hwy 286, 22.0 miles south of Robles Jct. at Las Delicias Ranch Road, 31.7899 N, -111.4452 W, 3200 m, 14 May 1988, *S. P. McLaughlin & J. E. Bowers 4563* (ARIZ); Santa Rita Experimental Range and Wildlife Area. Box Canyon Wash west of Forest Road #485, 31.8102 N, -110.8158 W, 4200 m, 17 May 1983, *Robert Mays, s.n.* (ARIZ); Arivaca Cienega, in upper zone with sacaton, 31.575 N, -111.3253 W, 3640 m, 27 Jul 1989, *S. P. McLaughlin & J. E. Bowers 5652-A* (ARIZ); Cabeza Prieta National Wildlife Refuge. Jose Juan Tank (Represo), an artificial dirt charco on San Cristobal Wash, 1.2 km W of the western boundary of Organ Pipe Cactus National Monument, 32.088889 N, -113.1 W, 1090 m, 14 Sep 1992, *R. S. Felger & P. Gierlach 92-709* (ARIZ); Cabeza Prieta National Wildlife Refuge, along road from Bates Well to Papago Well at 1.7 mi SW of west boundary of Organ Pipe Cactus National Monument, 32.116667 N, -113.1 W, 1060 m, 14 Sep 1992, *R. S. Felger & P. Gierlach 92-700* (ARIZ); Santa Rita Experimental Range and Wildlife Area. Box Canyon Wash west of Forest Road #485, 31.8102 N, -110.8158 W, 4200 m, 17 May 1983, *Robert Mays, s.n.* (ARIZ); Tucson. West Branch of the Santa Cruz River, 32.208333 N, -110.983333 W, m, 2 Aug 2001, *K. Mauz, 21-77* (ARIZ); Sabino Canyon, 32.3327 N, -110.7908 W, m, 14 Aug 1917, *Forrest Shreve, s.n.* (ARIZ); Flats below Baboquivari Canyon. Baboquivari Mountains, 31.7795 N, -111.6126 W, m, 30 Aug 1931, *M. F. Gilman, 44* (ARIZ); Organ Pipe Cactus National Monument; Aguajita Wash, vicinity of road to Quitobaquito and ca. 0.5 kilometers of Aguajita spring; major drainage for La Abra Plain to Sonoyta River, 31.943768 N, -113.010539 W, 1120 ft. m, 3 Mar 1992, *R. S. Felger & K. Cliffton 92-103* (ARIZ); Rincon Peak 7.5; USGS topographic Quadrangle; 12S 05 37 100mE 35 48 250mN. Rincon Mountains, Posta Quemada Canyon, 20 km southeast of the edge of Tucson, 2.5 km west-southwest of Papago Spring, 32.071667 N, -110.606667 W, 3620 ft. m, 6 Sep 2006, *Marc A. Baker, 16314* (ARIZ); Catalina Mountains. Sabino Canyon Recreational Area. At roadside near MP 1, 32.320133 N, -110.810317 W, m, 1 Apr 2008, *Joan Tedford, 644* (ARIZ); Catalina Mountains, Molino basin, roadside to campsites just before the stream crossing, 32.337033 N, -110.691567 W, 4350 m, 17 Sep 2009, *Joan Tedford, Frank Rose 978* (ARIZ); Growing in a sandy wash at the corner of Anklam and Speedway; T14S R13E S7, 2620 m, 30 Apr 1989, *P. Jenkins, 89-40* (ARIZ); Rosemont, 31.8343 N, -110.7329 W, m, 27 Jul 1918, *J. J. Thornber s.n.* (ARIZ); Rincon Mountains. Saguaro National Park, Rincon Mountain District; North of Picnic área, 32.13194 N, -110.51889 W, 19 May 1979, *C. E. Jenkins 1833* (ARIZ); Growing in a sandy wash at the corner of Anklam and Speedway, 798 m, 30 Apr 1989, *P. Jenkins*

89-40 (ARIZ); Saguaro National Park, 32.2787 N, -111.1826 W, 762 m, 8 Apr 1986, *D. Scheidemantel 22* (ARIZ); Rincon Mountains. Saguaro National Park, Rincon Mountain District, 32.1671 N, -110.7251 W, 29 Aug 1979, *C. E. Jenkins 2309* (ARIZ); Tumamoc Hill, 32.216667 N, -111.5 W, 749 m, 5 Aug 1983, *J. E. Bowers 2698* (ARIZ); Rincon Mountains. Saguaro National Park, Rincon Mountain District; Chilopsis wash. 0.7 mile from Visitors Center, 32.13194 N, -110.51889 W, m, 4 Sep 1978, *C. E. Jenkins 1088* (ARIZ); Rosemont, 31.8343 N, -110.7329 W, m, 27 Jul 1918, *J. J. Thornber s.n.* (ARIZ); Growing in a sandy wash at the corner of Anklam and Speedway, 798 m, 30 Apr 1989, *P. Jenkins 89-40* (ARIZ); Saguaro National Park, 32.2787 N, -111.1826 W, 762 m, 8 Apr 1986, *D. Scheidemantel 22* (ARIZ); Rincon Mountains. Saguaro National Park, Rincon Mountain District, 32.1671 N, -110.7251 W, 29 Aug 1979, *C. E. Jenkins 2309* (ARIZ); Tumamoc Hill, 32.216667 N, -111.5 W, 749 m, 5 Aug 1983, *J. E. Bowers 2698* (ARIZ); Rincon Mountains. Saguaro National Park, Rincon Mountain District; Chilopsis wash. 0.7 mile from Visitors Center, 32.13194 N, -110.51889 W, m, 4 Sep 1978, *C. E. Jenkins 1088* (ARIZ); Organ Pipe Cactus National Monument; Arroyo of Aguajita Spring, 1 mile by road east of Quitobaquito; ca. 50 m north of Mexican border, 31.940833 N, -113.013333 W, 13 Sep 1986, *R. S. Felger Linda Leigh 86-275* (ARIZ, MEXU); Saguaro National Park, 2500 m, 8 Apr 1986, *D. Scheidemantel, 22* (ARIZ, UCR); Picture Rock Canyon above Wade Rd., SW of Ina Road, 2500 m, 8 Apr 1986, *D. Scheidemantel, 22* (ARIZ, UCR). Cnty. Pima/Pinal, Santa Catalina Mountains, Oracle side, N-facing slope, 32.4773 N, -110.7193 W, 1219 m, 14 Dec 1962, *R. H. Whittaker W. & A. Niering s.n.* (ARIZ). Cnty. Pinal, Oracle, 32.6109 N, -110.7709 W, 1371 m, Sep 1905, *Thornber s.n.* (ARIZ); Sacatan Agency, 33.0767 N, -111.7393 W, *M. French Gilman 238* (ARIZ); Devils Canyon, 32.9801 N, -110.5987 W, m, 10 Oct 1926, *R. H. Peebles G. J. Harrison and T. H. Kearney 3174* (ARIZ); Near Winkelman, 32.9875 N, -110.77028 W, 30 Mar 1928, *R. H. Peebles G. J. Harrison and T. H. Kearney 5178* (ARIZ); Queen Creek, Pinal Mountains, 33.2895 N, -111.1073 W, 19 Jul 1931, *R. H. Peebles 7961* (ARIZ); Lower San Pedro Basin, 32.5496 N, -110.5429 W, 914 m, 28 Aug 1962, *S. B. Bingham 504* (ARIZ); 6 miles Northeast of Oracle, 32.665 N, -110.6838 W, 1219 m, 6 Aug 1956, *C. R. Hungerford 177* (ARIZ); Smelter Wash, north of San Manuel, northeast base of Santa Catalina Mountains, west side of San Pedro River Valley, 32.6362 N, -110.6624 W, 1006-1021 m, 12 Sep 1993, *T. R. Van Devender et al. 93-780* (ARIZ); West of Winklman, 32.9545 N, -110.7841 W, 2200 m, 19 Sep 1990, *Marc Baker, Jim Tress and David Shaari 8078* (ARIZ); Running through Hohokam archaeological site. Olberg, northwest of trading post, 33.066667 N, -111.666667 W, 13 May 1987, *Amadeo M. Rea, 1450* (ARIZ); Table Top Mountains. Maricopa/Pinal County Line Road north of Antelope Peak, 32.8188 N, -112.1776 W, 1920 m, 20 Mar 1994, *G. Bolton, 94-71* (ARIZ); Page Ranch, west of house, 32.6211 N, -110.8874 W, 1127 m, 9 Jun 1941, *Jack Kaiser 1511* (ARIZ); Smelter Wash, north of San Manuel, northeast base of Santa Catalina Mountains, west side of San Pedro River Valley, 3300 - 3350 m, 12 Sep 1993, *T. R. Van Devender et al. 93-780* (ARIZ, UCR). Cnty. Santa Cruz, Salero Ranch: W of Tejano Spring, Unit 4; 8mi ESE of Tubac, 31.57726 N, -110.9213 W, 4025 m, 15 Aug 2013, *Sue Carnahan, SC 116* (ARIZ); Patagonia Mountains, 31.4157 N, -110.7304 W, 1371 m, 24 Aug 1940, *T. H. Kearney R. H. Peebles 14814* (ARIZ); Nogales, 31.3404 N, -110.9343 W, 7 Aug 1927, *R. H. Peebles G. J. Harrison and T. H. Kearney 4628* (ARIZ); Tubac, 31.6126 N, -111.0459 W, 18 May 1928, *G. J. Harrison 5295* (ARIZ); Patagonia-Nogales road, 31.4403 N, -110.8431 W, 18 Aug 1928, *R. H. Peebles, G. J. Harrison, T. H. Kearney 5627* (ARIZ); Roadside, 23 miels north of Sonoita on Highway 83, 31.67944 N, -110.65472 W, 6 Aug 1979, *J. E. Bowers S. P. McLaughlin*

1773 (ARIZ); Tumacacori National Historical Park; Tumacacori Unit., º´ 31.569167 N, -111.051056 º´ W, 3260 m, 15 Mar 2001, *Patricia P. Guertin, 123* (ARIZ); Sonoita Creek State Natural Area; Old railroad right-of-way, 31.47961 N, -110.91808 W, 2 Mar 2001, *Steven P. McLaughlin, Betsy Lewis 8907* (ARIZ); Sonoita Creek State Natural Area; Drainage, southern part of park, 31.48303 N, -110.89944 W, 31 Jul 2001, *Steven P. McLaughlin, 9550* (ARIZ); Cnty, Yavapai, Castle Creek, 33.8995 N, -112.2963 W, 25 Jun 1892, *J. W. Toumey s.n.* (ARIZ); Canyon Bottom and slopes; Burro Creek Crossing, 34.5372 N, -113.5727 W, 701 m, 10 Apr 1947, *R. A. Darrow F. W. Gould 3691* (ARIZ); 14 mi NE Seven Springs, "51 Ranch" along Lime Creek, 34.006671 N, -111.785859 W, m, 1 Jun 1976, *T. Reeves & E. Lehto L-20174* (ARIZ); Tributary of Little Shipp Wash, ca. 14.5 km SE Bagdad, ca. 5.6 km W Santa Maria River, 2.4 km north AZ 96, 34.539 N, -113.0839 W, 825 m, 4 Apr 1996, *A. L. Reina-G. T. R. Van Devender 96-166* (ARIZ). Cnty. Yuma, Upper De La Osa Wash, Kofa Mountains, 33.33 N, -113.9535 W, 853 m, 13 Sep 1938, *A. A. Nichol s.n.* (ARIZ); Daniels Arroyo; Cabeza Prieta NWR, º´ 32.39464 N, -113.05597 º´ W, 1310 ft. m, 27 Sep 1992, *Annita Harlan, Victor Steinmann 343* (ARIZ). **New Mexico:** Cnty. Luna, Florida Mtns, Mahoney Park, 5000 to 5100 feet (1529 to 1555 meters), 8 May 1983, *Richard D. Worthington 10356* (UCR).

***Aristolochia* sp. nov. inedit. (El Chavarín)** Paizanni & S. Wanke sp. nov. inedit.—Type: MEXICO. Colima: Mun. Manzanillo, El Chavarín, en las faldas del cerrito rocoso, camino hacia la Cruz, 19°12'10.5" N, 104°33´26.5" W, 38 m, 26 September 2014, A. *Paizanni Guillén et al. 292* (holotype: IEB!; isotypes: IBUG!, DR!, MEXU!, ZEA!)

Aristolochia sp. nov. inedit. (El Chavarín) differs from *Aristolochia emiliae* by the limb, which is ovate, apiculate at the base; obtuse at the apex, purplish red covered by whitish to hyaline trichomes, and which is cymbiform from a lateral view.

Geophytic herb, procumbent, **perennial subterranean organs** 15-20 x 0.6-0.8 cm; **stems** with 1-2 subterranean internodes, nodes swollen, 5-15 mm long; **aerial internodes,** slightly geniculate, (1-)1.5-2.5(-3) cm long, and sparsely covered with septate trichomes. **Petioles** terete, (0.5-)1-1.4 x 0.2-0.5 cm, green, sparsely covered with septate trichomes. **Leaf blades** rare entire, usually trisect, discolorous, base obtuse, hastatus, apex acuminate to caudate, (2.0-) 4.5-7(-9) x (1-) 2-4.6(-5.5) cm, palmatinerved actinodromous, margin entire; **adaxial leaf** side green, with septate trichomes, 0.7-1 mm long, sparse and with acroscopic orientation; **abaxial leaf** side pale green, with dense septate trichomes, especially along the veins. **Flowers** axillary, placed in a racemose inflorescence growing in the basal part of the plant, **flower pedicels** 0.3-0.5 x 0.02-0.03 mm. **Bract** lanceolate-ovate, (0.3-) 0.5-0.7 x 0.2-0.4 cm. **Perianth geniculate**, 2-2.5(-3) cm long, yellowish, becoming purple toward the limb with, veins brown-orange, dense septate trichomes along

the veins; **utricle** spheroid to obloid 0.5-0.6 (-9) x 0.4-0.6 (-7) cm, internal surface yellow with purple dots, covered with moniliform mucilaginous trichomes; **tube** bent (110°-)150°-170°, basally straight, (7-) 10 x 3-4.5 mm, internal surface glabrous; **limb** ovate, apiculate at the base, obtuse at the apex, 1- 1.3 x 0.6-0.7 cm; purplish red covered by whitish to hyaline trichomes, in lateral view cymbiform; **throat**, ovoid dark-purple, minutely granular and glabrous; **syrinx** infundibuliform, eccentric, 2.5 mm long, 2.5-3 mm wide at base and 2 mm wide at apex. **Gynostemium** coroniform, stipitate, 2.5-3 x 1.3 mm wide; **stipe** 0.5-1 mm long, **stigmatic lobes** 5, 0.5-1.5 mm long, **anthers** 5, 1 mm long. **Ovary** inferior, lanceolate 5 x (0.8-) 0.9 mm, with sparse to dense septate trichomes. **Fruit** a capsule, obloid to ellipsoid, 1.5 x 0.7-0.9 cm, green when young, brown-yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 3.5-4 x 3-4.5 mm, upper part black, lower part yellow-brown, with granular surface. Figure 18I, J.

Phenology—This species flowers from late September to January, and has been collected in fruit in September.

Distribution and Habitat—*Aristolochia* sp. nov. inedit. is known only from the municipality of Manzanillo in the location of El Chavarín, in tropical dry perturbated forest, along a rocky path and hill sides.

Additional Specimens Examined—MEXICO. **Colima**: Mun. Manzanillo, A 2.8 km al SE de El Chavarín, carr. Manzanillo-Barra de Navidad. Pendientes que dan al W, 20 January 1986, *E. J. Lott & T.H. Atkinson 2769* (MEXU).

***Aristolochia* sp. nov. inedit. (Sierra Verde)** Santana Michel & Paizanni sp. nov. inedit.—Type: MEXICO. Jalisco: Mun. Atengo, Sierra Verde, 16-20 km por la brecha de Los Tablones (esta comienza a partir de la carr. 90 a 5 km al NE de Mixtlán) hacia Tenamaxtlán 20°25′12.78″ N, 104°18′0.39″ W, 2139 m., 5 August 2013, *J. G. González-Gallego, et al. 1532* (holotype: IBUG!; isotypes: IEB!, MEXU!).

Aristolochia sp. nov. inedit. (Sierra Verde) differs from *A. flexucosa* and *A. bracteosa* its lanceolate leaves and obloid utricle, as well as the lanceolate limb, which is maroon colored and covered with a clavate appendix, and the obovoid throat which is yellowish with purple dots.

Geophytic herb, procumbent, **perennial subterranean organs** not seen; **stems** with 1 subterranean internode, nodes swollen, 6-12 mm long; **aerial internodes**, straight (1.3-)2.1-4(-6) cm long, sparsely covered with septate trichomes. **Petioles** terete (0.3-)0.5-0.7(-

0.9) x 0.04-0.1 cm, green sparsely covered with septate trichomes. **Leaf blades** lanceolate, discolorous, base cordate, apex acuminate, (2.4-) 4.4-10.6 x (0.6-) 1.5-2.7 cm; palmatinerved actinodromous, basal veins pink-purple, margin entire, **adaxial leaf** side green with, septate trichomes, 0.05 mm long, sparse and with acroscopic orientation; **abaxial leaf side** pale green with dense septate trichomes. **Flowers axillary,** solitary or placed in a racemose inflorescence, growing in the apical part, **flower pedicels** 0.45-1.3 x 0.2-0.3 mm. **Bract** lanceolate-ovate, (0.4-) 1-1.7 x 0.2-0.45 cm. **Perianth** slightly geniculate, 2.4-3.3 cm long, green-yellowish, becoming purple toward the limb, veins brown-orange, dense septate trichomes along the veins; **utricle** obloid gibbous**,** 0.35-0.65 x 0.3-0.4 cm, internal surface yellow with purple dots covered with moniliform mucilaginous trichomes; **tube** bent 35°, basally straight, 10-16 x 2-2.5 mm, interal suface with black trichomes cover the half of the tube; **limb** lanceolate, cordate at the base, retuse at the apex, 1.7- 2.3 x 0.3-0.5 cm; maroon covered by clavatus shape appendix, in lateral view flat, bent 90° respect the tube and utricle; **throat**, obovoid, yellowish with purple dots, covered by small white septate trichomes; **syrinx** infundibuliform, eccentric, 0.7-1 mm long, 2 mm wide at base and 1 mm wide at apex. **Gynostemium** coroniform, stipitate, 2.5 x 1 mm; **stipe** 0.5 mm long, **stigmatic lobes** 5, 0.7-1 mm long, **anthers** 5, 1.5 mm long. **Ovary** inferior, lanceolate 6-8 x (0.6-) 0.9 mm, with sparse to dense septate trichomes. **Fruit** a capsule, obovoid to ellipsoid, 1.3 x 1.12 cm, green-purplish red when young, brown yellow when mature, with septicidal dehiscence at the apex. **Seeds** deltoid, 3-3.5 x 2.5-3 mm, upper part black, lower part yellow-brown, with granular surface. Figure 16H, I.

Phenology—This species flowers from August to September, and has been collected in fruit in September.

Distribution and Habitat— *Aristolochia* sp. nov. inedit. is known from the municipalities of Atengo and Pihuamo in the state of Jalisco, in pine-oak forest vegetation with *Quercus magnoliifolia, Lysiloma acapulcense,* and *Verbesina*.

Additional Specimens Examined—MEXICO. Jalisco: Mun. Pihuamo, 5km al SE de Pihuamo, 13Q 672060-2149189, 1,302 m. 23 September 2009, *A. Paizanni Guillén et al. 67* (IBUG, IEB, MEXU).

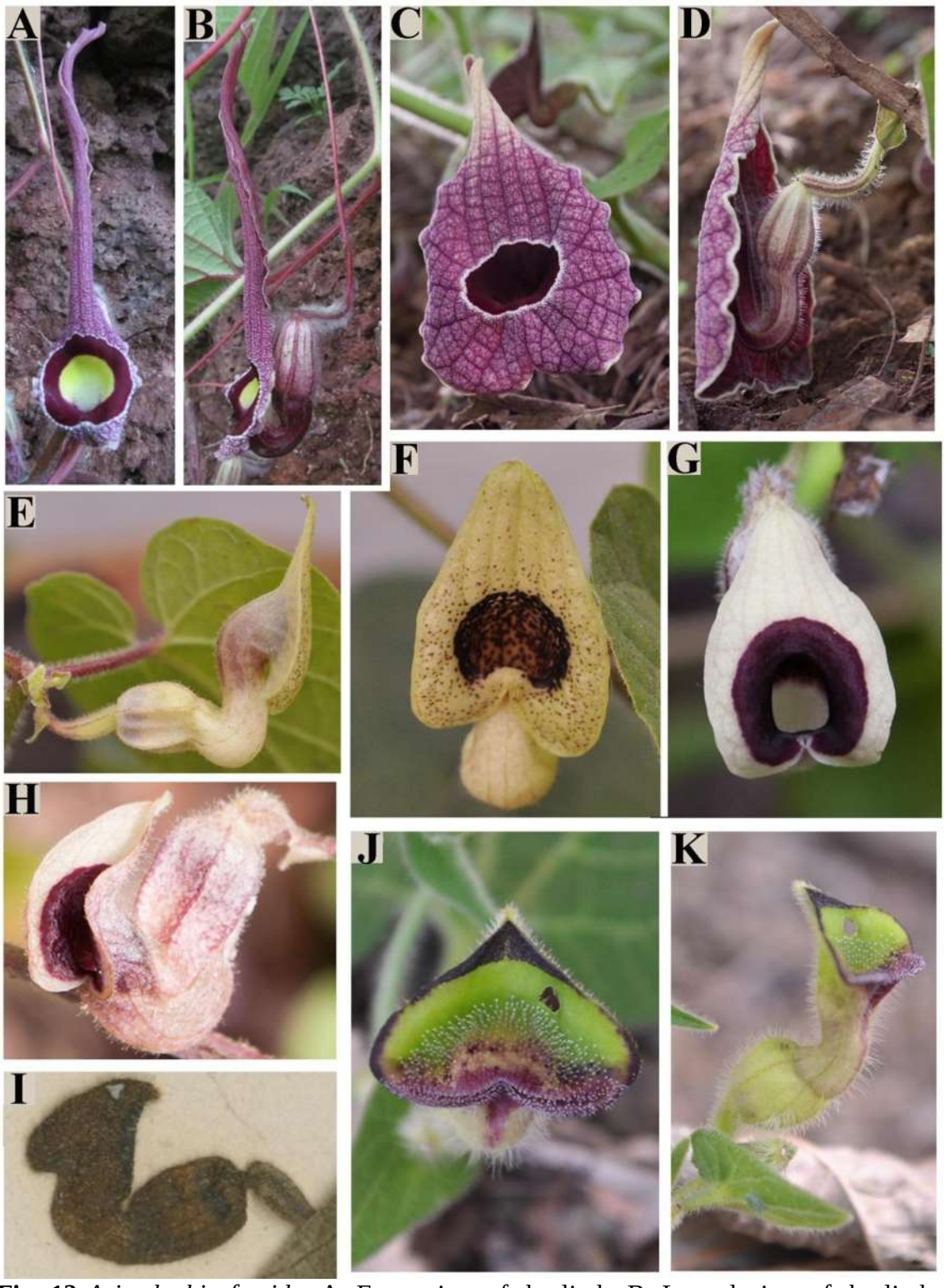

Fig. 12 *Aristolochia foetida*, A. Front view of the limb. B. Lateral view of the limb. *A. cardiantha*, C. Front view of the limb. D. Lateral view of the limb. *A. tequilana*, E. Lateral view of the limb. F. front view of the limb. *A. conversiae*, G. Front view of the limb. H. Lateral view of the limb. *A. tresmariae*, I. Lateral view of the limb. *A. mutabilis*, J. Front view of the limb. K. Lateral view of the limb. (I, picture from herbarium MEXU).

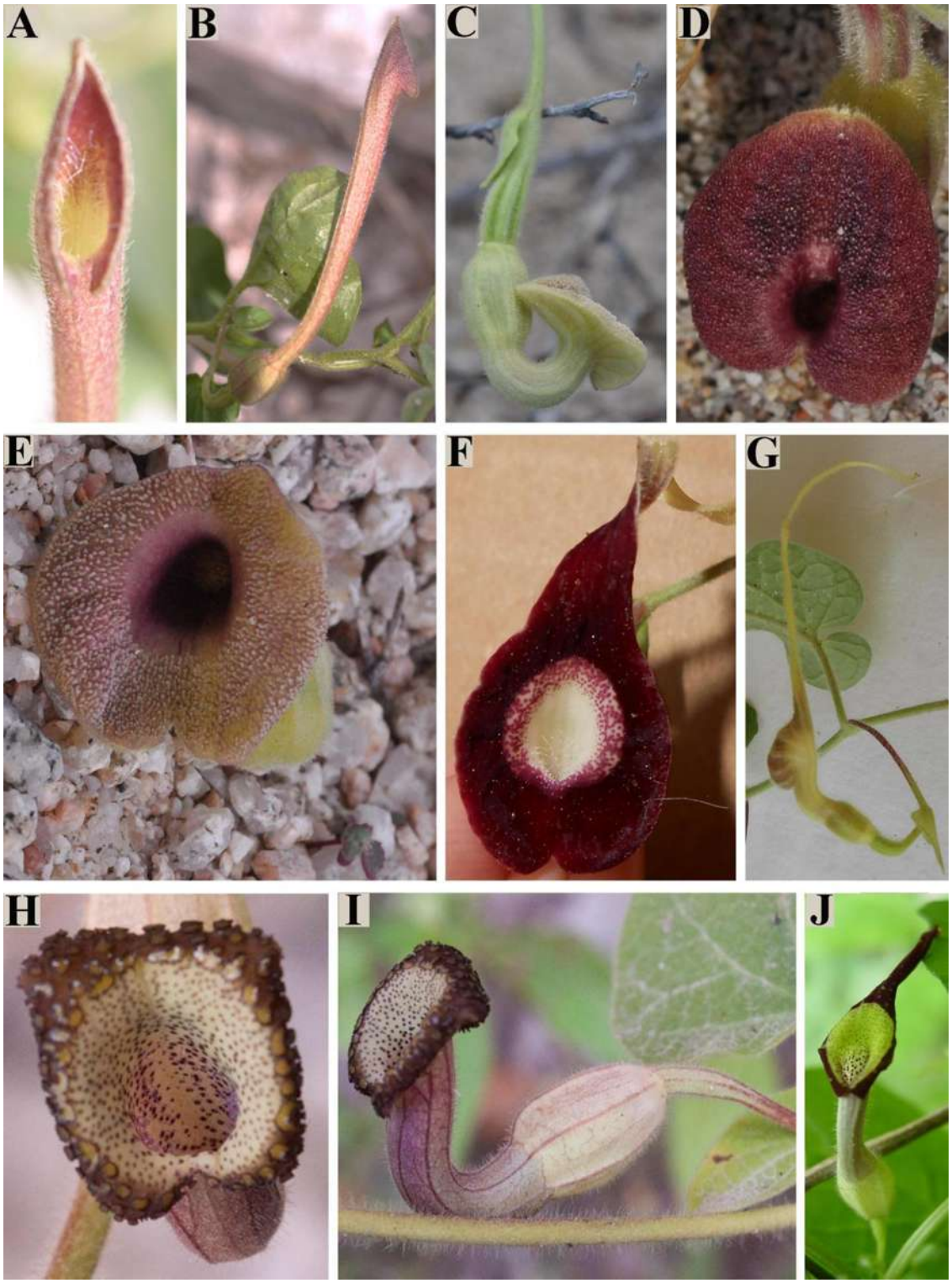

Fig. 13 *Aristolochia teretiflora*, A. Front view of the limb. B. Lateral view of the limb. *A. monticola*, C. Lateral view of the limb. D-E. Front view of the limb. *A. nelsonii*, F. Front view of the limb. G. Lateral view of the limb. *A. durangensis*, H. Front view of the limb. I. Lateral view of the limb. *A. pentandra*, J. front view of the limb. (F, G, pictures from M.-S.Samain).

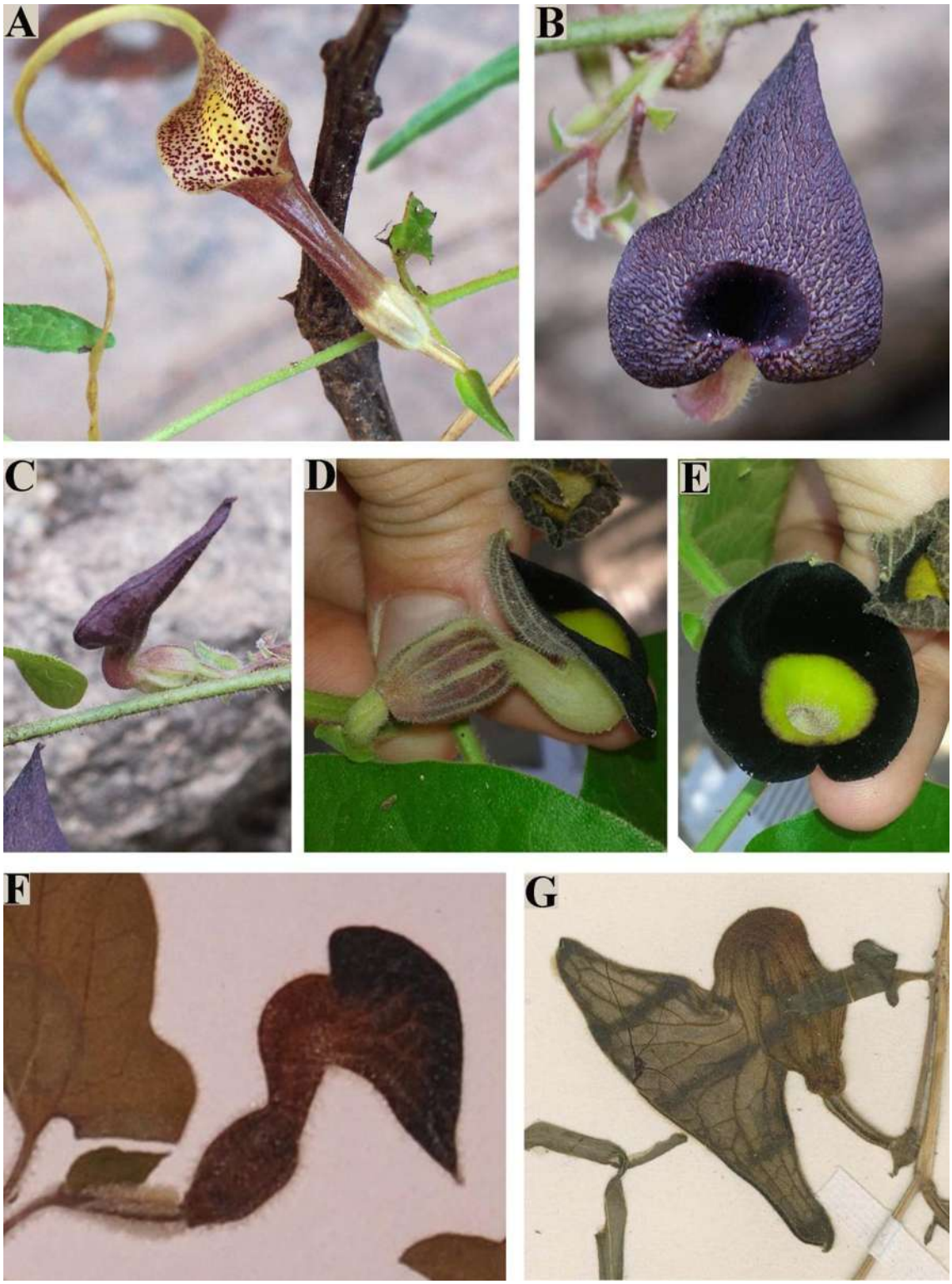

Fig. 14 *Aristolochia palmeri*, A. Front view of the limb. *A. rzedowskiana* B. Front view of the limb. C. Lateral view of the limb. *A.* sp. nov. inedit. (Tlalcoyunque), D. Lateral view of the limb., E. Front view of the limb. *A. variifolia*, F. Lateral view of the limb. *A. acontophylla*, G. Lateral view of the limb. (D, E, pictures from Amandine Bourg; F. from herbarium MEX and G. from herbarium US).

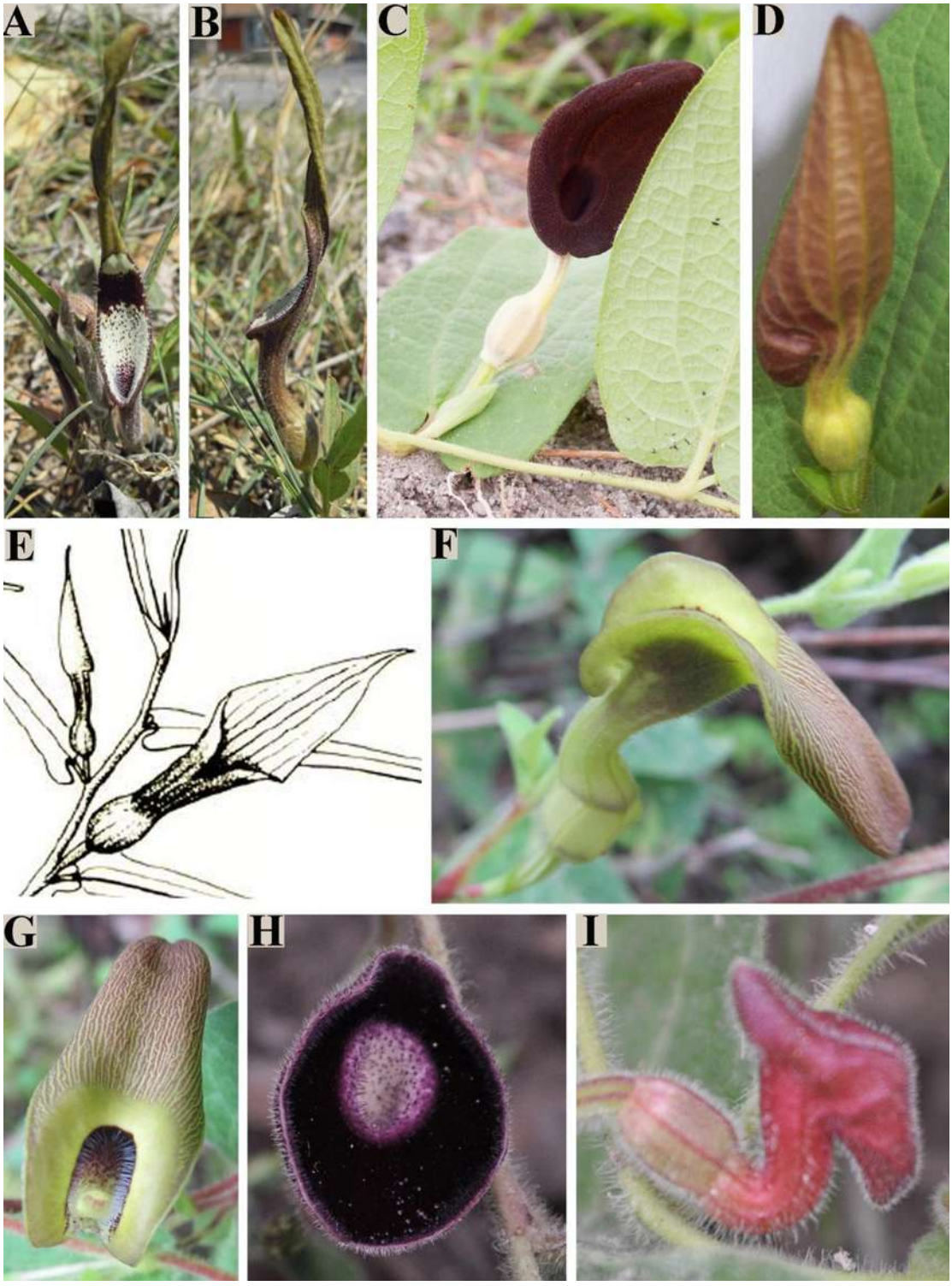

Fig. 15 *Aristolochia erecta*, A. Front view of the limb. B. Lateral view of the limb. *A. bracteosa* C. Front view of the limb. D. Lateral view of the limb. *A. davilae*, E. Front view of the limb. *A. flexuosa*, F. Lateral view of the limb. G. Front view of the limb. *A. secunda*, H. Front view of the limb. I. Lateral view of the limb. (A, B. pictures from (http://www.thedauphins.net/id111.html); E. from Calzada et al. 1997 and F, G. from J. M. Ramírez Amezcua).

Fig. 16 *Aristolochia brevipes*, A. Front view of the limb. B. Lateral view of the limb. *A. occidentalis* C. Front view of the limb. *A. oaxacana*, D. Front view of the limb. E. Lateral view of the limb. *A.* sp. nov. inedit. (Quila), F. Front view of the limb. G. Lateral view of the limb. *A.* sp. nov. inedit. (Sierra Verde), H. Front view of the limb. I. Lateral view of the limb. (C. pictures from J.F. Santana Michel and H, I. from J. González Gallegos).

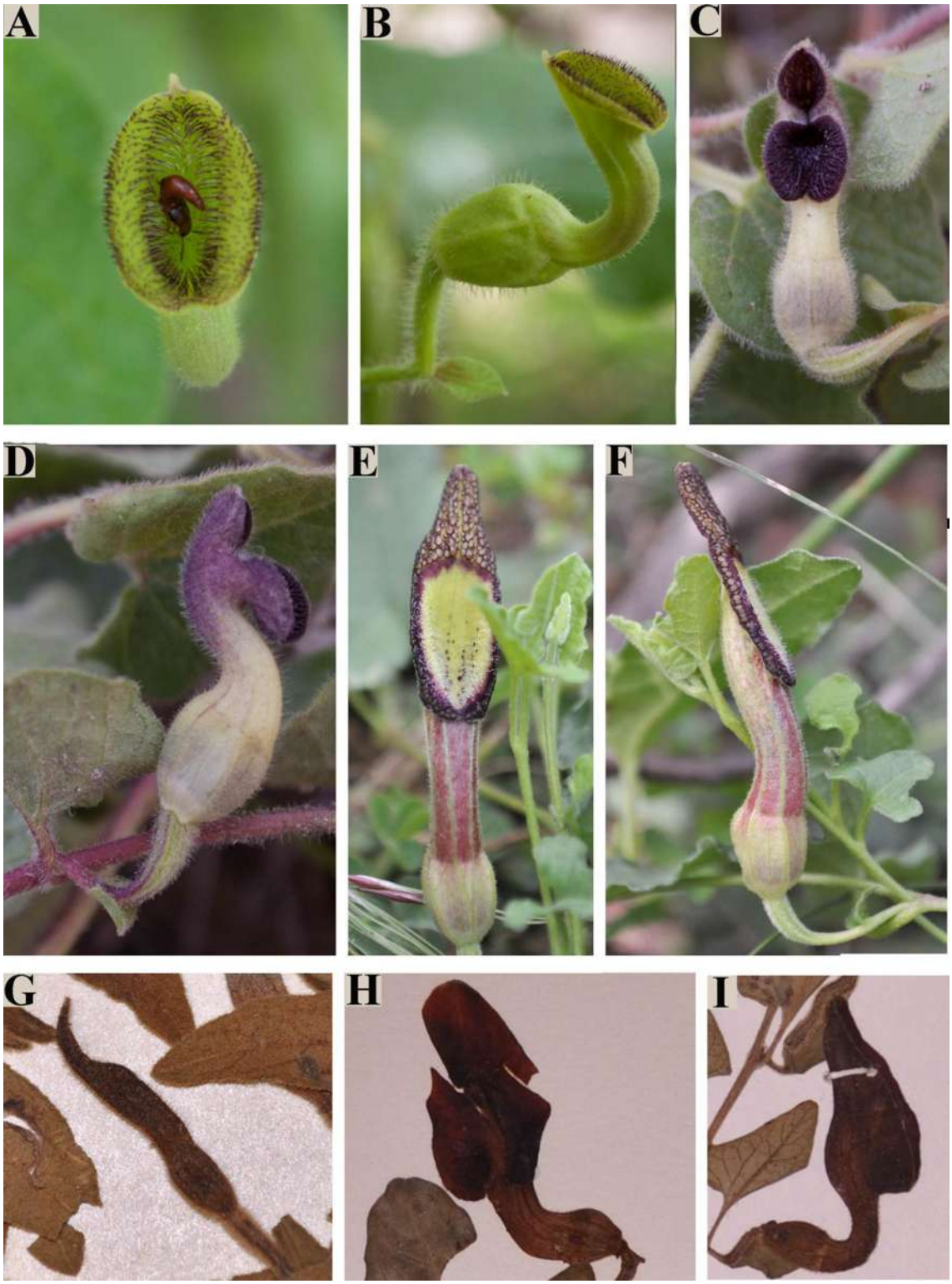

Fig. 17 *Aristolochia* sp. nov. inedit. (Huetamo), A. Front view of the limb. B. Lateral view of the limb. *A. cordata*, C. front view of the limb. D. Lateral view of the limb. *A. versabilifolia*, E. front view of the limb. F. Lateral view of the limb. *A. whitei*, G. Lateral view of the limb. *A. karwinskii*, H. Front view of the limb. I. Lateral view of the limb. (G-I, pictures from herbarium MEXU).

Fig. 18 *Aristolochia nana*, A. Front view of the limb. B. Lateral view of the limb. *A. luzmariana* C. front view of the limb. D. Lateral view of the limb. *A. wrightii*, E. Front view of the limb. F. Lateral view of the limb. *A. emiliae*, G. Front view of the limb. H. Lateral view of the limb. *A.* sp. nov. inedit. (Chavarín), I. Front view of the limb. J. Lateral view of the limb. (J, G, pictures from J.F. Santana Michel).

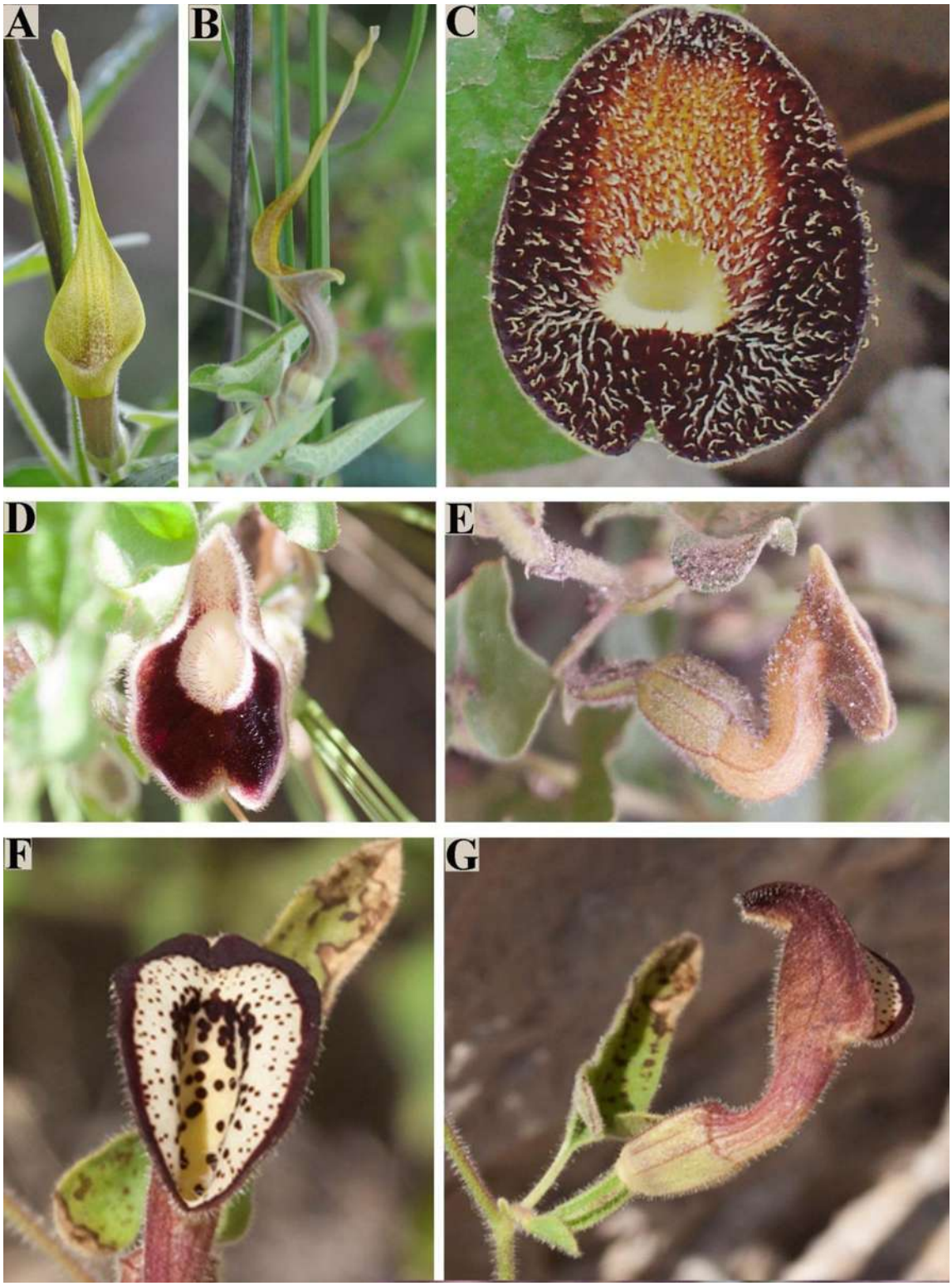

Fig. 19 *Aristolochia pringlei*, A. Front view of the limb. B. Lateral view of the limb. *A. manantlanensis* C. Front view of the limb. *A. lassa*, D. Front view of the limb. E. Lateral view of the limb. *A. coryi*, F. Front view of the limb. G. Lateral view of the limb. (C, pictures from J.F. Santana Michel; F, G, from http://www.forestventure.com/speciesdetail.cshtml?id=93087).

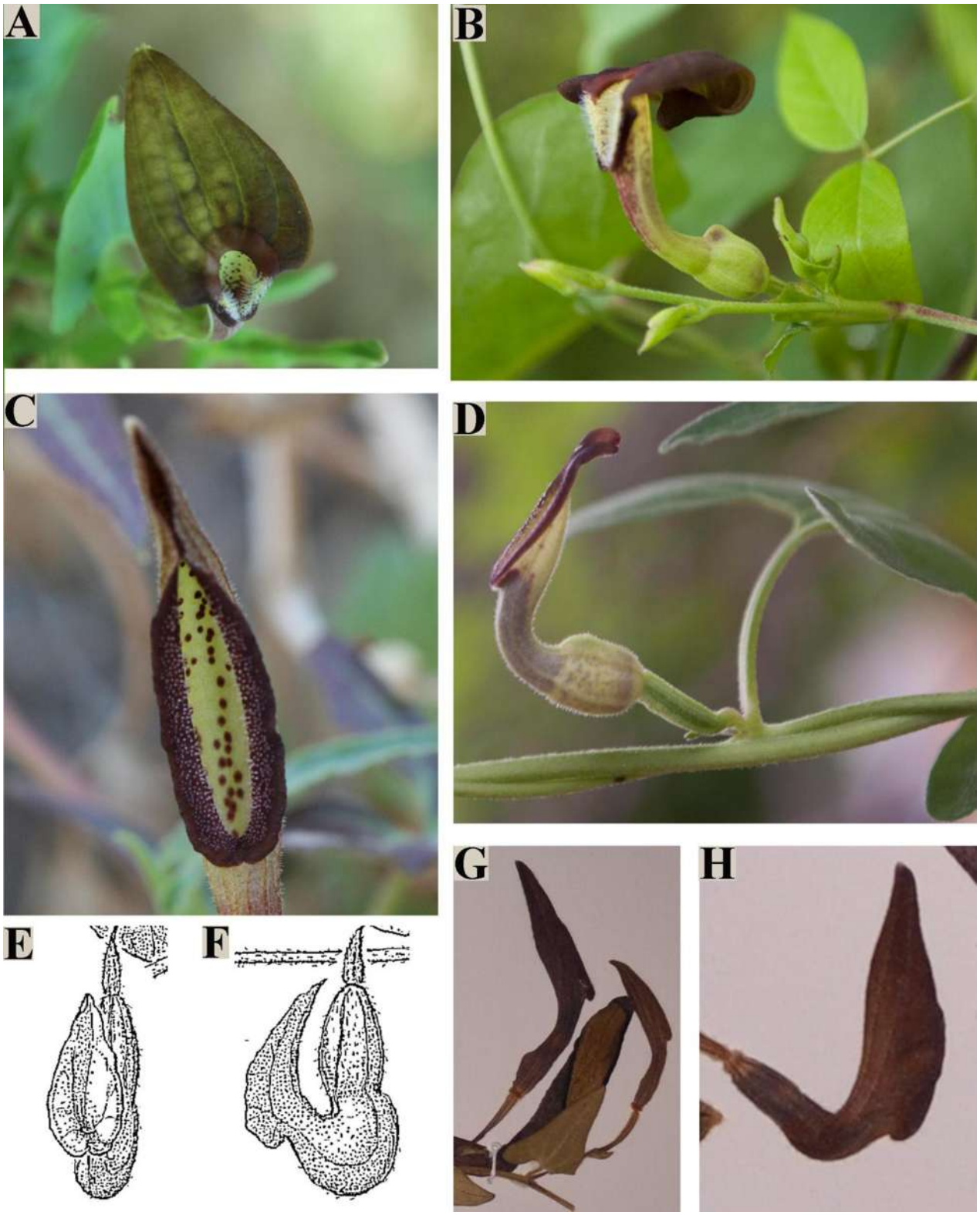

Fig. 20 *Aristolochia colimensis*, A. Front view of the limb. B. Lateral view of the limb. *A. watsonii*, C. Front view of the limb. D. Lateral view of the limb. *A. micrantha*, E. Front view of the limb. F. Lateral view of the limb. *A. sinaloae*, G, H. Lateral view of the limb. (C, D, pictures from S. Wanke; E, F, from Pfeifer, 1970; E. from herbarium MEXU).

1.4 DISCUSSION

An important result of this project is the substantial increase in knowledge of *Aristolochia* subsection *Pentandrae*, based on a molecular study (see chapter 2) and a taxonomic revision, using the broadest sampling ever, including many field collections, field observations and an extensive study of herbarium material.

We observed and collected 40 species in the field, 17 of which in their type locality. Additionally, new records of six species were found, such as *A. durangensis* in Nayarit, and *A. nana* and *A. lassa* in Coahuila. Species circumscriptions were clarified and amended by documenting the morphological variation which is especially common in the leaves, as well as the coloration of the limb and throat. For instance, *Aristolochia watsonii* is here reported with very variable limb coloration. Moreover, *Aristolochia wrightii* shows variation in the color of the throat throughout its distribution area. Confusion between the species of the *A. buntingii* complex because of the similar flowers shapes also have been clarified (Paizanni et al. 2016). Another example of morphological variation that should be disentangled in future studies is the potential synonymy of *Aristolochia foetida* and *A. cardiantha*. Both species show in their respective type location notable morphological differences; however, during fieldwork it was observed that both species fall within a range of morphological characters. This observation is relatively common in several species as much more morphological variation is present within a particular species than originally described. This is most likely due to the fact our studies are based on the broadest and most representative sampling that has ever been carried out in *Pentandrae*.

Furthermore, in this study we have made a detailed list of all diagnostic characters, such as the ornamentation and tridimensionality of the limb, the indument inside the perianth and its texture, the front view and coloration of the limb, the throat shape, color and indument, the utricle shape as well as the leaf shape. All of which has allowed us to complete historical descriptions and provide emended descriptions.

It should also be stated here that species of this group are relatively difficult to find, as they are quite rare, hence, a lot of time was spent on exploration during the fieldwork. Because of these difficulties, the collections are not so well represented in the herbaria. Additionally, new species are expected in unexplored areas throughout Mexico. Within *Pentandrae*, vegetative characters such as leaf shape might be prone to parallel evolution as an adaptation of recurring abiotic factors. The aforementioned attributes, specific characteristics and correlations with respect to the phylogenetic hypothesis of *Pentandrae*,

as well as the taxonomic revision, are the first attempt to increase the general knowledge of this group. Therefore, it is a suitable study group to address some major questions concerning variation ranges, anatomy of the subterranean organs, dispersal, population genetics, chemical components and risks of using this plant as medicine, among others.

CHAPTER 2 PHYLOGENETIC RELATIONSHIP IN *ARISTOLOCHIA* SUBSECTION *PENTANDRAE*

2.1 INTRODUCTION

Aristolochiaceae is together with Asaraceae, Lactoridaceae, and Hydnoraceae part of a Piperales clade that is characterized by the presence of a perianth, in contrast to the perianth-less families Piperaceae and Saururaceae (Naumann *et al.* 2013). Currently, Aristolochiaceae comprise only two genera (e.g. Horner et al. 2015): *Aristolochia* L. with about 500 species and a nearly worldwide distribution, and *Thottea* with 35 species distributed in Asia (Neinhuis et al. 2005; Wanke et al. 2006).

The highest species richness of *Aristolochia* is found in the tropics and subtropics (Kelly 2000; Neinhuis et al. 2005; Ohi-Toma et al. 2006; Wanke et al. 2006; González et al. 2010). It has been subdivided in three subgenera: subgenus *Siphisia* occurring in Asia, North and Central America (Wagner *et al.* 2014, González et al. 2014), subgenus *Pararistolochia* present in tropical Africa and Australasia (Buchwalder et al. 2014) and subgenus *Aristolochia* occurring in America, Europe, Australasia, Africa and the Middle East. The latter subgenus is further subdivided into several monophyletic groups, but the relationships of these remain largely unknown and are currently treated as operational taxonomic units (OTUs). Six OTUs are currently recognized: "Howardia" p.p. (including the former genera *Euglypha* and *Holostylis*), *Aristolochia* s.str., *Thyrsicae*, the "*Aristolochia lindneri* group", subsection *Pentandrae* (former genus *Einomeia* according to Rafinesque (1828) and Huber (1985, 1993)), as well as the "*Aristolochia grandiflora* complex" (Neinhuis et al. 2005).

In Mexico *Aristolochia* is present with about 70 species belonging to two subgenera (i.e. *Siphisia* and *Aristolochia*) (Pfeifer 1966, 1970; González et al. 2014). The subsection *Pentandrae* (hereafter shortened to *Pentandrae*) is traditionally placed in section *Gymnolobus*, proposed by Duchartre (1854, 1864) and is the most species-rich *Aristolochia* lineage in Mexico. The clade is characterized by two morphological synapomorphies: five stamens and five carpels (a 5-merous gynostemium), whereas all other Mexican species have six stamens (Pfeifer 1966, 1970). The representatives of *Pentandrae* are further characterized by a geophytic and procumbent habit (occasionally climbing), as well as a perennial subterranean organ. Although *Pentandrae* is a comparatively species-rich lineage, it is the least studied *Aristolochia* lineage on worldwide scale. Its distribution area ranges from the southern United States to Colombia

(San Andrés Island), including Guatemala, Belize, Honduras (Swan Islands) and the West Indies, but it is most diverse in Mexico (Pfeifer 1970; González et al. 2010). Thirty-five species have been recognized by Pfeifer (1970), who has published the only available, broad scale taxonomic revision of the subsection. Subsequently, eight additional species have been described from Mexico by Ortega and Ortega (1995), Santana-Michel (1995, 2002, 2007), Santana-Michel and Lemus-Juárez (1996), Calzada et al. (1997), Santana-Michel and Solís-Magallanes (2007), and Santana-Michel and Guzmán-Hernández (2014), resulting in a total of 43 species known today. Additionally, four new species have been published recently (Paizanni et al. 2016).

Since the treatment of Pfeifer (1970), relatively little research has been carried out on *Pentandrae* as a whole. Molecular studies within the family Aristolochiaceae have especially focused on genus and subgenus level and only few studies have addressed particular lineages in more detail (e.g. Neinhuis et al. 2005; Ohi-Toma et al. 2006; Wanke et al. 2006). Molecular phylogenetic studies indicated that it is a monophyletic group although only a few species of *Pentandrae* were included. The species that are most closely related to *Pentandrae* are an informal group consisting of five species, named the *Aristolochia lindneri* group (Wanke et al. 2006, Ohi-Toma et al. 2006; González et al. 2010). Until now, only morphological evidence exists that the group is is a monophyletic entity sister to *Pentandrae* (González et al. 2010).

Until today, limited molecular-genetic data are available for *Pentandrae* species and their natural relationships remain largely unknown. A first molecular phylogenetic hypothesis of this clade is presented in this study. Together with the morphological data, this information will serve as the basis for more extensive research, including the biogeographic history and adaptive radiation of this group.

In the past only few species of *Pentandrae* have been sequenced for the plastid *trnL-F* region as well as the *trnK-matK-trnK-psbA* region. Both regions have been recommended as good candidates for molecular reconstructions among angiosperms. These regions are also among the most variable regions of the plastid chromosome (e.g. Shaw et al. 2007, 2013). A preliminary study of the TU Dresden research group, including a handful accessions of *Pentandrae* using the *trnK-matK-trnK-psbA* region, indicated rather low variability between the studied accessions. Therefore, a first step was to test the variability of plastid regions on a small but representative sampling. Because we were well aware that variability might be too low to resolve species level relationships and potentially also the

backbone of the phylogenetic tree. We decided to increase the dataset with additional non-coding plastid regions, including the *rpL32-trnL*$^{(UAG)}$ spacer, the *rpL16* intron and the *psbJ-petA* spacer, following the results from Wagner et al. (2012) and suggestions published by Shaw et al. (2005, 2007).

The chloroplast *trnK-matK-trnK-psbA* region consists of the *tRNA* ^{LYS} *(UUU)* gene (*trnK*) containing a group II intron (*trnK* intron). The latter also encodes the *matK* open reading frame (ORF) and is the only maturase of the plastid genome involved in splicing of group II introns (Vogel et al. 1997, Hausener et al. 2005) (Fig.1, 2). This region has become one of the most frequently used chloroplast gene markers in angiosperm phylogenetic studies. Since *matK* can easily be co-amplified with the flanking non-coding intron parts, the complete *trnK* intron is increasingly used, expanding the dataset to 2400-2700 bp. As a consequence, the utility of this region extends to inter- and intra- species level (e.g., Wanke et al. 2006). The *trnK-psbA* spacer was sequenced as well as three additional regions: *rpL32-trnL*$^{(UAG)}$ spacer, *rpL16* intron and *psbJ-petA* spacer (Figures 21, 22).

The *rpL32-trnL*$^{(UAG)}$ spacer region is located in the small single copy region (SSC) of the plastid genome. It is an intergenic spacer that has been used by Timme et al. (2007) who noted that it is a rather variable region. Shaw et. al. (2007) showed large indels in *Hibiscus* (Malvaceae) and *Magnolia* (Magnoliaceae) among others, and it has been used in *Aristolochia* subgenus *Siphisia* resulting in good support on species level (Wagner et al. 2012). The average length of *rpL32-trnL*$^{(UAG)}$ spacer is 1018 bp, and it ranges from 543-1417 bp due to its indels (Figs. 21, 22).

The *rpL16* intron region consists of a group II intron that divides the coding region into a shorter and a longer exon. The region has been shown to have high sequence divergence in flowering plants (Wolfe et al. 1987; Shaw et al. 2005). However, the first attempt to use the *rpL16* intron was published by Jordan et al. (1996) who reported little variation. This region has also been applied to the genus *Aristolochia* before (Wagner et al. 2012). The average length of the *rpL16* intron is 1002 bp, ranging from 811 to 1208 bp, and is especially indel prone in the D3 loop region (Baum et al. 1998; Kelchner 2000; Kelchner 2002; Pfeil et al. 2002; Shaw et al. 2005) (Figs. 21, 22).

The *psbJ-petA* spacer is located in the large single copy region (LSC). The use of this region resulted in good statistical support on species level in *Aristolochia* subgenus *Siphisia* (Wagner et al. 2012). It was also recommended as a potentially useful microsatellite region in *Castanea* (Fagaceae; Sebastiani et al. 2004). However, Shaw et al.

(2007) showed that more distantly related species were difficult to align and they observed several poly-A/T repeats in all the investigated taxa. The average length of this intergenic spacer is 1040 bp, and it ranges from 734-1261 bp. (Figs. 21, 22).

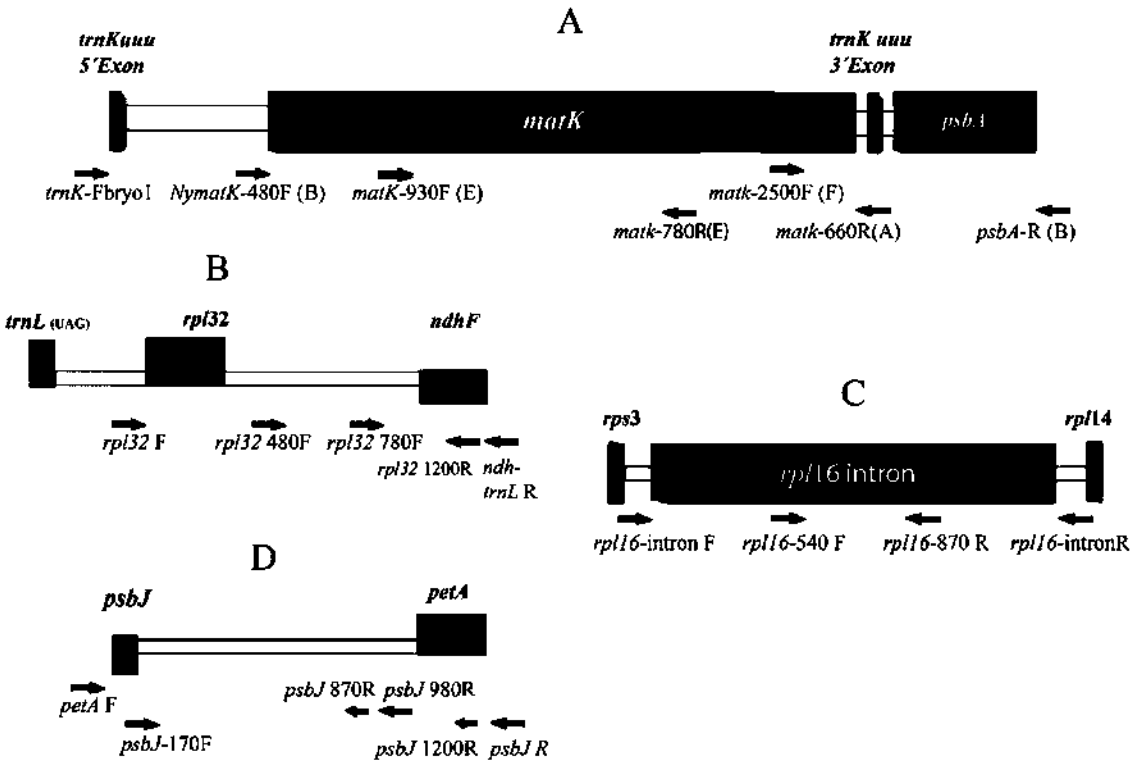

Fig. 21 Plastid regions tested for molecular phylogenetics (all regions at the same scale) A. *trnK-matK-trnK-psbA*. B. *rpL32-trnL*$^{(UAG)}$ spacer. C. *rpL16* intron and D. *psbJ-petA* spacer, with approximate primer annealing sites indicated (arrows) and from the plastid *trnK-matK-trnK-psbA* the letter in brackets represent the combination of primers that were used, see Tables 3, 4.

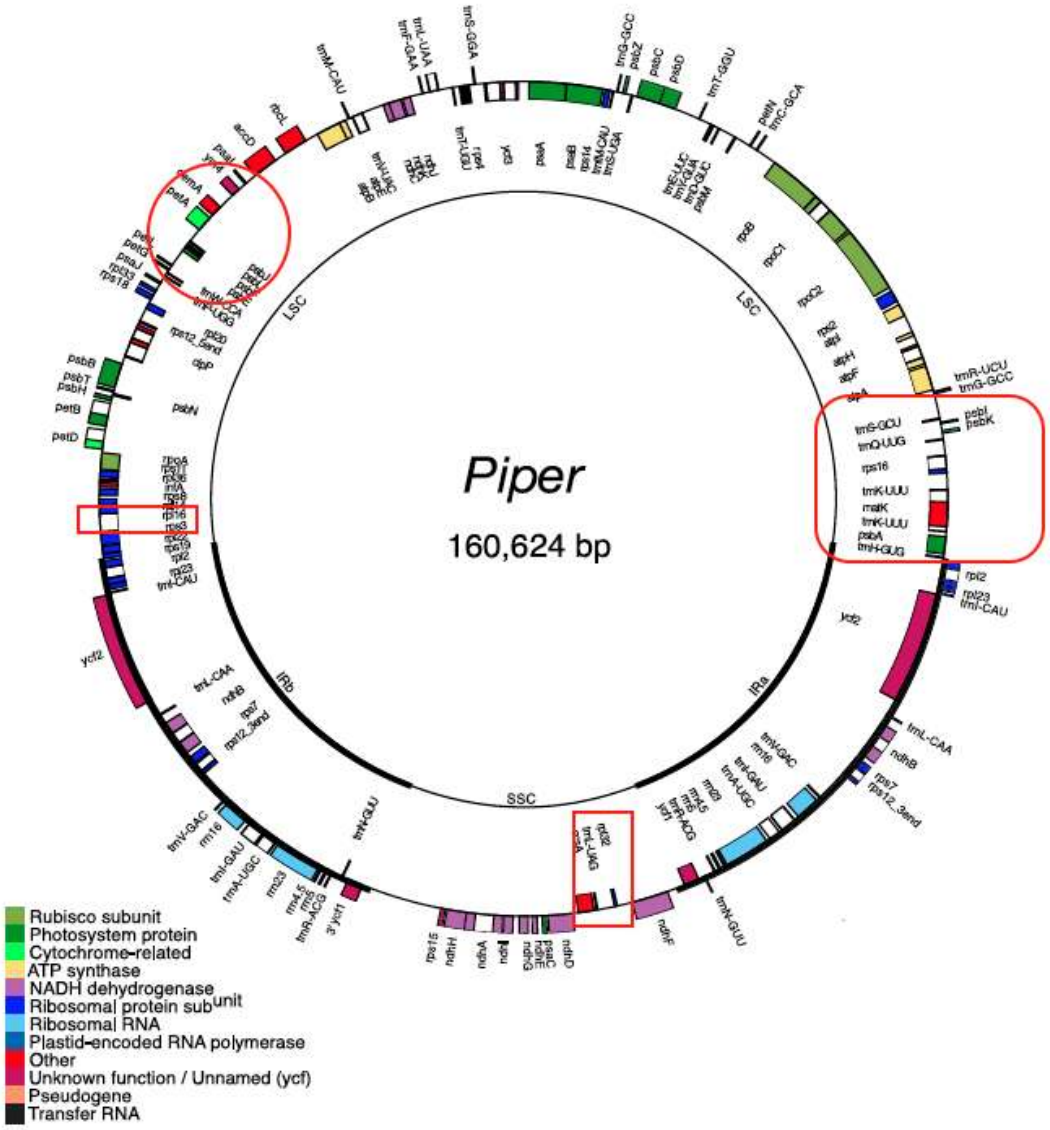

Fig. 22 Plastid genome of *Piper coenoclatum*. The relative positions of the plastid regions used for phylogenetics in this study are shown (red squares and circles) (taken from Cai et al. 2006).

In molecular phylogenetics, plastid and nuclear ribosomal DNA (nrDNA) have mainly been used in flowering plants. However, it has been shown that other loci from the nuclear genome might be preferred over these traditionally used regions. The challenge is to identify informative markers that allow the reconstruction of the species relationships or where lineages split in quick succession. Nuclear single copy genes and especially their introns have the potential to overcome these disadvantages. Access to genome scale, massively paralleled sequenced data was provided by Sebastian Müller, a Master student in the Dresden research group, who is part of the collaborative project between Mexico and Germany. This project also includes a first test of two loci selected from the data of Müller

(2015). The possibilities to explore alternative nuclear encoded regions will be studied in the future.

The phylogenetic objectives are:

- Perform a first molecular phylogenetic study of *Pentandrae* that will not only provide the basis for a revision of the clade, but will also allow obtaining future insight in the evolutionary biology of the species including their diversification and biogeography.
- Obtain a first molecular phylogenetic hypothesis based on two plastid regions: *trnK-matK-trnK-psbA* and *rpL16* intron.
-Testing two variable nuclear loci L26 and L144.

2.2. Material and Methods

Plant material was collected during a field trip: from mid June to early October 2014, and during 2015 in January (Yucatán) and in September (Baja California Sur). The fieldwork was supported by the Technical University of Dresden (Germany) through financing by the German Academic Exchange Service (DAAD), as well as funding by the Instituto de Ecología, A.C. During fieldwork, leaves were conserved in silica gel for molecular lab work, and whole plant material was collected for further study and herbarium vouchers.

The phylogenetic work was carried out in collaboration with Dr. Stefan Wanke of the Technical University of Dresden (Germany). The research stays in Dresden were supported by the Instituto de Ecología, A.C. through financing by CONACyT. The methodological background was gained during a first visit of two months (end of November 2014 to end of January 2015) while most of the practical work was done during a second stay (mid April to begin of July 2015).

Table 1. List of the currently known species of *Aristolochia* subsection *Pentandrae*, including unpublished species new to science. The numbers in the columns of DNA material and field collection represent the different material we obtained in different locations through the fieldwork. Some of the species were previously collected. Consequently, we do not have the same number of DNA collections as field collections. The numbers in the line of the herbarium material (HB material) represent the ones that were taken for DNA. The collector APG- means Anna Paizanni Guillén and in the origin column part, the abbreviation of the states means: Baja California Sur, B.C.S.; Chihuahua, Chih.; Coahuila Coah.; Colima Col.; Distrito Federal D. F.; Durango Dgo.; Guanajuato Gto.; Guerrero Gro.; Hidalgo Hgo.; Jalisco Jal.; Michoacán Mich.; Morelos Mor.; Nayarit Nay.; Nuevo León N. L.; Oaxaca Oax.; Puebla Pue.; Querétaro Qro.; San Luis Potosí S. L. P.; Sinaloa Sin.; Tamaulipas Tamps.; Yucatán Yuc.

	Sample	**Collector and number**	**DNA Material**	**Field collection**	**HB material**	**Origin**
1	*Aristolochia acontophylla* Pfeifer					Col.
2	*A. bracteosa* Duch.	APG 238	1	1		Jal.
3	*A. brevipes* Benth	APG 119, 318	1	2		Edo., Mex., Mich.
4	*A. buntingii* Pfeifer	APG 113	1	1		Mich.
5	*A. cardiantha* Pfeifer	APG 191, 192	2	2		Gro.
6	*A. colimensis* J. Santana	APG 287, 66	1	2		Col.
7	*A. conversiae* Pfeifer	APG 239	1	1		Edo. Mex.
8	*A. cordata* Eastw.	APG 223	1	1		Dgo.
9	*A. coryi* I.M. Johnst.		1		1	Texas, USA
10	*A. davilae* Calzada, G. Flores & O. Téllez					Nay.
11	*A. durangensis* Pfeifer	APG 217, 218	2	2		Dgo., Nay.
12	*A. emiliae* Santana Mich. & Solís	F. Santana s.n	1		1	Jal.
13	*A. erecta* L.					
14	*A. flexuosa* Duch.	APG 67, 118, 194, 297	3	4		Mich.
15	*A. foetida* Kunth	APG 286, 290, 205, 294, 195, 207	6	6		Col., Jal.
16	*A. islandica* Pfeifer					
17	*A. karwinskii* Duch.					Tamps.

18	*A. luzmariana* J. Santana	APG 199, 201, 202, 282, 283, 285	6	6		Jal.
19	*A. manantlanensis* Santana Mich.					Jal.
20	*A. micrantha* Duch.					Jal.
21	*A. monticola* Brandegee	APG 298, 302, 304, 312, 313, 315, 317_MS 2015-08,09,14,15	11	11		BCS
22	*A. mutabilis* Pfeifer	APG 112, 114	1	2		Mich.
23	*A. nana* S. Watson	APG 255, 256, 269, 270	4	4		S.L.P., Coah.
24	*A. nelsonii* Eastw.	Samain 2014-017	1	1		Oax.
25	*A. oaxacana* Eastw.	APG 249, 250	2	2		Oax.
26	*A. occidentalis* J. Santana & S.Lemus	APG 196, 197, 198, 280	4	4		Col., Jal.
27	*A. palmeri* S. Watson	APG 68, 69, 102, 211	4	4		Jal.
28	*A. pentandra* Jacq.	APG 266, 262, 263; Samain	4	4		N.L., Yuc.
29	*A. lassa*	APG 267, 268, 261	3	3		Coah.
30	*A. pringlei* Rose	APG 70, 75, 76, 111, 206, 208, 209	7	7		Jal.
31	*Aritolochia pueblana* J.F. Ortega & R.V. Ortega	APG 242	1	1		Pue.
32	*A. secunda* Pfeifer	APG 260	1	1		Coah.
33	*A. sinaloae* Brandegee		1		1	Sin.
34	*A. socorroensis* Pfeifer	APG 123, 124, 129, 128, 147, 149	3	6		Isla Socorro
35	*A. tequilana* S. Watson	APG 108, 212, 213	3	3		Jal.
36	*A. teretiflora* Pfeifer	APG 248, 251	2	2		Oax.
37	*A. tresmariae* R.S. Ferris					
38	*A. variifolia* Duch.		1		1	Gro.
39	*A. versabilifolia* Pfeifer	APG 122, 252, 253, 271, 254	3	5		Qro.,Gto.

40	*A. watsonii* Wooton & Standl.	APG 214, 215, 237, 222, 305, 306, 308_MS 2015-07,10, 11, 12, 13, 16_SW(4)ARIZ	12	17		Sin., Nay. B.C.S.
41	*A. whitei* I.M. Johnst.					Chih.
42	*A. wrightii* Seem.	APG 230, 231, 232, 233, 234	3	5		Dgo, N.L
43	*A. rzedowskiana* J. Santana & Guzmán Hernández	APG 62, 63, 71, 274, 275	2	5		Jal.
44	*A. nahua* Paizanni & J. Santana	APG 278	1	1	1	Jal.
45	*A. pacifica* J. Santana & Paizanni	APG 296	1	1		Jal., Col.
46	*A. savannoidea* Paizanni & J.M. Ramírez	APG 219, 220, 221	3	3		Nay.
47	*A. tuitensis* J. Santana & Paizanni	Santana number			1	Jal.
48	*A.* sp.1 (Huetamo)	APG 190	1			Gro.
49	*A.* sp.2 (Quila)	APG 59, 77, 78	1	3		Jal.
50	*A.* sp.3 (Roca Tlacoyunque)				1	Gro.
51	*A.* sp.4 (Sierra Verde)	APG 210	1	1		Jal.
52	*A.* sp.5 (El Chavarín)	APG 292	1	1		Col.
53	*A.* aff. *tequilana* (Tamazula Dgo.)	APG 236	1	1		Dgo.
54	*A.* sp.7 (Pihuamo)				1	Jal.
55	*A.* sp.8 (Purhepecha)				1	Gro.
56	*A.* sp.9 (Cabo Corrientes)	APG 273	1	1		Jal.
57	*A.* affinis	APG 204, 279, 203, 293	4	4		
	TOTAL		111	127	9	

2.2.1 Taxon Sampling and Plastid DNA sequences

The plastid regions *trnK-matK-trnK-psbA*, *rpL16*-intron, and the two spacers *rpL32-trnL*$^{(UAG)}$ and *psbJ-petA* were sequenced for 39 *Pentandrae* species (fresh material) representing about 73% of the species diversity. *Thottea*, the sister genus of *Aristolochia*, was chosen as outgroup. In order to test the effect of outgroup choice, additional outgroups were considered: representatives of subgenus *Pararistolochia* (*Aristolochia promissa* from Africa), subgenus *Siphisia* (*Aristolochia arborea*, *A. asclepiadifolia*, *A. chiapensis*, *A. impudica*, *A. kalebii*, *A. malacophylla*, *A. salvadorensis*, *A. serpentaria*, *A, tomentosa*, *A. veracruzana* from Mexico, Central America and *A. westlandii* from Asia), and multiple OTU's of subgenus *Aristolochia*: *Aristolochia s. str*. (*A. bottae* from the Mediterranean), the *Thyrsicae* group, the "*Aristolochia grandiflora* complex" (*A. grandiflora* from Mexico and Central America), the "Howardia" p.p. group (*Aristolochia gigantea* from Central and South America), as well as the "*Aristolochia lindneri* group" (*A. lindneri and A. burelae* from South America) (Table2).

Table 2. List of all samples used for phylogenetic reconstruction. The sequence information from the outgroup (*Thottea*), the subgenus *Siphisia* and other OTU´s (see above) was provided from previous studies from the TU Dresden laboratory group (grey area). The collector APG- means Anna Paizanni Guillén and in the origin column part, the abbreviation BG means Botanical Garden and of the countries means: Bolivia, BO; Germany, DE; Jordan, JO; India, IN; Mexico, MX; Spain, ES; United States of America, USA.

#	**Species**	**Collection #**	**Lab #**	**Origin**	*trnK - psbA*	*psbJ-petA*	*rpL32-trnL*$^{(UAG)}$	*rpL16* intron	L26	L144
1	*Aristolochia aff. lassa*	267 APG	P1748	La Sandía, Nuevo León, MX	1	1		1		
2	*A. aff. Tequilana*	236 APG	P1825	Tamazula, Durango, MX	1	1	1	1		
3	*A. bracteosa*	238 APG	P1757	Tala, Jalisco, MX	1	1	1	1		
4	*A. brevipes*	119 APG	P1776	Pátzcuaro, Michoacán, MX	1	1	1	1	1	1
5	*A. buntingii*	113 APG	P1758	Arteaga, Michoacán, MX	1	1	1	1	1	1
6	*A. cardiantha*	191 APG	P1752	Coyuca de Catalán, Michoacán, MX	1	1	1	1	1	1
7	*A. colimensis*	287 APG	P1777	Ixtlahuacán, Colima, MX	1	1	1	1		
8	*A. conversiae*	239 APG	P1778	Colorines, Mexico, MX	1	1	1	1		
9	*A. cordata*	223 APG	P1754	Otinapa, Durango, MX	1	1	1	1	1	1
10	*A. durangensis*	218 APG	P1751	El Naranjo, Nayarit, MX	1	1	1	1		
11	*A. emiliae*	Santana s.n.	P1826	Chamela, Jalisco, MX	1	1	1	1		
12	*A. flexuosa*	194 APG	P1753	Uruapan, Michoacán, MX	1	1	1	1	1	1
13	*A. foetida*	205 APG	P1779	Tlajomulco, Jalisco, MX	1	1	1	1		
14	*A. lassa*	261 APG	P1791	Saltillo, Coahuila, MX	1	1	1	1		
15	*A. luzmariana*	199 APG	P1780	El Terrero, Colima, MX	1	1	1	1		
16	*A. mutabilis*	112 APG	P1756	Arteaga, Michoacán, MX	1	1	1	1		
17	*A. nahua*	278 APG	P1821	Tamazula, Jalisco, MX	1	1	1	1		
18	*A. nahua*	Santana 15309	P1824	Ejutla, Jalisco, MX	1	1	1	1		
19	*A. nana*	256 APG	P1781	Guadalcazar, SLP, MX	1	1	1	1		
20	*A. nelsonii*	2014-016 Marie	P1792	Oaxaca, MX	1	1	1	1		
21	*A. oaxacana*	250 APG	P1782	San Cristolbal	1	1	1	1		

				Suchixtlahuaca, Oaxaca, MX							
22	*A. occidentalis*	198 APG	P1750	Minatitlán, Colima, MX	1	1	1	1			
23	*A. pacifica*	279 APG	P1822	Minatitlán, Colima, MX	1	1	1	1			
24	*A. pacifica*	296 APG	P1823	Casimiro Castillo, Jalisco, MX	1	1	1	1			
25	*A. palmeri*	211 APG	P1783	Zapopan, Jalisco, MX	1	1	1	1			
26	*A. pentandra*	262 APG	P1745	Allende, Nuevo León, MX	1	1	1	1	1	1	
27	*A. pringlei*	208 APG	P1746	Tlajomulco, Jalisco, MX	1	1	1	1			
28	*A. pueblana*	242 APG	P1785	Xochitlán, Puebla, MX	1	1	1	1			
29	*A. rzedowskiana*	275 APG	P1749	San Sebastián del Oeste, Jalisco, MX	1	1	1	1			
30	*A. rzedowskiana*	274 APG	P1803	Pto. Vallarta, Jalisco, MX					1	1	
31	*A. savannoidea*	219 APG	P1818	Acaponeta, Nayarit, MX	1	1	1	1			
32	*A. savannoidea*	220 APG	P1819	Acaponeta, Nayarit, MX	1	1	1	1			
33	*A. savannoidea*	221 APG	P1820	Acaponeta, Nayarit, MX	1	1	1	1			
34	*A. secunda*	260 APG	P1744	Galeana, Nuevo León, MX	1	1	1	1			
35	*A. socorroensis*	147 APG	P1786	Isla Socorro, Archipielágo de Revillagigedo, MX	1	1	1	1	1	1	
36	*A.* sp. nov. inedit. *(Cabo Corrientes)*	273 APG	P1774	Cabo Corrientes, Jalisco, MX	1	1	1	1			
37	*A.* sp. nov. inedit. *(El Chavarín)*	293 APG	P1775	Manzanillo, Colima, MX	1	1	1	1			
38	*A.* sp. nov. inedit. *(Huetamo)*	190 APG	P1788	Huetamo, Michoacán, MX	1	1	1	1			
39	*A.* sp. nov. inedit. *(Purhepecha)*	Santan s.n.	P1793	Guerrero, MX	1	1	1	1			
40	*A.* sp. nov. inedit. *(Sierra Verde)*	210 APG	P1787	Sierra Verde, Jalisco, MX	1	1	1	1			
41	*A. tequilana*	213 APG	P1789	Tequila, Jalisco, MX	1	1	1	1	1	1	
42	*A. teretiflora*	251 APG	P1790	Suchixtlahuaca, Oaxaca, MX	1	1	1	1			
43	*A. versabilifolia*	271 APG	P1747	Ocampo, Guanajuato, MX	1	1	1	1			

44	*A. watsonii*	237 APG	P1784	Tamazula, Durango, MX	1	1	1	1		
45	*A. watsonii*	222 APG	P1799	Culiacán, Sinaloa, MX	1	1	1	1	1	1
46	*A. wrightii*	232 APG	P1755	Mipimi, Durango, MX	1	1	1	1	1	1
47	*A. arborea*	Samain, MS 20.08.2010	P1174	Teapa, Tabasco, MX		1	1	1		
48	*A. arborea*		P1741	93, Hamburg (oo1680-15), DE						1
49	*A. asclepiadifolia*	Isnard, S. 09.09.2009	P1153	Remudadero, Veracruz, MX.		1	1	1		
50	*A. baetica*	B. Oelschlägel	P1739	06, Gaudin (017265-21), ES					1	1
51	*A. burelae*	SBW 2008-045	DP37			1	1	1		
52	*A. chiapensis*	Samain, MS 18.08.2009	P1170	Ocosingo, Chiapas, MX		1	1	1		
53	*A. gigantea*		P1189	BG Dresden, DE		1		1		
54	*A. impudica*	Granados, C. 27.08.2009	P1155	Soteapan, Veracruz, MX		1	1	1		
55	*A. kalebii*	Samain, MS 14.08.2009	P1158	Arriaga, Chiapas, MX		1	1	1		
56	*A. lindneri*	Unknown	P156	BG Bonn, DE		1	1	1		
57	*A. lindneri*	P. Ibisch	P1734	12/91, San José de Chiquitos (013979-29), BO					1	1
58	*A. malacophylla*	Samain, MS 15.08.2009	P1164	Comitán de Dominguez, Chiapas, MX		1		1		
59	*A. praevenosa*		P1428	BG Dresden, DE		1	1			
60	*A. promissa*		P1426	BG Dresden, DE		1	1	1		
61	*A. salvadorensis*		P1188	BG Dresden 10720, DE		1	1	1		
62	*A. serpentaria*	C.Neinhuis	P74	BG BN 16072; BG DD 013307-14, DE		1	1			
63	*A. serpentaria*	B. Westlund	P22	Travis, Texas, USA			1	1		
64	*A. tomentosa*		P14	75 / taschkent 699; 006146-17 Dresden, DE		1	1	1		
65	*A. tricaudata*	Isnard, S. 17.09.2009	P1156	Tehuacán, Oaxaca, MX		1	1	1		

66	*A. veracruzana*	Samain, MS 23.08.2010	P1177	San Andres Tuxtla, Veracruz, MX		1	1	1		
67	*A. westlandii*		P1191	BG Dresden, DE		1	1	1		
68	*A. asclepiadifolia*	Isnard, S. 09.09.2009	P1153	Remudadero, Veracruz, MX	1					
69	*A. bottae*	Mahfoud (HM 029/2)	P274	As, Sarih, JO	1					
70	*A. grandiflora*	Isnard, S. 01.09.2009	P1157	Los Tuxtlas, Veracruz, MX	1					
71	*Thottea siliquosa*	Oelschlägel (01/2008)	P929	Pradeep, IN	1					
72	*T. siliquosa*	S. Isnard 17, 07/04/2010	P1258	TBGRI, IN		1	1	1		
				TOTAL	49	63	61	62	13	14

2.2.2 DNA extraction, amplification and sequencing

Genomic DNA was extracted using a CTAB method (Borsch et al. 2003) from leaves of 45 accessions collected in silica gel (Table 2). PCR was done following the protocol of Wanke et al. (2007). Primer sequences were obtained from previous studies (Table 3, 4). Two primer combinations were selected to obtain two amplicons for the *trnK-matK-trnK-psbA* chloroplast region with an overlap of about 410 bp (Table 3). It would have been possible to amplify the *rpL32*-trnL$^{(UAG)}$ spacer, the *rpL16* intron and the *psbJ-petA* spacer (Table 4) in a single PCR each, but a conservative approach was chosen given that some accessions derive from herbarium material that might potentially have lower DNA quality hampering amplification.

Table 3. Primer list used for amplification and sequencing of the *trnK-matK-trnk-psbA* chloroplast region.

PCR-Matk	**Primer name**	**Sequence**	**Region**	**Design**
A	**trnK-Fbryo1**	GGGTTGCTAACTCAATGGTAGAG	Chloroplast	Quandt et al. (2004)
	AR-matK-660R	CCCATTTGYTATTTCGGAT	Chloroplast	Wanke et al. (2007)
B	**NymatK-480F**	CATCTGGAAATCTTGSTTC	Chloroplast	Borsch (2000)
	psbA-R	CGCGTCTCTCTAAAATTGCAGTCAT	Chloroplast	Steele and Vilgalys_(1994)
E	**AR-matK-930F**	TATTAGTACCTGATGCGGG	Chloroplast	Wanke et al. (2007)
	AR-matK-780R	GGTCTTCTGAAAATGATTAC	Chloroplast	Wanke et al. (2007)
F	**AR-matK-2500F**	ATCAGGACATCCCATTAGTAAG	Chloroplast	Oelschlägel et al. (2011)
	psbA-R	CGCGTCTCTCTAAAATTGCAGTCAT	Chloroplast	Steele and Vilgalys_(1994)

Table 4. Primer list used for amplification and sequencing of the *rpL16* intron, *psbJ-petA* spacer, *rpL32-trnL*$^{(UAG)}$ spacer chloroplast regions.

Primer name	Sequence	Region	Design
***rpL16* intron-F**	ATGCTTAGTGTGCGACTCGTT	Chloroplast	Wagner at al. 2012
***rpL16* intron-R**	CATCCTTCCTCTATGTTGTTTAC	Chloroplast	Wagner at al. 2012
PEN-*rpL16*-870R	TTGACCTTTACTGAATAGCCA	Chloroplast	Wanke 2015
PEN-*rpL16*-540F	TAGAGACTGAGAAGATTGACT	Chloroplast	Wanke 2015
***petA*-Fnew**	TGGCACAAATCTTTTTGGTTCT	Chloroplast	Wagner at al. 2012
***psbJ*-Rnew**	ATTCCTCTTTGGCTGATAGG	Chloroplast	Wagner at al. 2012
PEN-*psbJ*-1200R	TTTCAACCCGCTGTGTTCTTT	Chloroplast	Wanke 2015
PEN-*psbJ*-980F	AAGTGGTATAGAAAGGATGTC	Chloroplast	Wanke 2015
PEN-*psbJ*-870R	ATGTTCTTCTTAATGTTCCC	Chloroplast	Wanke 2015
PEN-*psbJ*-170F	GCTTATTTTGTTTATACTCTGT	Chloroplast	Wanke 2015
***rpL32*-F**	ATGGCGGTTCCAAAGAAAC	Chloroplast	Wagner at al. 2012
***ndhF-trnL*-R**	TTCCTAAGAGCAGCGTGTCTA	Chloroplast	Wagner at al. 2012
PEN-*rpL32*-480F	ACAAAAGCAGTTCCATACAAGA	Chloroplast	Wanke 2015
PEN-*rpL32*-780F	GCATCCATGAAATCAGATAAAA	Chloroplast	Wanke 2015
PEN-*rpL32*-1200R	TTCAAAAATRGTTAACTTAGTATCAT	Chloroplast	Wanke 2015

PCR was done as a 50 µl reaction. The master mix consisted of 26.75 µl H_20, 10 µl 1x GoTaq buffer (Promega), 2µl forward and reverse primer each (10 pmol/µl), 8 µl dNTP (each 1.25mM) and 0.25 µl GoTaq DNA polymerase. 1 µl genomic DNA was added to individual tubes. A Biometra Thermocycler was used with the program matk-Uni to run the PCR with the following cycles: pre-denaturation at 96° C for 1.5 min., first primer annealing at 50° C for 1 min., first elongation at 68° C for 2 min., followed by 45 amplification cycles at 95° C for 30 sec., 50° C for 1 min. and 68° C for 2 min. PCR products were tested via gel electrophoresis using a 0.8% agarose gel conducted at 80-100V. The gel was loaded with 2-4 µl PCR-product, 5 µl of 1x loading buffer and 1-2 µl of Gelstar to visualize DNA under UV light. In order to estimate the size of PCR products and concentration, a peqGold 100bp DNA-Ladder Plus (PeqLab) was included in a separate lane. A BioDocAnalyze (Biometra) was used for gel documentation. To obtain sufficient PCR product for later sequencing of multiple primers, 2-4 PCR products of the same sample where pooled. Purification of target products was performed applying a gel electrophoresis with 1.2% agarose gel. 2.4g/150 ml TAE 1x) 20 µl concentrated PCR product, 5 µl 6x loading buffer and 2 µl Gelstar were charged in the gel slots and run at 80-90 V for 3-4 h. Bands were visualized under blue light using a transiluminator (Biostep) and cut out with cover slips. Bands were purified with a NucleoSpin® Extract II kit (Macherey-Nagel) following the standard protocol, except that elution was done for 10 min. at 50°C. Purified products were sent to and sequenced by Macrogen.

2.2.3 Alignment and phylogenetic reconstruction

Sequences were edited and aligned by eye using PHYDE (Müller et al. 2005) and Sequencher 4.2.1 (Gene Codes Corp., Ann Arbor, Michigan, USA). In case that overlapping signal was observed in pherograms, the IUPAC nucleotide ambiguity code was applied.

The best model for each dataset was determined with JModelTest 64. maximum likelihood (ML) analyses were performed using 10 000 Bootstrap replicates in RaxML (Stamatakis et al. 2005), using the RAxML GUI and the IQ-Tree (Nguyen et al. 2015) using the ultrafast bootstrap approximation approach (Minh et al. 2013). Bayesian Inference analyses were performed using Mr. Bayes version 3.2 (Ronquist and Huelsenbeck, 2003). Four parallel Markov chains were run and calculated simultaneously with 10,000,000 to 15,000,000 generations. In total two runs were calculated and the consensus tree was selected. Trees were saved every 250 generations and the first 0.25% generations were discarded as burn-in from

each run as evaluated with Tracer version 1.5 (Rambault and Drummond, 2007). The CIPRES Science Getaway platform (Miller et al. 2010) was used to perform these calculations. The datasets were concatenated using the program Mesquite 2.75 (Maddison and Madison, 2011). Obtained trees were edited using TreeGraph version 2.0 (Stöver and Müller, 2010). Figures 23, 24

2.2.4. Nuclear loci

Access to genome scale, massively paralleled sequenced data was provided by Sebastian Müller, a Master student in the Dresden research group, who is part of the collaborative project between Mexico and Germany.

These data were generated using an anchored enrichment process based on universal probes (Fig. 3) (Lemmon et al. 2012) and sequenced using the Illumina HiSeq platform. This dataset consisted of 20 *Aristolochia* species including several *Pentandrae* accessions. 20 most informative loci (MIL) were ranked according to their phylogenetic informativeness (Townsend, 2007) and two loci were selected randomly to test if they can be amplified from genomic DNA in regular PCR and sequenced with Sanger.

2.2.4.1 The two selected nuclear loci

We selected the region L26 that encodes the gamma tocopherol O-methyltransferase gene consisting of three introns and three exons, and is involved in vitamin E biosynthesis. The entire gene has a length of 2024 bp in the *Arabidopsis thaliana* homolog (AT1G64970) (TAIR, http://arabidopsis.org). The second region (L144) represents a gene that encodes the F-box protein two, consisting of one intron and two exons. Currently, it is only known that the resulting protein interacts physically with BHLH32 and appears to be involved in mediating phosphate starvation responses (TAIR). Its expression is responsive to phosphate (Pi) and not phosphite (Phi) in roots and shoots. The entire gene has a length of 3600 bp in the *Arabidopsis thaliana* homolog (AT5G21040) (TAIR) (Table 5). Of these two regions, only small parts were chosen for subsequent tests.

Table 5. Showing the 20 MIL(Most Informative Loci), their average (maximum) phylogenetic informativeness and the time when the maximum phylogenetic informativeness was reached. For every locus the maximum phylogenetic informativeness was reached before the split of *Pentandrae* indicating low phylogenetic noise. ATG numbers for the respective *Arabidopsis thaliana* homologues were obtained from TAIR. Sequences from the respective loci were blasted using the nucleotide blast implemented in TAIR; the hit with the lowest E-value was

considered as homologue. (Müller, 2015).-The gray marks are the locus I used for single copy gene.

Locus	ATG Number of *Arabidopsis* homologue	Average Phylogenetic Informativeness	Maximum Phylogenetic Informativeness	Max value reached at
L144	AT5G21040	145	168	0.37
L125	AT4G33030	121	141	0.51
L171	AT4G04610	121	136	0.44
L170	AT4G02700	114	130	0.26
L16	AT1G31730	110	128	0.46
L68	AT2G46890	109	126	0.46
L26	AT1G64970	108	121	0.34
L132	AT5G08720	103	120	0.48
L19	AT1G45150	103	117	0.31
L115	AT3G57630	102	117	0.37
L35	AT4G30600	101	116	0.32
L165	AT5G61850	96	110	0.34
L101	AT3G49080	95	111	0.31
L100	AT3G48500	94	103	0.61
L40	AT2G17510	93	105	0.36
L134	AT5G10920	90	104	0.44
L82	AT3G12080	89	106	0.4
L65	AT2G44760	89	97	0.32
L9	AT1G09010	87	97	0.45
L128	AT2G48121	83	96	0.45

2.2.4.2 Sampling and molecular work with nuclear single copy genes (NSCG).

Taxon sampling includes 10 species from *Aristolochia* subsection *Pentandrae* (Table 6) according to the species used in the anchored hybrid gene enrichment study by Müller (2015).

DNA extraction, amplification, sequencing (Table 7) and analyses were carried out as mentioned above.

Table 6. List of all samples and accessions used for testing with nuclear single copy genes, including species name, lab number, collector and origin. An asterisk after the name of the species indicates the samples are growing in the Dresden Botanical Garden; hence, the accession number is given in brackets.

Sample	Lab. Number	Origin
Aristolochia socorroensis	P1723	APG 147, Isla Socorro, Mexico
A. cordata	P1724	APG 223, Otinapa, Durango, Mexico
A. pentandra	P1725	APG 262, Allende, Nuevo León, Mexico
A. cardiantha	P1727	APG 191, Coyuca de Catalán, Michoacán, Mexico
A. flexuosa	P1728	APG 194, Uruapan, Michoacán, Mexico
A. tequilana	P1729	APG 213, Tequila, Jalisco, Mexico
A. durangensis	P1730	APG 217, Nayarit, Mexico
A. watsonii	P1731	APG 222, Culiacán, Sinaloa, Mexico
A. wrightii	P1732	APG 232, Mapimí, Durango, Mexico
A. buntingii	P1743	APG 113, Arteaga, Michoacán, Mexico
A. brevipes	P1776	APG 119, Pátzcuaro, Michoacán
A. rzedowskiana	P1733	APG 274, Jalisco, Mexico
A. lindneri	P1734	P. Ibisch 12/91, San José de Chiquitos, Bolivia (013979-29)
A. baetica	P1739	B. Oelschlägel 06, Gaudin, Spain (017265-21)
A. grandiflora	P1740	CR680 San José, Costa Rica (017181-18)
A. arborea	P1741	93, Hamburg (oo1680-15)
Thottea sp.	P1742	J. Bogner 9/97, Thrissur, Kerala, India

Table 7. Primer list used for amplification and sequencing of the two nuclear Single Copy Gene.

Primer name	Sequence	Region	Design
AHGE_L26_3F	TTTCTCCYTCCGA	Nuclear	Müller (2015)

	GAYGTGT		
AHGE_L26_350R	CCTGCGAATTTGA GAGTTTC	Nuclear	Müller (2015)
AHGE_L144_78F	ATTCTCCATTGCC TTGATGC	Nuclear	Müller (2015)
AHGE_L144_1000R	TGCAAGGGAAAG AACTGGAC	Nuclear	Müller (2015)

2.3 RESULTS

2.3.1 The plastid regions (DNA Chloroplast).

The chloroplast loci showed most variability in the *trnK-matK-trnK-psbA* region and the *rpL16* intron, the first with a data matrix comprised 2940 bp of which 160 bp highly variable regions (hotspots) were identified, and excluded. The same was applied to the second data matrix that consists of 1365 bp and 120 bp of hotspots that were excluded. Moreover, the data were concatenated and analized of two combinations: one with 2 regions and the other with 4 regions, see Fig. 24.

The regions *rpL32-trnL*$^{(UAG)}$ and *psbJ-petA* spacer were excluded from the analysis because of their inconsistency, as well as the 4 regions analysis (Table 8), showing low support in the topologies obtained by ML and Mr Bayes. In contrast, the combination of 2 regions (Table 8, Fig. 23) provided high statistical support for the nodes of interest besides the results from trnK-matK-trnK-psbA (Fig. 24).

Table 8. Concatenated datasets, showing the regions used and their respective length.

Concatenated data sets	***Total length***
2 Regions *trnK-matK-trnK psbA+rpL16* intron	3978 bp
4 Regions *trnK-matK-trnK psbA+rpL16* intron+*rpL32-trnL+psbJ-petA*	7142 bp

Bayesian inference is in accordance with the topologies obtained from the maximum likelihood analyses, although the values are low in the latter. The resulting phylogenetic hypotheses support the monophyly of *Pentandrae* but not of the *Aristolochia lindneri* group. The latter, included with two species, forms a successive grade with respect to *Pentandrae*.

Within *Pentandrae*, three clades are recovered and two clades receive maximum support (Fig. 23).

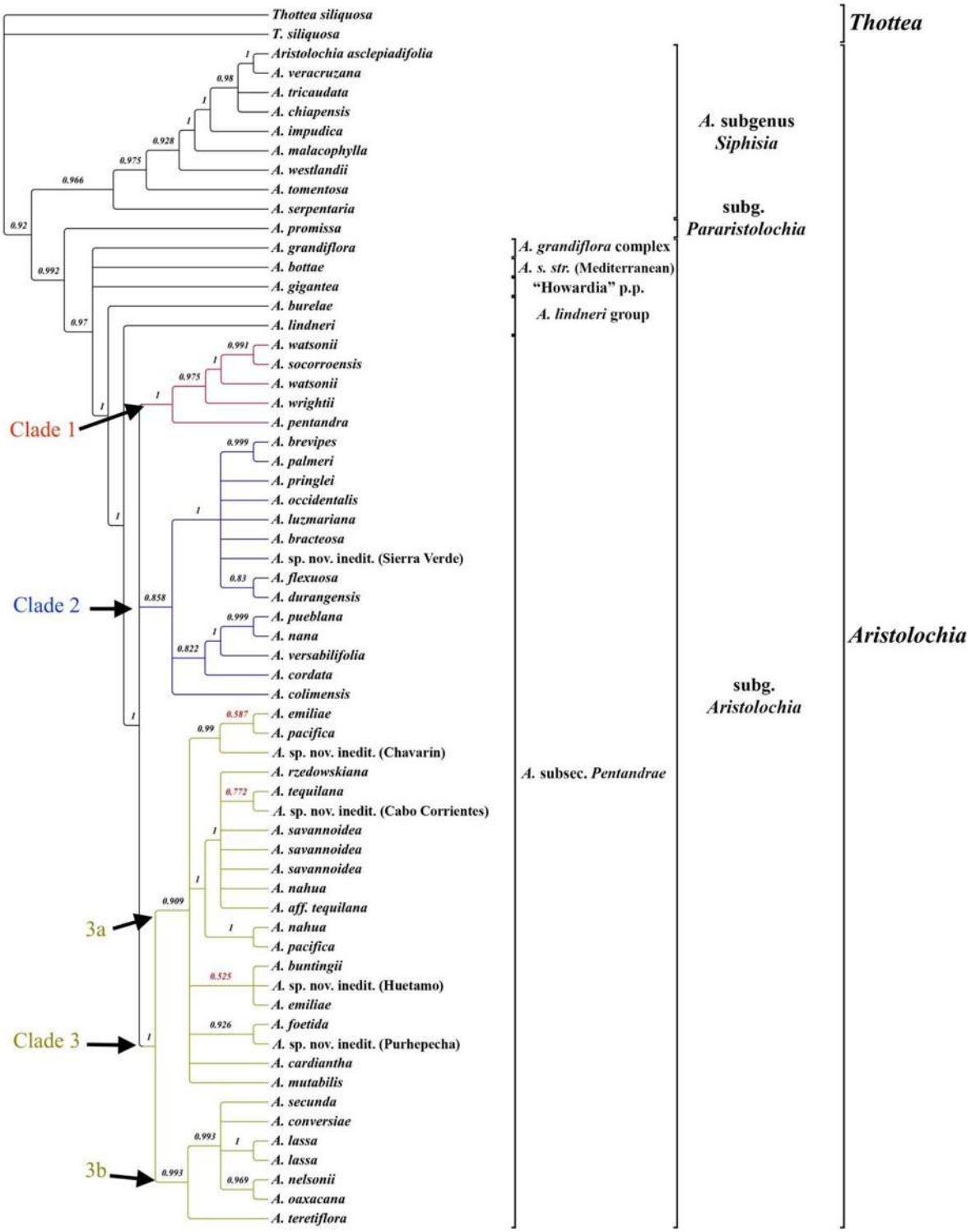

Fig. 23 Plastid regions *trnK-matK-trnK-psbA* and *rpL16* intron consensus tree generated by MrBayes running with 15 million generations. The subgenera included are *Siphisia*,

Pararistolochia and *Aristolochia*. Into the latter subgenus the following OTU's were included: "*Aristolochia grandiflora* complex" (*A. grandiflora*), *Aristolochia* s. str. (*A. bottae*), "Howardia" p.p., "*Aristolochia lindneri* group" (*A. lindneri* and *A. burelae*) and *A.* subsect. *Pentandrae.* The posterior probabilities marked in red are the clades with low support. The different colors represent the three clades into *Pentandrae* and the letters the subclades.

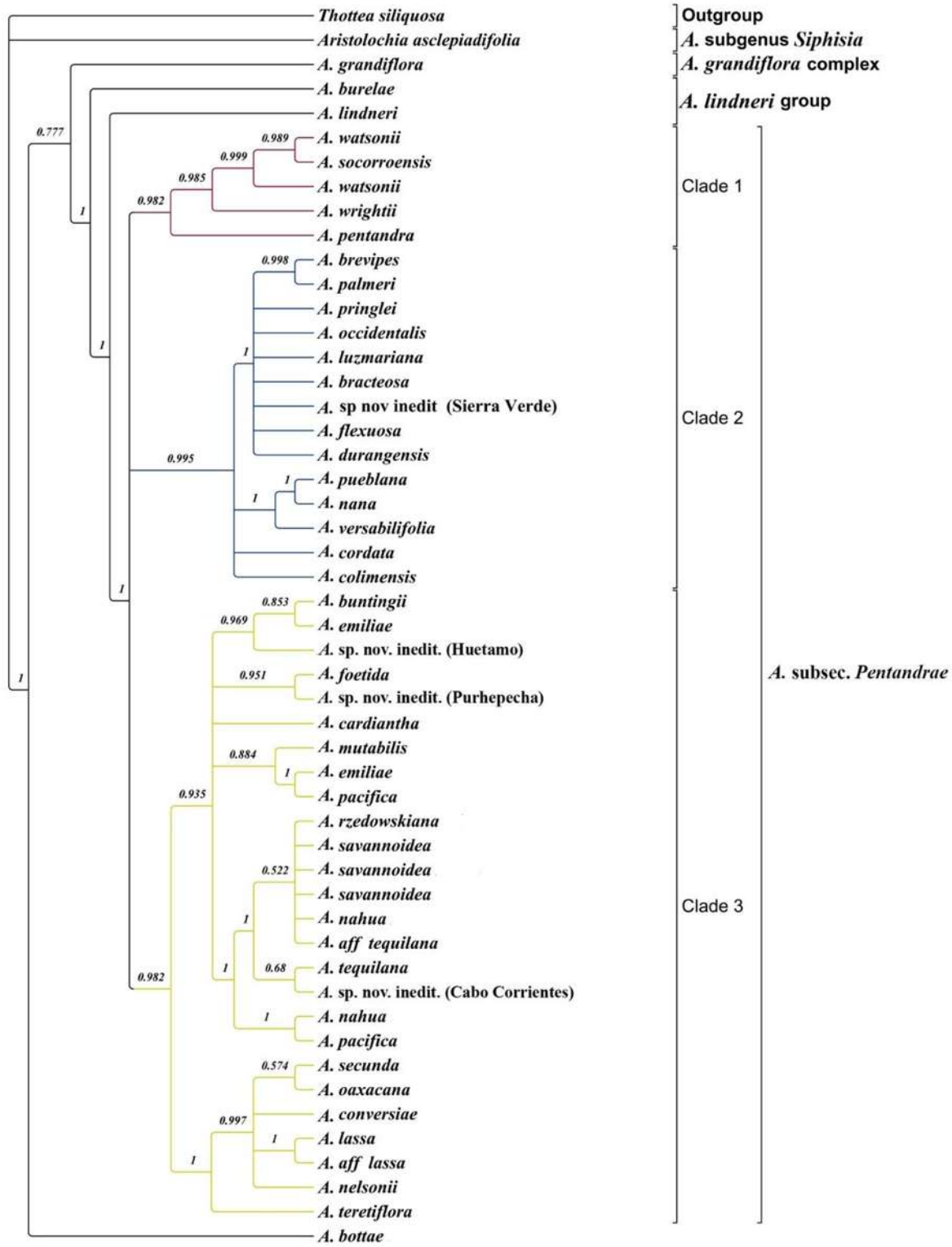

Fig. 24 *TrnK-matK-trnK-psbA* - consensus tree generated by MrBayes with 15 million

generations. The subgenera included are *Siphisia* and *Aristolochia*. Into the latter subgenus the following OTU's were included: "*Aristolochia grandiflora* complex" (*A. grandiflora*), *Aristolochia* s. str. (*A. bottae*), "*Aristolochia lindneri* group" (*A. lindneri* and *A. burelae*) and *A.* subsect. *Pentandrae*. The probability mark in red is the only clade with low support. The different colors represent the three clades into *Pentandrae.*

2.3.2 Nuclear Single Copy Genes

The L144 data matrix comprised 1090 bp of which 160 bp highly; L26 that consists of 465 bp of which 35 bp highly variable regions (hotspots) were identified, and excluded. The first attempt of using the two NSCG in *Pentandrae* was successful, providing good amplification and sequencing results (Figs.25, 26).

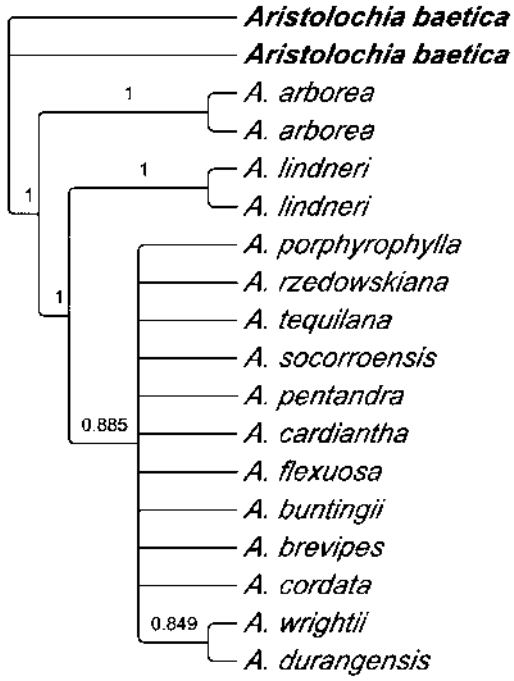

Fig. 25 Nuclear Single Copy Gene L 144. Tree generated by MrBayes running with 3 million generations, including 15 accessions. The outgroups include seq1 and seq2 which represent both assumed alleles produced by assembling the data of each sample (Müller, 2015). The subgenera included were *Siphisia* (*A. arborea*) and *Aristolochia*. Into the latter subgenus the following OTU's were included: *Aristolochia* s. str. (*A. baetica*), "*Aristolochia lindneri* group" (*A. lindneri*) and the rest of the species are part of *A.* subsect. *Pentandrae.*

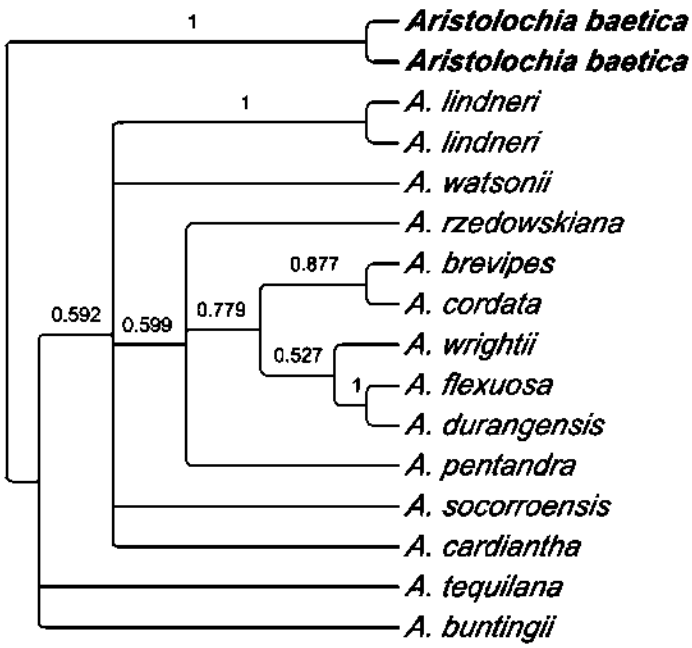

Fig. 26 Single Copy Gene L26. Tree generated by MrBayes running with 3 million generations, including 14 accessions. The outgroups include seq1 and seq2 which represent both assumed alleles produced by assembling the data of each sample (Müller, 2015). From *Aristolochia subgenus Aristolochia* the following OTU's were included: *Aristolochia* s. str. (*A. baetica*), "*Aristolochia lindneri* group" (*A. lindneri*) and the rest of the species are part of *A.* subsect. *Pentandrae.*

2.4 DISCUSSION

The molecular phylogenetic results confirm the monophyly of *Aristolochia* subsection *Pentandrae* for the first time, using a broad sampling. Including about 73% of the species, three clades were recovered within *Pentandrae*. In accordance with the phylogenetic hypotheses, habitats, altitudinal ranks of occurrence, patterns in distribution and morphological characters are also discussed.

Clade 1 (Figs. 23, 27) comprises the species with widest distribution and the morphological characters shared are the following: color of the limb purplish red, maroon to dark violet and the throat yellow with purplish red dots (except *Aristolochia wrightii*, which in some locations can have a dark violet throat). The first subclade shows *A. soccoroensis* nested between *A. watsonii* accessions. *A. socorroensis* was described by Pfeifer (1970) as endemic of the Socorro Islands, belonging to the Revillagigedo Archipelago 400 km off the coast of the state of Baja California Sur. On the other hand, *A. watsonii* (which is an older name) has a wide distribution from Arizona (USA) to the states of Sonora, Sinaloa, Nayarit, and Baja California Sur. The comparison of herbarium material, diagnostic characters, field observations, and collections in

the type localities leads to propose *A. socorroensis* as a synonym of *A. watsonii* (see Notes under *A. watsonii* description). This is also supported by the results of the phylogenetic analysis using two plastid regions concatenated (*trnK-matK-trnK-psbA* and *rpL16* intron) (Fig. 23).

Clade 2 (represented with triangles, see Figs. 23, 27) shows some correlation between the habitat and altitude as most of the species of this clade grow in pine-oak forest and oak forest (*Aristolochia pueblana*, and *A. versabilifolia* grow in xerophyte areas). The majority of the species are found at 1,500 to 2,500 m elevation, with few exceptions (*A. durangensis* and *A. colimensis*) and the leaf shapes observed are lanceolate to ensiform, lanceolate-ovate to hastate.

Clade 3 has two patterns of distributions: Subclade 3a (Figs.23, 27) is distributed in the central west of Mexico, such as the states of Colima, Durango, Jalisco and Michoacán. These species also share a similar habitat and grow in tropical deciduous forest and subtropical deciduous forest. The majority of the species grow between 50-1,000 m elevation and their leaf shapes are orbicular to cordiform, except for *A. emiliae* and *A.* sp. nov. (El Chavarín). Subclade 3b (Figs. 23, 27) includes species distributed in the states of Coahuila, Mexico, Nuevo León and Oaxaca and its species are found between 1,000 and 2,300 m elevation. Their habitat is either grasslands, xerophyte dominated vegetation or pine-oak forest (Figs. 23, 27) and their leaf shapes lanceolate-ovate to hastate and deltate to trilobate or sagittate.

Because the majority of the species are confined to the western part of Mexico, this area can be considered as the center of diversity of *Pentandrae*. According to Morrone (2005), this region is the core of the confluence of three important biogeographic provinces (Sierra Madre Occidental, Transmexican Volcanic Belt and Sierra Madre del Sur). A similar pattern also occurs for other flowering plant lineages. One example is the genus *Cosmos* (Asteraceae) that has been shown to be particularly diverse in the central west of Mexico (Vargas-Amado et al. 2013).

Furthermore, the results obtained by analyses of Nuclear Single Copy Gene, prove that it is possible to mine genome scale data for molecular phylogenetic studies in *Pentandrae*. Unfortunately, the lengths of both regions did not span the introns of these genes (Figures 25, 26) and thus only less variable coding regions were amplified and sequenced. Although the proof of principle was successful, a new attempt, spanning intron regions will demonstrate if the regions are useful for shallow scale phylogenetics in *Pentandrae*.

We present here the basis for further phylogenetic studies of *Pentandrae*. There are plenty of possibilities to test additional NSCG as well as more non-coding chloroplast regions.

Nevertheless, it would be favorable continuing searching with the strategy to test the variability into some of the clades well supported such as the clade 1 (Figs. 23, 27), wich is the one with wide distributions and able to collect different population to be evaluated. Alternatively, particular species with a remarkable range of morphological variability. Additionally, good candidates for including in the "ingroup" besides the *Aristolochia lindneri* group are the species of the "Howardia" p.p. group (distributed in America) such as *A. styoglossa*, because it is one of the species which shares with *Pentandrae* capsular fruits which open at the apex, in contrast to the majority of "Howardia" p.p. group which is characterized by capsular fruits that open at their base (like baskets). This is especially needed because it has been complicated to resolve the relationships between the OTUs within the subgenus *Aristolochia.*

2.5. General conclusions

The results of this thesis largely depended on fresh material collected and observations made during the extensive fieldwork. For the first time ever in *Pentandrae*, a broad range of intra- and interspecific morphological variation has been observed, allowing the preparation of very detailed descriptions of species new to science, amending concise historical descriptions, as well as synonymizing several species. The careful preparation of plant material in the field has also resulted in the high-quality photos illustrating the species descriptions.

The availability of fresh field-collected material was essential for providing DNA of sufficient quality for the phylogenetic study, which reveals, also for the first time ever, the natural relationships between a highly representative sampling of *Pentandrae* species.

Although additional work needs to be carried out to finish the taxonomic revision of this clade and complete the phylogenetic hypothesis with additional markers and samples, the results of the current thesis allow integrate both, and as such forming the basis for further studies. Some burning questions about relationships within the subsection *Pentandrae* as well as between this clade and closely related OTUs of subgenus *Aristolochia* have now been preliminary answered, but many more questions arose during this study, such as the role of certain factors like the variability presented in the coloration of the limb, their different shape of leaves into some species, their dispersion, habitat and altitudinal range in evolution and radiation of *Pentandrae*. Despite the fact that nearly all pentandrous species are difficult to find and a lot of time need to be spent on exploration, future field studies including observations of pollinators and dispersers will lift an additional corner of the veil of the fascinating morphology and evolutionary history of subsection *Pentandrae*.

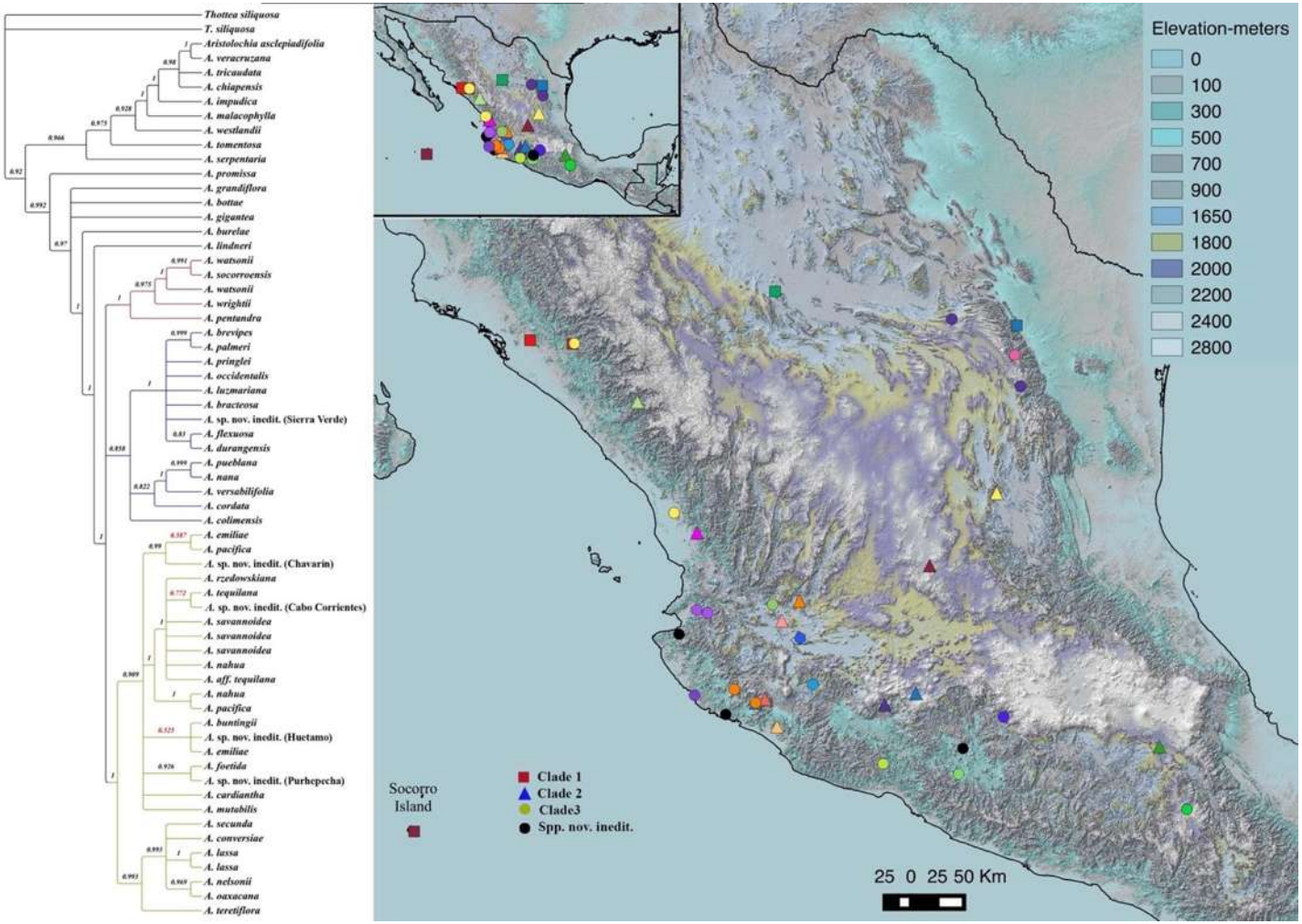

Fig. 27 Correlation between the clades of *trnK-matK-trnK-psbA* and *rpL16* intron consensus tree generated by MrBayes running with 15 million generations. *Aristolochia* subsect. *Pentandrae* and its distribution in Mexico. The different colors represent the three clades into *Pentandrae* and in the map every color represents a different species in the topology to the left. The dots, squares and triangles on the map refer to a specific location of every clade.

LITERATURE CITED

Borsch T. 2000. Phylogeny and evolution of the Nymphaea (Nymphaeaceae). Doctoral Thesis. PhD Thesis University of Bonn.

Borsch T., K.W. Hilu, D. Quandt, V. Wilde, C. Neinhuis and W. Barthlott. 2003. Noncoding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology*. 16:558–576.

Buchwalder K., M.S. Samain, G. Sankowsky, C. Neinhuis and S. Wanke. 2014. Nomenclatural updates of *Aristolochia* subgenus *Pararistolochia* (Aristolochiaceae). *Australian Systematic Botany*. 27:48–55.

Cai, Z., C. Penaflor, J. V. Kuehl, J. Leebens-Mack, J. E. Carlson, C. W. dePamphilis, R. K. Jansen. 2006. Complete plastid genome sequences of *Drimys*, *Liriodendron*, and *Piper*: implications for the phylogenetic relationships of magnoliids. *BMC Evolutionary Biology*. 6, 77. http://doi.org/10.1186/1471-2148-6-77

Calzada, J. I., G. Flores F. and O. Téllez V. 1997. *Aristolochia davilae* (Aristolochiaceae), a new species from Nayarit, Mexico. *Contr. Univ. Michigan Herb.* 21:143-146.

Duarte, J.M., P.K. Wall, P.P. Edger, L.L. Ma, H. Landherr, J.C. Pires, J. Leebens-Mack. 2010. Identification of shared single copy nuclear genes in Arabidopsis, Populus, Vitis and Oryza and their phylogenetic utility across various taxonomic levels. BMC *Evolutionary Biology*. 10:61.

Duchartre, P. 1854. Tentamen Methodicae Divisionis Generis Aristolochia. *Annales de Sciences Naturelles* 54:29–76.

Duchartre, P. 1864. Aristolochiaceae. Pp. 421-498 in A. P. De Candolle (ed.), *Prodromus Systematics Naturalis Regni Vegetabilis*. 15 (1):421-498 Treuttel at Würtz, Paris.

Gene Codes Corp. 2014. Sequencher, versión 4.2.1 Gene Codes, Ann Arbor, Michigan, USA

González F., H. E. Esquivel, G. A. Murcia, N. Pabón-Mora. 2010. *Aristolochia pentandra* (Aristolochiaceae) in Colombia: Biogeographic implications and proposed synapomorphies between the Pentandrous species of *Aristolochia* and its South American sister group. *Rev. Acad. Colomb. Cienc.* 34 (133):467-478.

González, F. A., Stevenson, D.W. 2002. A phylogenetic analysis of the subfamily Aristolochioideae (Aristolochiaceae). *Rev. Acad. Colomb. Cienc.* 26:25–60.

González F. & D. W. Stevenson. 2002. A phylogenetic analysis of the subfamily Aristolochioideae (*Aristolochiaceae*). *Rev. Acad. Colomb. Ci. Exact. Fís. Nat.* 26(98):25-60.

González, F., S.T. Wagner, K. Salomo, L. Symmank, M-S Samain, S. Isnard, N.P. Rowe, C. Neinhuis, S. Wanke. 2014. Present trans-Pacific disjunct distribution of *Aristolochia* subgenus *Isotrema* (Aristolochiaceae) was shaped by dispersal, vicariance and extintion. *Journal of Biogeography* 41:380-391

Hausner, G., R. Olson., D Simon., I. Johnson., E. R. Sanders., K.G. Karol., R.M. McCourt and S. Zimmerly. 2006. Origin and Evolution of the Chloroplast *trnK* (*matK*) Intron: A Model for Evolution of Group II Intron RNA Structures. *Mol. Biol. Evol.* 23(2):380-391.

Horner, H.T., M.S. Samain, S.T. Wagner, S. Wanke. 2015. Towards uncovering evolution of lineage-specific calcium oxalate crystal patterns in Piperales. *Botany* 93:159-169.

Huber, H. 1985. Samenmerkmale und Gliederung der Aristolochiaceen. *Botanisches Jahrbuch Systematik* 107:277–320.

Huber, H., 1993. Aristolochiaceae, in: Kubitzki, P.D.K. D.J.G. Rohwer, D.V. Bittrich (Eds.). Flowering plants Dicotiledons. The Families and Genera of Vascular Plants. Springer Berlin Heidelberg. 129-137 pp.

Jordan, W. C., M. W. Courtney, and J. E. Neigel. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeeds (Lemnaceae). *American Journal of Botany* 83:430–439.

Jarvis, A., H.I. Reuter, A. Nelson, E. Guevara. 2008, Hole-filled SRTM for the globe Version 4, available from the CGIAR-CSI SRTM 90m Database (http://srtm.csi.cgiar.org).

Kelly L. M. 2000. Aristolochiaceae. Flora del valle de Tehuacán-Cuicatlán. 29:1-8.

Lemmon, A.R. and E.M. Lemmon. 2012. High-Throughput Identification of Informative Nuclear Loci for Shallow-Scale Phylogenetics and Phylogeography. Syst Biol 61, 745–761. doi:10.1093/sysbio/sys051

Maddison, W. P. and D. R. Maddison. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75 http://mesquiteproject.org

Miller, M.A., W. Pfeiffer and T. Schwartz. 2010. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA 1-8 pp.

Minh, B.Q., M.A.T. Nguyen and A. von Haeseler. 2013. Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.*, 30:1188-1195. DOI: 10.1093/molbev/mst024

Morrone, J.J. 2005. Toward a synthesis of Mexican Biogeography. *Revista Mexicana de Biodiversidad* 76:207-252

Müller, K., J. Müller, C. Neinhuis and D. Quandt. 2005. PhyDe® Phylogenetic data editor. http:// www.phyde.de

Müller, S. 2015. Anchored hybrid gene enrichment illuminates molecular phylogeny of Aristolochia (Aristolochiaceae) and first insights into subsection Pentandrae, Master of Science thesis. Technische Universität Dresden. 52 pp.

Naumann J., K. Salomo, J.P. Der, E.K. Wafula, J.F. Bolin, E. Maass, L. Frenzke, M-S Samain, C. Neinhuis, C.W. dePamphilis, S. Wanke. 2013. Single-copy nuclear genes place haustorial Hydnoraceae within Piperales and reveal a Cretaceous origin of multiple parasitic angiosperm lineages. *PLOS One.* 8:e79204.

Nguyen, L.-T., H.A. Schmidt, A. von Haeseler and B.Q. Minh. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.*, 32:268-274. DOI:10.1093/molbev/msu300

Oelschlägel, B., S. Wagner, K. Salomo, N.S. Pradeep, T.L. Yao, S. Isnard, N. Rowe, C. Neinhuis, S. Wanke. 2011. Implications from molecular phylogenetic data for systematics, biogeography and growth form evolution of *Thottea* (Aristolochiaceae). *Gardens' Bulletin, Singapore.* 63:259–275.

Ohi-Toma, T., T. Sugawara, H. Murata, S. Wanke, C. Neinhuis and J. Murata, 2006. Molecular Phylogeny of *Aristolochia* sensu lato (Aristolochiaceae) based on Sequences of *rbc*L, *mat*K, and *phy*A Genes, with Special Reference to Differentiation of Chromosome Numbers. *Systematic Botany.* 31:481–492. doi:10.1043/05-38.1

Ortega O., J. F. and R. V. Ortega O. 1995. Contribución al estudio de Aristolochiaceae de México, I. Una especie nueva de *Aristolochia* de Puebla, México. *Acta Bot. Mex.* 32:39-45.

Pfeifer, H.W. 1966. Revision of the north and central American *Hexandrous* species of *Aristolochia* (Aristolochiaceae). *Ann. Missouri Bot. Gard.* 53(2):115-196.

Pfeifer, H. W. 1970. A revision of the pentandrous species of *Aristolochia*. The University of Connecticut Publication Series. Storrs, Connecticut. 134 pp.

Pfeifer, H. W. 1976. An error in the typification of Aristolochia watsoni Wooton and Standley. *Brittonia*. 28 (3):352.

Quandt, D., K. Müller., M. Stech., J.-P. Frahm., W. Frey., K. W. Hilu and T. Borsh. 2004. Molecular Evolution of the chloroplast trnL-F region in land plants. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98:13-37.

QGIS Development Team. 2014. QGIS 2.8 Wien. Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org. Accessed 1 November 2015.

Rambaut A. and A. Drummond. 2007. Tracer 1.5, available from http://beast.bio.ed.ac.uk/Tracer.

Rafinesque, C.S. 1828. Medical Flora; Or, Manual of the Medical Botany of the United States of North America: Containing a Selection of Above 100 Figures and Descriptions of Medical Plants, with Their Names, Qualities, Properties, History, &c.: and Notes or Remarks on Nearly 500 Equivalent Substitutes. Atkinson & Alexander. 61-65 pp.

Ronquist F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics.* 19 (12): 1572-1574.

Santana-Michel, F. J. 1995. Una especie nueva de *Aristolochia* (subsección *Pentandrae*) del estado de Jalisco, México. *Boletín IBUG.* 3(1-3):87-90.

Santana-Michel, F. J. and S. Lemus J. 1996. *Aristolochia occidentalis* (Aristolochiaceae), una especie nueva de la subsección *Pentandrae* de Colima y Jalisco, México. *Acta Bot. Mex*. 36:53-57.

Santana-Michel, F. J. 2002. Una especie nueva de *Aristolochia* L., subsección *Pentandrae* (Aristolochiaceae) del estado de Colima, México. *Acta Bot. Mex*. 58:63-67.

Santana-Michel, F. J. 2007. Una especie nueva de Aristolochia L., subsección *Pentandrae* (Aristolochiaceae) de la reserva de la biosfera Sierra de Manantlán, Jalisco, México. *Acta Bot. Mex.* 79:81-87.

Santana-Michel, F. J. and J. A. Solís-Magallanes. 2007. *Aristolochia emiliae* (Aristolochiaceae: subsección *Pentandrae*), una especie nueva de la costa de Jalisco, México. *Acta Bot. Mex.* 82:7-13.

Santana-Michel, F. J. and L. Guzmán-Hernández. 2014. *Aristolochia rzedowskiana* (Aristolochiaceae), una especie nueva para la subsección *Pentandrae* del estado de Jalisco, México. *Acta Bot. Mex.* 106:1-7.

Schmidt, O. C. 1935. Aristolochiaceae, in A. Engler & K. Prantl. *Die Natürlichen Planzenfamilien*. ed.2 16b:204-242.

Sebastiani, F., S. Carnevale, and G. G. Vendramin. 2004. A new set of mono- and dinucleotide chloroplast microsatellites in Fagaceae. *Molecular Ecology Notes.* 4:259–261.

Shaw, J., and R. L. SMALL. 2005. Chloroplast DNA phylogeny and phylogeography of the North American plums (Prunus subgenus Prunus section Prunocerasus, Rosaceae). *American Journal of Botany* 92: 2011–2030.

Shaw, J., E.B. Lickey, Schilling, E.E. R.L. Small, 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in

angiosperms: the tortoise and the hare III. *American Journal of Bot.* 94:275–288. doi:10.3732/ajb.94.3.275

Shaw, T.I., Z. Ruan, T.C. Glenn, L. Liu, 2013. STRAW: Species TRee Analysis Web server. *Nucl. Acids Res*. gkt377. doi:10.1093/nar/gkt377

Stamatakis, A., Ludwig, T. and H. Meier. 2005. RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics*. 21:456-463.

Steele, KP., R. Vigalys, 1994. Phylogenetic analysis of the Polemoniaceae using nucleotide sequences of the plastid gene matK. *Systematic Botany.* 19:126-142.

Stöver, B.S. and K.F. Müller. 2010. TreeGraph 2: Combining and visualizing evidence

from different phylogenetic analyses. *BMC Bioinformatics*. 11:7.

TAIR. (The Arabidopsis Information Resource, http://arabidopsis.org) july/2015

Townsend, J.P., 2007. Profiling Phylogenetic Informativeness. *Syst Biol.* 56:222–231. doi:10.1080/10635150701311362

Truong, V.D. 2015. Systematics and Evolution of *Aristolochia* (Aristolochiaceae) from Vietnam and adjacent areas. PhD thesis Technische Universität Dresden, Germany. 126 pp.

Vargas-Amado G., A. Castro-Castro, M. Harker, J.L. Villaseñor, E. Ortiz and A. Rodríguez. 2013. Geographic distribution and richness of the genus Cosmos (Asteraceae: Coreopsidae). *Revista Mexicana de Biodiversidad.* 84:536–555.

Vogel, J., T. Hübschmann, T. Börner and W.R. Hess. 1997. Splicing and intron-internal RNA editing of trnK-matK transcripts in barley plastids: support for mat*K* as an essential splice factor. *J.Mol. Biol*. 270:179-187.

Wagner T. S., L. Hesse, S. Isnard, M.S. Samain, J. Bolin, E. Maass, C. Neinhuis, N.P. Rowe, and S. Wanke 2014. Major trends in stem anatomy and growth forms in the perianth-bearing Piperales, with special focus on Aristolochia. *Annals of botany.* 113 (7):1139-1154.

Wagner T. S., S. Isnard, NP Rowe, M.S. Samain, C. Neinhuis, S. Wanke 2012. Escaping the lianoid habit: evolution of shrub-like growth forms in *Aristolochia* subgenus *Isotrema* (Aristolochiaceae). *American Journal of Botany.* 99:1609–1629.

Wanke S. J. U. 2006. Evolution of the genus *Aristolochia* Systematics, Molecular Evolution and Ecology. Technische Universität Dresden. 159 pp.

Wanke S, F González, C. Neinhuis. 2006. Systematics of pipevines: combining morphological and fast-evolving molecular characters to investigate the relationships within subfamily Aristolochioideae (Aristolochiaceae). *International Journal of Plant Sciences.* 167:1215–1227.

Wanke, S., M.A. Jaramillo, T. Borsch, M. S. Samain, D. Quandt and C. Neinhuis. 2007

Evolution of Piperales – *matK* gene and *trnK* intron sequence data reveal lineage specific resolution contrast. *Molecular Phylogenetics and Evolution*. 42:477–497.

Wolfe, K. H., W. H. Li and P. M. Sharp. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences*. USA 84:9054–9058.

Zhao, Y., J. Yin, H. Guo, Y. Zhang, W. Xiao, C. Sun, J. Wu, X. Qu, J. Yu, X. Wang, and J. Xiao. 2015. The complete chloroplast genome provides insight into the evolution and polymorphism of *Panax ginseng. Front. Plant. Sci.* 5:696. doi: 10.3389/fpls.2014.00696

Appendix 1

PAIZANNI GUILLÉN, A., SANTANA MICHEL, F.J., RAMÍREZ AMEZCUA, J.M., WAGNER, S.T., MÜLLER, S., MONTERO CASTRO, J.C., WANKE, S. & SAMAIN, M.S. (2016). Four new species of *Aristolochia* subsection *Pentandrae* from Western Mexico. *Systematic Botany*. 41(1) doi: 10.1600/036364416X690651

(I will place the pdf of the publication at the end of the final version of the thesis)

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Four New Species of *Aristolochia* subsection *Pentandrae* from Western Mexico

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Abstract—Four new *Aristolochia* species from the Mexican states of Colima, Jalisco, and Nayarit are described and illustrated. The four new species, ***Aristolochia nahua***, ***A. pacifica***, ***A. savannoidea***, and ***A. tuitensis***, belong to *Aristolochia* subsection *Pentandrae*, and are morphologically similar to *A. buntingii* and *A. tresmariae*. All of these species grow in the biogeographic province of the Mexican Pacific Coast, an important area of diversity and species richness of *Aristolochia* subsection *Pentandrae*.

Keywords—*Aristolochia nahua*, *Aristolochia pacifica*, *Aristolochia savannoidea*, *Aristolochia tuitensis*, Mexican Pacific Coast, taxonomy.

The genus *Aristolochia* L. comprises approximately 500 species, making it the most species-rich genus of Aristolochiaceae; the majority of these are found in the tropics and subtropics (Kelly 2000; Neinhuis et al. 2005; Ohi-Toma et al. 2006; Wanke et al. 2006; González et al. 2010). In Mexico, *Aristolochia* is represented by approximately 70 species belonging to two subgenera (*Siphisia* and *Aristolochia*) (Pfeifer 1966, 1970; González et al. 2014). All subgenera are monophyletic (Wanke et al. 2006) and subgenus *Aristolochia* consists of several subclades. These clades partially correspond to earlier taxonomic groups of different rank (González and Stevenson 2000; Wanke et al. 2006). Subsection *Pentandrae* together with species belonging to the *Aristolochia lindneri* group form one of these clades (Ohi-Toma et al. 2006, Wanke et al. 2006, González et al. 2010). Subsection *Pentandrae* Duchartre (hereafter shortened to *Pentandrae*) is the most species-rich *Aristolochia* lineage in Mexico; it is characterized by five stamens, whereas all other Mexican species have six stamens (Pfeifer 1966, 1970). The representatives of *Pentandrae* are further characterized by a geophytic and procumbent habit (occasionally climbing), as well as perennial subterranean organs, which occasionally are corky (Pfeifer 1970). Although *Pentandrae* is a comparatively species-rich lineage, it is the least-studied *Aristolochia* lineage on a worldwide scale. It can be found from the southern U. S. A. to Colombia (San Andrés Island), including Guatemala, Belize, Honduras (Swan Islands), and the West Indies, but it is most diverse in Mexico (Pfeifer 1970; González et al. 2010). Thirty-five species have been recognized by Pfeifer (1970), who published the only available broad-scale taxonomic revision of the subsection. Since Pfeifer's revision, eight additional species have been described from Mexico by Ortega Ortiz and Ortega Ortiz (1995), Santana-Michel (1995, 2002, 2007), Santana-Michel and Lemus-Juárez (1996), Calzada et al. (1997), Santana-Michel and Solís-Magallanes (2007), and Santana-Michel and Guzmán-Hernández (2014), resulting in a total of 43 species.

During fieldwork in the framework of our research on *Aristolochia* subsection *Pentandrae*, we discovered four new species, morphologically similar to *Aristolochia buntingii* Pfeifer and *A. tresmariae* Ferris, in the Mexican states of Colima, Jalisco and Nayarit, which are here described and illustrated. An emended description of *Aristolochia buntingii* is provided for ease of comparison. A comparative table and identification key for distinguishing the four new species from *A. buntingii* and *A. tresmariae* are provided, as well as a distribution map.

Materials and Methods

Extensive field work was performed in the states of Colima, Jalisco, Michoacán, and Nayarit, coinciding with the flowering seasons of the species studied. Individuals were carefully observed and herbarium material and flowers and fruits were collected for further study. Additionally, herbarium material of subsection *Pentandrae* from the herbaria CHAPA, DR, IBUG, IEB, MEXU, MICH, UCR, and ZEA has been studied and compared with our own collections.

Measurements were performed on dried specimens to allow comparison to the type material of *A. buntingii*. Throughout the manuscript, measurements are mentioned in the following format: length/height × width. Figure 1 shows the general flower architecture of a typical *Pentandrae* representative for ease of reference, introducing the terminology used in the descriptions. Botanical terminology follows Moreno (1984) and Harris and Harris (2006). Distribution maps were made with the software Quantum GIS version 2.6 Brighton (QGIS Development Team 2014) with the projection WGS84, using the digital elevation model from INEGI (2014) as base map.

Taxonomic Treatment

Aristolochia buntingii Pfeifer,Taxon. Re. Pentand. Aristolochia, 45. 1970.—TYPE: MEXICO. Michoacán: Mun. Arteaga, 8.6 miles from Arteaga (just beyond km 150) on road to Playa Azul, 750 m, 11 Sept 1961, *Moore & Bunting 8773* (Holotype: MO!; isotype: UC!).

Emended description: Geophytic herb, procumbent, subterranean organs 25–35 cm long; stems slightly ribbed with 1–2 subterranean internodes, swollen nodes, 10 mm long; aerial internodes slightly geniculate, (3–)4–5 cm long, and sparsely covered with septate trichomes. Petioles terete, (0.7–)0.8–1 × 0.1–0.2 cm, green, sometimes pink-purple, sparsely covered with septate trichomes. Leaf blades ovate, discolorous, base cordate, apex obtuse slightly acute, (4.0–)5.0–6.5(–8.0) × (3.2–)4.5–5.5(–6.8) cm; palmatinerved, actinodromous,

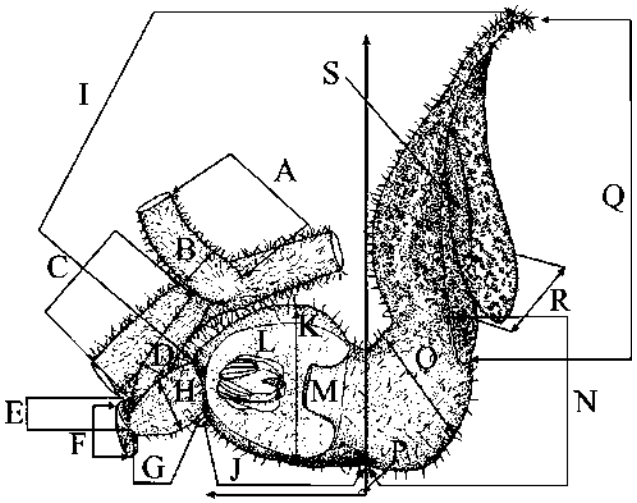

FIG. 1. Flower architecture of a typical *Aristolochia* subsection *Pentandrae* species, indicating the floral characters measured. A. Petiole length. B. Petiole width. C. Peduncle/pedicel length. D. Peduncle/pedicel width. E. Bracteole length. F. Bracteole width. G. Ovary length. H. Ovary width. I. Perianth length. J. Utricle length. K. Utricle width. L. Gynostemium. M. Syrinx. N. Tube length. O. Tube width. P. Curvature angle of the tube with respect to the utricle. Q. Height of the front part of the limb. R. Limb width. S. Throat. Based on *F. J. Santana-Michel & J. Bernal 14993*; Drawing by José Manuel Ramírez Amezcua.

in young leaves basal veins purple-pink, margin entire; adaxial leaf side green, bullate, with sparse tomentose tufts and sparse septate trichomes, 0.7–1 mm long with acroscopic orientation; abaxial leaf side pale green, reticulate, veins pale yellow with dense septate trichomes, especially along the veins. Flowers axillary, solitary or placed in a racemose inflorescence, flower pedicels 9–15 × 0.4–0.6 mm. Bract lanceolate, 0.3–0.4 × 0.15–0.3 cm. Perianth geniculate, (1.1–)1.5–2 cm long, pale yellow becoming purple toward the limb, veins brown-orange, sparse septate trichomes along the veins; utricle obloid, (0.37–)0.4–0.5 × (0.2–)0.3–0.5 cm, internal surface yellow with purple dots, covered with moniliform mucilaginous trichomes; tube bent 70°–90°, basally straight, widened toward the limb, 3–4(–9) × 1–2 mm, internal surface with white uniseriate trichomes, 0.4–0.3 mm long; limb obovate, slightly cordate at the base, apiculate at the apex, 0.7–2 × (0.5–)0.7–1.25 cm, dark purple, covered with fimbriate trichomes, in lateral view cup-shaped; throat ovate, dark purple, covered by small white trichomes, 0.5 mm long; syrinx infundibuliform, slightly eccentric, 2.7 mm long, 2.5 mm wide at the base and 0.8 mm wide at the apex. Gynostemium coroniform, stipitate, 4 × 2 mm, stipe 0.8–0.3 mm long, stigmatic lobes 5, 0.6 mm long, anthers 5, 0.7 mm long. Ovary inferior, lanceolate, 7–9 × 3.5–5 mm, with sparse septate trichomes. Fruit a capsule, purple when young, mature fruit not seen; seeds not seen. Figure 2A–E.

Phenology—*Aristolochia buntingii* has been found flowering from August to September and has been collected in fruit in August.

Distribution and Habitat—This species is only known from the municipality of Arteaga in Michoacán (Fig. 3). It grows in oak forest on hillsides, formed by ferrous volcanic rocks, from 750–800 m altitude.

Additional Specimens Examined—MEXICO. Michoacán: Mun. Arteaga, road to Arteaga near La Lagunita, 18°25′51″N, 102°06′16.2″W, 809 m, 1 Aug 2013, *A. Paizanni Guillén et al.* 113 (IEB).

Aristolochia nahua Paizanni & Santana-Michel sp. nov.—TYPE: MEXICO. Jalisco: Mun. Tamazula, around Palmillas de Arriba, close to the ranch of Javier del Toro, 19°40′08.4″ N, 103°11′53.5″W, 1,178 m, 24 September 2014, *A. Paizanni Guillén et al. 278* (holotype: IEB!; isotypes: IBUG!, DR!, MEXU!, ZEA!,)

Aristolochia nahua differs from *Aristolochia buntingii* and *A. tresmariae* by the orbicular to cordiform leaves and the tube widening at the basal part. Additionally, it can be distinguished by the ovate to cordiform limb, which is acuminate at the apex, with a yellowish area covered with purplish red-papillose protuberances, and the throat reniform, minutely granular and glabrous.

Geophytic herb, procumbent, subterranean organs 18–25 × 0.5–0.8 cm; stems with 2–3 subterranean internodes, swollen nodes, 4–10 mm long; aerial internodes slightly geniculate, (1.8–)2.5–3.5(–5.5) cm long, sparsely covered with septate trichomes. Petioles terete, (0.6–)1.1–1.7(–2.5) × 0.1–0.6(–0.9) cm, green, sparsely covered with septate trichomes. Leaf blades orbicular to cordiform, discolorous, base cordate, apex obtuse, rarely apiculate, (2.0–)2.8–4.5(–7.5) × (1.7–)3.3–4.6(–7.4) cm; palmatinerved, actinodromous, margin entire; adaxial leaf side green, with septate trichomes, 0.7–1 mm long, sparse and with acroscopic orientation; abaxial leaf side pale green with dense septate trichomes, especially along the veins. Flowers axillary, solitary or placed in a racemose inflorescence, flower pedicels 5–10 × 0.5–0.8 mm. Bract ovate, 0.2–0.4 × 0.17–0.45 cm. Perianth geniculate, 1.3–1.9(–2.3) cm long, white-yellowish, becoming purple toward the limb, veins brown-yellow, dense septate trichomes along the veins; utricle obovoid to ellipsoid, 0.5–0.6 × (0.2–)0.3–0.5 cm, internal surface yellow, purple-dark toward the syrinx, covered with moniliform mucilaginous trichomes; tube bent 90°–120°, basally widened, 3.5–5(–6) × 3–5 mm, internal surface glabrous; limb ovate to cordiform, cordate at the base, acuminate at the apex, 0.5–0.7(–1) × 0.6–1 cm; with a yellowish area covered by purplish red-papillose protuberances, in lateral view cup-shaped; throat reniform, dark-purple, minutely granular and glabrous; syrinx infundibuliform, eccentric, 2 mm long, 2 mm wide at base and 1 mm wide at apex. Gynostemium coroniform, stipitate, 2–3 × 1–2 mm; stipe 2–5(–6) mm long, stigmatic lobes 5, 0.6–1.3 mm long, anthers 5, 0.9–1.5 mm long. Ovary inferior, lanceolate, 5 × 1.3 mm, with sparse to dense septate trichomes. Fruit a capsule, spheroid, 0.68–0.95 × (0.8–)1–1.6 cm, green when young, brown-yellow when mature, with septicidal dehiscence at the apex. Seeds deltoid, 3.5–4 × 3–4.5 mm, upper part black, lower part yellow-brown, with granular surface. Figures 2F–I, 4.

Phenology—This species has been found flowering from July to September, whereas fruits have been collected in September.

Distribution and Habitat—*Aristolochia nahua* is known from the municipalities of Tamazula and Ejutla in the state of Jalisco (Fig. 3), in secondary vegetation near sugar cane plantations, in gallery vegetation with *Trichilia* P. Browne and *Lysiloma* Benth., as well as elements of tropical forest, such as *Bursera* Jacq. ex L., *Lasiocarpus* Liebm., *Acacia* Mill. and *Lysiloma* Benth.

Common Name and Uses—"Hierba del indio" (herb of the indian). The root of this species is boiled and drunk. It is used to treat stomach pain and diarrhea.

Etymology—The species epithet refers to the indigenous Nahua community near the type locality. Thanks to their

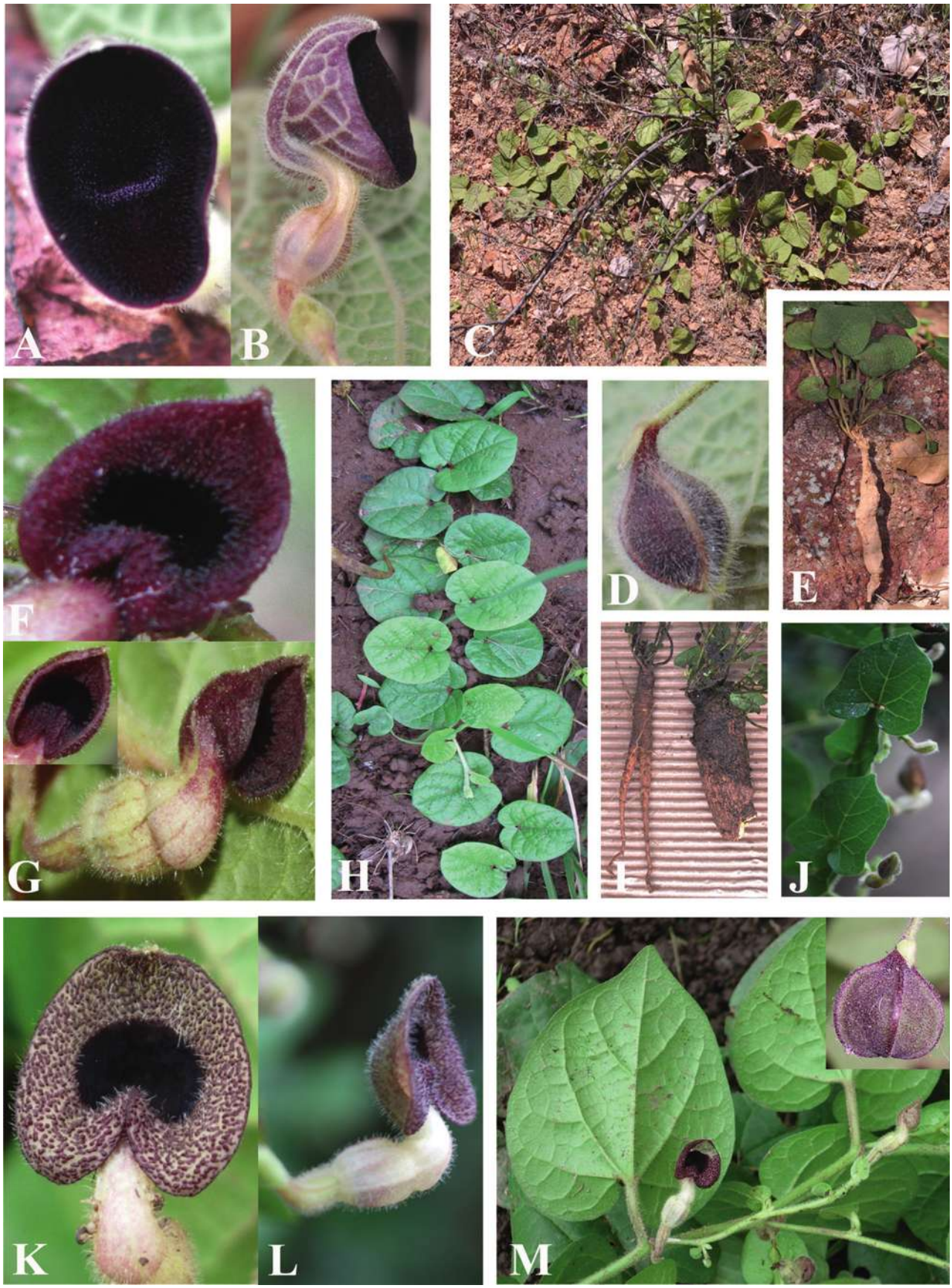

Fig. 2. General morphology of *Aristolochia buntingii*. A. Front view of the limb. B. Lateral view of the limb. C. Habit. D. Immature fruit. E. Subterranean organ. General morphology of *A. nahua.* F. Front view of the limb. G. Lateral view of the limb and inset close-up of the limb. H. Habit. I. Subterranean organ. General morphology of *A. pacifica.* J. Leaves. K. Front view of the limb. L. Lateral view of the limb. M. Habit, figure inset (upper right) fruit. Photographs by A. Paizanni Guillén: A–E, H–M, from the type locality; and by J. F. Santana Michel: F, G, from the Municipality of Ejutla, Jalisco.

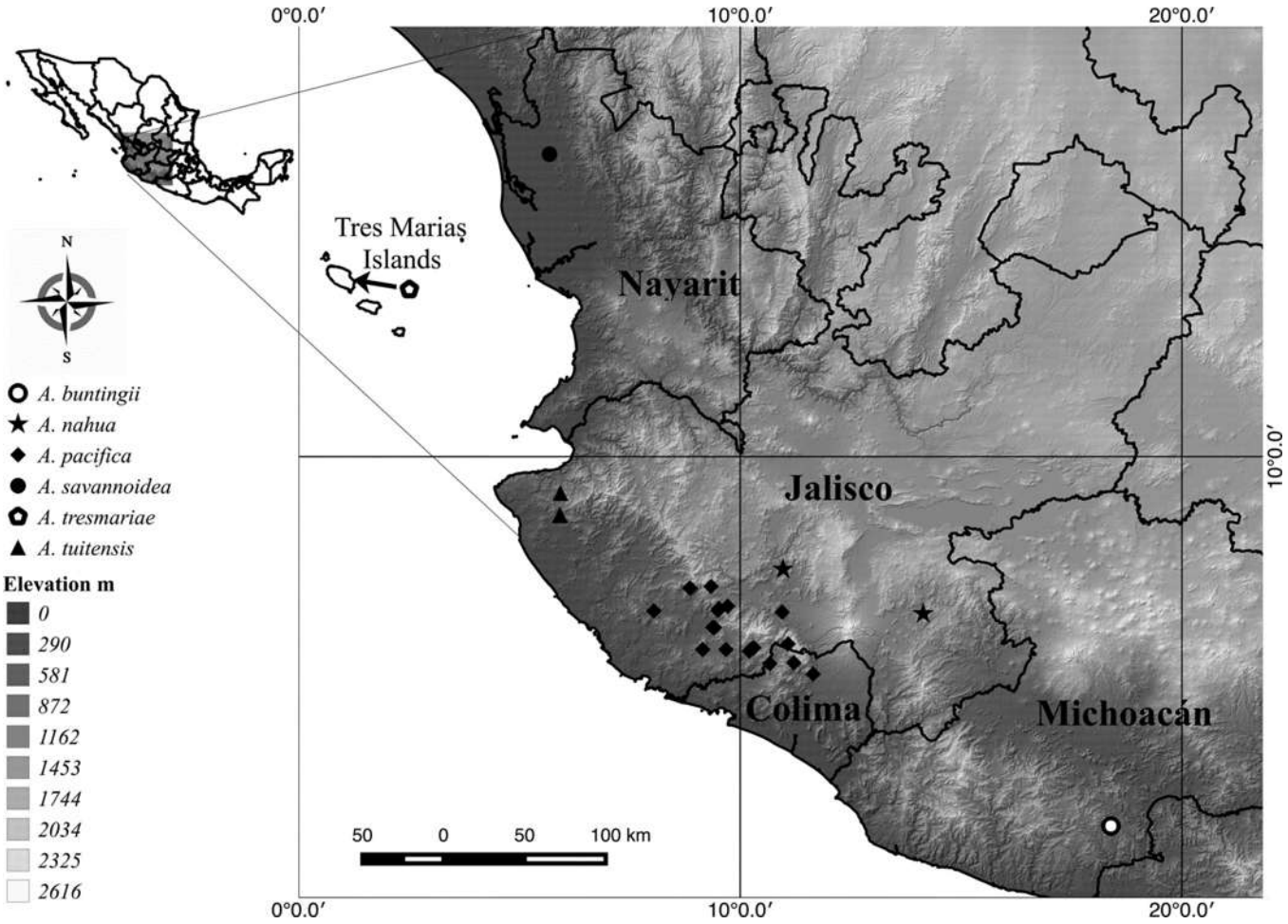

FIG. 3. Distribution map of four new *Aristolochia* species. Map of Mexico with the states in the upper left, grey shading indicates close up shown in the main map, focusing on the states of Nayarit, Jalisco, Colima, and parts of Michoacán. The *Aristolochia buntingii* complex consists of *A. buntingii*, *A. nahua*, *A. pacifica*, *A. savannoidea*, *A. tresmariae*, and *A. tuitensis*. Map projection WGS84, using the Digital Elevation Model from INEGI (2014), base map.

customs, beliefs and respect for nature, this area has been preserved.

Additional Specimens Examined—MEXICO. Jalisco: Mun. Ejutla, 2 km al SE de La Labor, 9–10 km al E de San Miguel Hidalgo por la brecha a Coatlancillo, 19°55′20″N, 104°0′30″W, 1,000 m, 6 Aug 1996, *F. J. Santana M. 8063* (ZEA); 1–2 km al NW de La Labor, 8 km al E de San Miguel Hidalgo, 19°56′36″N, 104°0′43″W, 1,000 m, 7 Aug 1996, *F. J. Santana M. 8037* (ZEA); 1 km al SW de La Labor, 20 km al ESE de El Limón, 19°55′40″N, 104°0′50″W, 950 m, 20 July 1996, *F. J. Santana M. 8007* (ZEA); poblado Palmillas de abajo 3 km al E del municipio por la brecha San Juan Nigromante, 1,500 m, Sept 1990, *O. Zepeda 42* (IBUG).

Aristolochia pacifica Santana-Michel & Paizanni sp. nov.—TYPE: MEXICO. Jalisco: Las Marías, between Cuzalapa and Ayotitlán, Sierra de Manantlán 19°26′20″N, 104°14′10″W, 812 m, 11 Nov 2011, *F. J. Santana Michel & J. Bernal 14993* (holotype: ZEA!; isotypes: DR!, IEB!, MEXU!,).

Aristolochia pacifica differs from *Aristolochia buntingii* and *A. tresmariae* by the auriculiform-ovate leaves, the cordiform bract, the perianth with a spheroid utricle, and the tube widening at the basal part. Additionally, it can be distinguished by the cordiform limb, which is obtuse or slightly apiculate at the apex, with a yellowish area covered with dark purple-tuberculate reticulate protuberances, sparsely white deciduous trichomes, and the throat reniform, dark purple, and with white trichomes at the border, minutely granular.

Geophytic herb, procumbent, subterranean organs 20–25 cm long; stems with 1–2 subterranean internodes; aerial internodes, slightly geniculate, (2.0–)2.5–5.5(–6) cm long, sparsely covered with septate trichomes. Petioles terete, 1–2.5 × 0.1 cm, green, densely covered with septate trichomes. Leaf blades auriculiform-ovate, discolorous, base cordate, apex acuminate, (3.5–)4–9 × 3–6.5 cm; palmatinerved, actinodromous, margin entire; adaxial leaf side green with septate trichomes, 0.6–1 mm long, with acroscopic orientation; abaxial leaf side pale green with dense septate trichomes, especially along the veins. Flowers axillary, placed in a racemose inflorescence, flower pedicels 5–10 × 0.5–0.7 mm. Bract cordiform, (0.2–)0.3–0.5 × 0.3–0.4 cm. Perianth geniculate, 2–2.6 cm long, white-yellowish becoming purple toward the limb, veins brown-orange, dense septate trichomes along the veins; utricle spheroid, 0.65–0.74 × 0.5–0.55 cm, internal surface yellow with purple dots, covered with moniliform mucilaginous trichomes; tube bent 90°, basally widened, 1–1.5 × 3–3.5 mm, internal surface glabrous; limb cordiform, cordate at the base, obtuse or slightly apiculate at the apex, 1.3–1.7 × 1.2–1.6 cm, with a yellowish area covered by dark purple tuberculate reticulate protuberances, sparse white trichomes deciduous, in lateral view flat; throat reniform, dark-purple, white trichomes at the margin, minutely granular; syrinx infundibuliform, eccentric, 1.7–2.4 mm long, 1.4–2 mm wide at base and

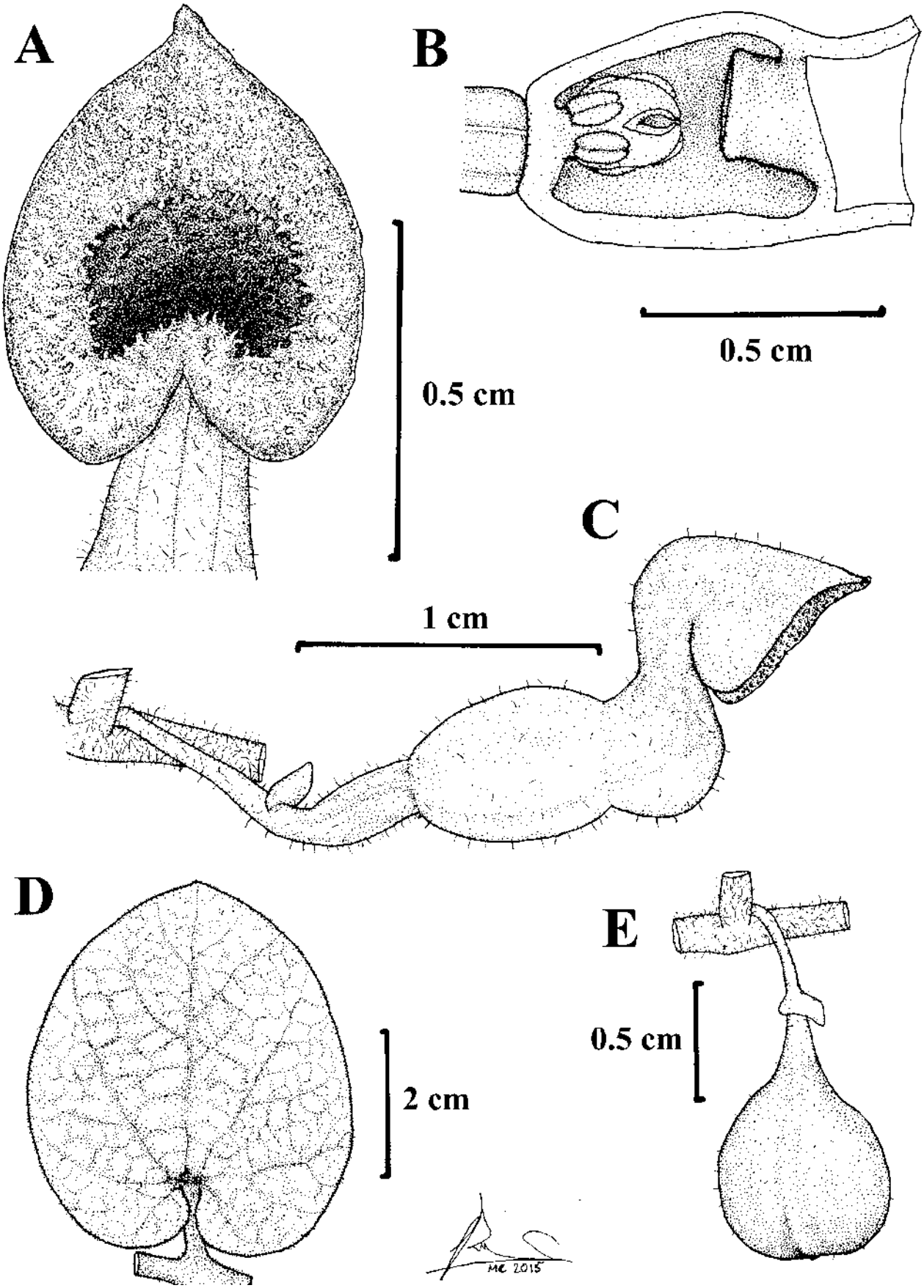

FIG. 4. *Aristolochia nahua*. A. Front view of the limb. B. Interior view of the utricle, which is composed of the gynostemium and the syrinx. C. Perianth, inferior ovary, bract and pedicel. D. Leaf blade. E. Fruit. Based on *A. Paizanni Guillén 278 et al.* and *F. J. Santana-Michel 8007, 8037, 8063*; Drawing by José Manuel Ramírez Amezcua.

1 mm wide at apex. Gynostemium coroniform, stipitate, 1.8–2.5 × 1.5–1.9 mm wide; stipe, rarely subsessile, 0.5–0.9 mm long; stigmatic lobes 5, 0.8–1.2 mm long, anthers 5, 0.9–1.5 mm long. Ovary inferior, lanceolate, 5.0 × 1.0 mm, with sparse to dense septate trichomes. Fruit a capsule, spheroid, 1.5–1.8 × 1.2–1.5 cm, purple when young, brown-yellow when mature, with septicidal dehiscence at the apex. Seeds deltoid, 4–4.5 × 4–5 mm, upper part black, lower part yellow-brown, with granular surface. Figures 2J–M, 5.

Phenology—This species is reported to flower from June to early December and has been collected in fruit from August to September.

Distribution and Habitat—*Aristolochia pacifica* is distributed along the Pacific Coast of the Mexican states of Jalisco (municipality of Autlán) and Colima (municipality of Comala) (Fig. 3). This species grows in open secondary vegetation near tropical subdeciduous forest, oak forest, pine-oak forest, cloud forest and gallery forest, on flat areas or in canyons, from 500–1,900 m altitude.

Common Name and Uses—"Camote de indio" (sweet potato of the indian; Santana-Michel et al. 5469), referring to the shape of the subterraneous part of this species. In the municipality of Autlán the root is used to treat diseases such as stomach pain and diarrhea. It is prepared by boiling the root and the infusion is drunk.

Etymology—The species epithet refers to the distribution area along the Pacific Coast of Mexico in the states of Colima and Jalisco.

Notes—Santana-Michel and Cuevas Guzmán (2014) included *Aristolochia buntingii* in a revision of the genus *Aristolochia* for the state of Colima; however, some of the material refers to the here described *A. pacifica.*

Additional Specimens Examined—MEXICO. Colima: Mun. Comala, 2–3 km al SE de Campo Cuatro, 15–16 km al NW de Colima, 1,100 m, 15 Aug 1992, *F. J. Santana-Michel et al. 5292*, *5294* (IBUG, ZEA); Rancho El Jabalí, 22 km (airline) NNW of Colima in the SW foothills of the Volcán de Colima, 1,300 m, 20 May 1991, *A. C. Sanders 11076* (UCR); Rancho El Jabalí, 22 km (airline) NNW of Colima in the SW foothills of the Volcán de Colima, Hacienda San Antonio, 1,200 m, 02 Oct 1991, *L. Vazquez V. 1301, 1637* (UCR); Rancho El Jabalí near Hacienda San Antonio 20 km NNW of the city of Colima, 1,150 m, 25 Aug 1988, *A. C. Sanders 5495, 5414* (UCR); carretera Queserías, en el tramo crucero a San Antonio-Carrizalillos, 1,400 m, 17 July 1997, *R. Flores Virgen 579* (ZEA). Mun. Minatitlán, after the town Cerro Colorado, heading to the mine (Hill of Juanes) approx. 6 km, 19°22′40.1″N, 104°05′28.3″W, 894 m, 24 Sept 2014, *A. Paizanni Guillén et al. 279* (DR, IBUG, IEB, MEXU, ZEA). Jalisco: Mun. Autlán, mountain summits 9–10 miles southwest of Autlán, 1,300–1,600 m, 16 Nov 1956, *R. McVaugh 14193* (MICH); mountains above Ahuacapán, road to Corralitos, 10–12 miles south-southeast of Autlán, 1,500–1,800 m, 29 Sept 1960, *R. McVaugh 19585* (MICH); 3 km al NO de El Jalocote por la brecha a San Juan Cacoma, 19°49′40″N, 104°26′00″W, 1,450 m, 2 Aug 2000, *F. J. Santana Michel & L. Guzmán H. 10901* (ZEA); Las Mancornadas, 500 m al NO de Ayutita, por la brecha Autlán-Cacoma, 19°40′40″N, 104°01′10″W, elev. 1,100 m, 17 July 2002, *F. J. Santana Michel & A. A. Flores Flores 10940* (ZEA); Los Mazos, Torre de Microondas, 19°41′15″N, 104°23′36″W, 1,700 m, 26 Sept 1994, *F. J. Santana Michel & L. Guzmán H. 6938* (ZEA); 1–2 km al O de Ahuacapán, 19°42′45″N, 104°20′11″W, 1,200 m, 3 Oct 1991, *F. J. Santana M. et al. 5469* (ZEA); Los Mazos, 8–9 km al SSO de Autlán, 19°41′17″N, 104°23′30″W, 1,450 m, 01 Sept 1988, *F. J. Santana M. & P. Lorente A. 3832* (ZEA); Puerto Los Mazos por la carretera Autlán-Barra de Navidad, 1,100–1,200 m, 8 July 1997, *L. Guzmán H. & R. Delgado Cruz 1649* (ZEA). Mun. Casimiro Castillo, Los Mazos cerca de la torre de microondas, 19°41′15″N, 104°23′36″W, 1,700 m, 25 Oct 1995, *F. J. Santana Michel 7662* (ZEA); Los Mazos, 10 km SW of Autlán, Sierra de Manantlán, 19°41'N, 104°77'W, 1,800 m, 13 July 1992, *A. Leinberger 51* (ZEA); Los Mazos, Sierra de Manantlán, 9–10 km al SSO de Autlán, 19°40′36″N, 104°23′04″W, 750 m, 15 July 1992, *F. J. Santana Michel et al. 11185* (ZEA); Los Mazos, torre de microondas, 9–10 al SSO de Autlán, 19°40′36″N, 104°23′04″W, 1,700 m, 11 Dec 1995, *F. J. Santana M. & M. Vázquez 7702* (ZEA); 1–2 km al SE de Casimiro Castillo, 19°35′16″N, 104°24′48″W, 600–700 m, 11 Nov 1992, *J. Cevallos et al. 156* (ZEA); base del cerro La Petaca, 2 km al SE de Casimiro Castillo, 19°35′16″N, 104°24′48″W, 500 m, 10 June 1992, *F. J. Santana Michel & B. F. Benz 5720* (ZEA); cerro La Petaca, 2 km al SE de Casimiro Castillo, 19°35′16″N, 104°24′48″W, 750 m, 5 Aug 1993, *F. J. Santana Michel & B. F. Benz 6373* (ZEA); cerro La Petaca, 2 km al SE de Casimiro Castillo, 19°35′16″N, 104°24′48″W, 800 m, 22 Sept 1994, *F. J. Santana Michel & B. F. Benz 7605* (ZEA); following the street "5 de Mayo" in the town, to the pathway of the hill El Perote, 19°35'22.4"N, 104°25′26.2″W, 517 m, 29 Sept 2014, *A. Paizanni Guillén et al. 296* (DR, IBUG, IEB, MEXU, ZEA). Mun. Cuautitlán de García Barragán, 500 m al NO de Los Terreros de Ayotitlán por el camino a Telcrucito de la Piedra Pintada, 19°27′01″N, 104°12′41″W, 950 m, 23 July 1997, *F. J. Santana M. et al. 8660* (ZEA); 2 km al NO de Lagunillas de Ayotitlán, por el camino a Las Cañadas, 19°27′51″N, 104°11′54″, 1,200 m, 23 July 1997, *F. J. Santana M. et al. 8628* (ZEA); 1–2 km al NE de Ayotitlán, 19°28′13″N, 104°11′04″W, 750 m, 28 Aug 1991, vegetación secundaria, *F. J. Santana Michel & B. F. Benz 5327* (ZEA); 1–2 km al NE de Cuautitlán, 19°27′36″ N, 104°20′49″W, 760 m, 27 July 1989, *L. Guzmán H. & F. J. Santana M. 756* (ZEA); 3 km al N de Cerro El Otate, 19°27′35″N, 104°28′56″W, 700 m, 17 Dec 2000, *R. Cuevas et al. 7015* (ZEA). Mun. Tecalitlán, Rancho El Carrizalillo, km 142 de la carretera Tecalitlán-Pihuamo, 19°25′42″N, 103°21′44″W, 1,300 m, 19 Sept 2003, bosque de *Pinus* y *Quercus*, *F. J. Santana Michel & J. M. Michel Fuentes 11413* (ZEA). Mun. Tolimán, Los Encinitos, 19°29′33″N, 103°59′05″W, 1,700 m, 27 July 1995, *F. J. Santana M. & M. Vázquez 7251*(ZEA). Mun. Villa de Purificación, Villas de Cacoma, cerca de 16 km, al NE de Villa de Purificación, 19°49′05″N, 104°33′29″W, 1,022 m, 14 Aug 2009, *L. Guzmán H. et al. 5171* (ZEA); Villas de Cacoma, cerca de 16 km, al NE de Villa de Purificación, 19°49′ 02″N, 104°33′19″W, 1,070 m, 27 Sept 2009, *L. Guzmán Het al. 5233* (ZEA); 1 km al O de El Chino por la brecha Villa de Purificación-Talpitita, 19°41′05″N, 104°46′00″W, 500 m, 23 July 1997, *F. J. Santana Michel & R. Miranda Medrano 10698* (ZEA).

Aristolochia savannoidea Paizanni & M. Ramírez sp. nov.—TYPE: MEXICO. Nayarit, Mun. Acaponeta, San Felipe Aztatán, town La Presa, in the foothills of El Fileño, 22°20′18.7″N, 105°21′42.4″W, 52 m, 23 July 2014, *A. Paizanni Guillén et al. 219* (holotype: IEB!; isotypes: DR!, IBUG!, MEXU!, ZEA!).

Aristolochia savannoidea differs from *Aristolochia buntingii* and *A. tresmariae* by the elliptic to cordiform, rarely auriculiform leaves. Additionally, it can be distinguished by the widely ovate limb, which is deeply or slightly cordate at the base, and acuminate at the apex, with a yellowish area covered by a dark-purple, short vermiform appendage, and the throat reniform to deltate, minutely granular and glabrous.

Geophytic herb, procumbent, subterranean organs 30–40 cm long; stems slightly ribbed with 1–2 subterranean internodes, swollen nodes, 10 mm long; aerial internodes, slightly geniculate, (4–)4.5–6(–7) cm long, sparsely covered with septate trichomes. Petioles terete, (0.8–)1.0–1.5(–2.7) × (0.07–)0.1–0.15 cm, green, sometimes red-pink, sparsely covered with septate trichomes. Leaf blades elliptic to cordiform, rarely auriculiform, chartaceous, discolorous, base cordate with oblique lobes, apex acuminate and rarely obtuse, (2.3–)4.5–6.0(–8.6) × (2.3–)3.5–5.0(–7.2) cm; palmatinerved, actinodromous, margin entire; adaxial leaf side green with septate trichomes, 0.7–1 mm long, sparse and with acroscopic orientation; abaxial leaf side pale green with dense septate trichomes, especially along the veins. Flowers axillary, solitary, peduncle 8–25 × 0.2–0.7 mm. Bract ovate, 0.25–0.4 × 0.13–0.2 cm. Perianth geniculate, (1.2–)1.8–2.3 cm long, pale yellow becoming purple toward the limb with dense septate trichomes along the veins; utricle irregularly ellipsoid (0.4–)0.7–0.9 × (0.2–)0.3–0.4 cm, internal surface yellow with purple dots at the base, covered with moniliform mucilaginous trichomes; tube bent 30°–50°, basally straight, (2.5–)6–9 × 1.2–2 mm, internal surface

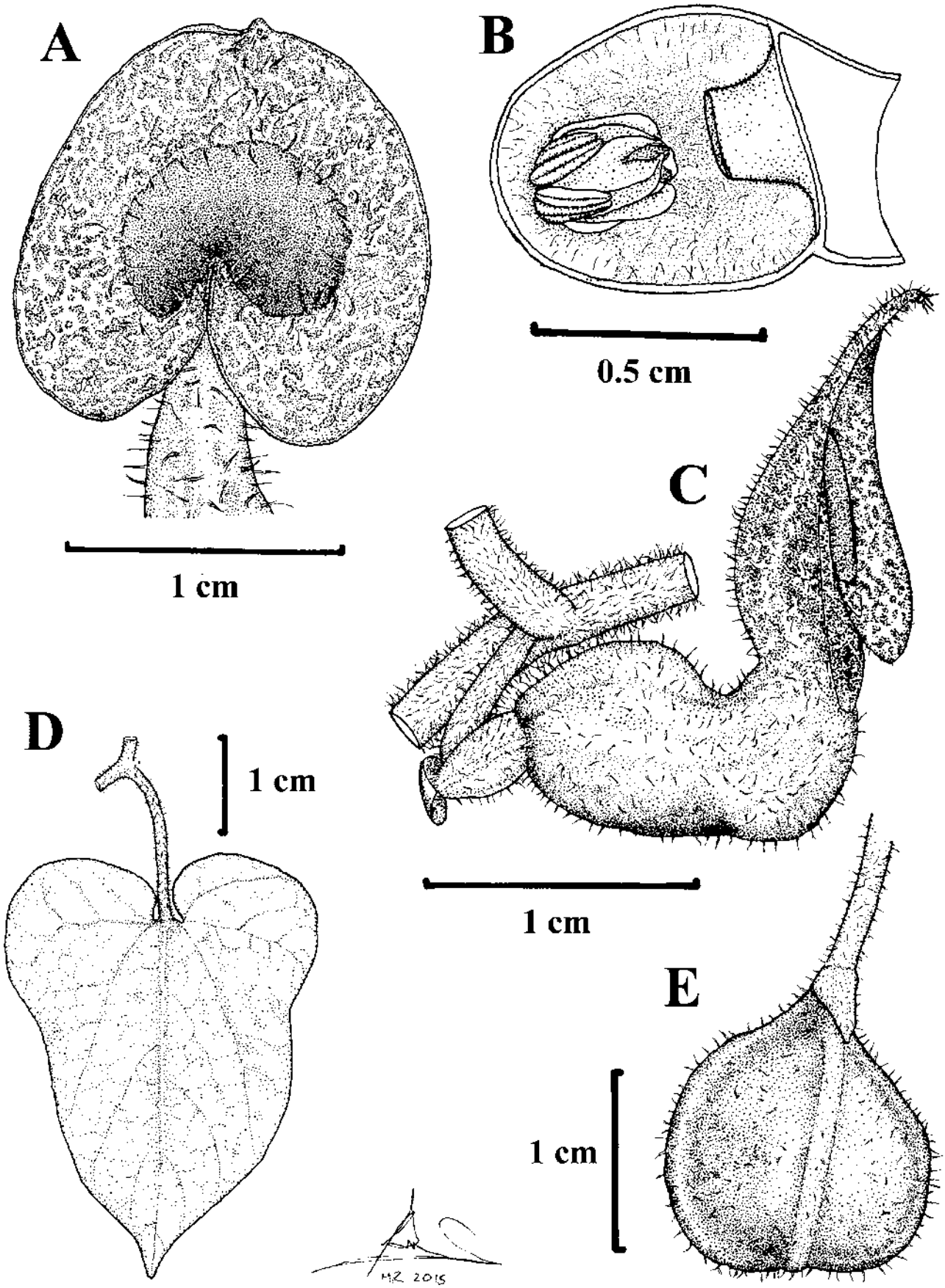

FIG. 5. *Aristolochia pacifica*. A. Front view of the limb. B. Interior view of the utricle, which is composed of the gynostemium and the syrinx. C. Perianth, inferior ovary, bract and pedicel. D. Leaf blade. E. Fruit. Based on *A. Paizanni Guillén 296* et al. and *F. J. Santana-Michel & J. Bernal 14993*; Drawing by José Manuel Ramírez Amezcua.

glabrous; limb widely ovate, deeply or slightly cordate at the base, acuminate at the apex, 0.45–0.6(–1) × (0.5–)0.65–0.9 cm, yellowish area covered by a dark-purple short vermiform appendage, in lateral view cup-shaped; throat reniform to deltate, dark-purple, minutely granular and glabrous; syrinx infundibuliform, eccentric, 1.8–2 mm long, 2–3 mm wide at the base and 0.8–1 mm wide at the apex. Gynostemium coroniform, stipitate, 1.5 × 0.8 mm, stipe 0.8–0.3 mm long, stigmatic lobes 5, 0.6 mm long, anthers 5, 0.7 mm long. Ovary inferior, penicillate, 6–7(–14) × 1–2 mm wide, with sparse septate trichomes. Fruit and seeds not seen. Figures 6, 7A–D.

Phenology—This species has been found flowering in July. The fruiting season remains unknown.

Distribution and Habitat—This species is currently only known from its type locality in the municipality of Acaponeta in the state of Nayarit (Fig. 3). It grows in savannoid vegetation on steep hill sides from 50–80 m altitude.

Etymology—The species epithet refers to the savannoid vegetation where this species has been collected.

Notes—As few collections of this species are currently available, it is yet unclear whether it only shows solitary flowers or whether a racemose inflorescence may develop as is the case in other pentandrous species such as *Aristolochia buntingii*, *A. nahua* and *A. tuitensis*.

Additional Specimens Examined—MEXICO. Nayarit: Mun. Acaponeta, San Felipe Aztatán, Town La Presa, at the foothills of El Fileño, 22°20′18.7″N, 105°21′42.4″W, 52 m, 23 July 2014, *A. Paizanni Guillén et al. 220* (DR, IBUG, IEB, MEXU, ZEA); San Felipe Aztatán, town La Presa, at the foothills of El Fileño, 22°20′18.7″N, 105°21′42.4″W, 52 m, 23 July 2014, *A. Paizanni Guillén et al. 221* (DR, IBUG, IEB, MEXU, ZEA); La Presa 4 km al SW de San Felipe Aztatán, cerro El Fileño, 22°20′52.5″N, 105°22′29″W, 80 m, 29 Nov 2008, *A. Castro-Castro et al. 1435* (IBUG).

Aristolochia tuitensis Santana-Michel & Paizanni sp. nov.—TYPE: MEXICO, Jalisco, Mun. Cabo Corrientes, 4–5 km of El Tuito, follow the road of Minas of Zimapan, Sierra of El Cuale, 19°21′08″N, 105°18′26″W, 830 m, 21 Sept 2003, *F. J. Santana-Michel & J. M. Michel Fuentes 11444* (holotype: ZEA!; isotypes: IEB!, MEXU!).

Aristolochia tuitensis differs from *Aristolochia buntingii* and *A. tresmariae* by the cordiform leaves, which are acuminate at the apex and the perianth with a tube widening at the basal part. Additionally, it can be distinguished by the orbicular limb, which is slightly cordate at the base and apiculate at the apex, in lateral view cup-shaped, with a purplish red area covered with dark-purple setose hairs, and the throat reniform and dark purple.

Geophytic herb, procumbent, subterranean organs 15–20 cm long; stems with 2–3 subterranean internodes, swollen nodes not seen; aerial internodes slightly geniculate, 2–5.5 cm long, sparsely covered with septate trichomes. Petioles terete, (1–)1.5–3 × 0.1 cm, green, densely covered with septate trichomes. Leaf blades cordiform, discolorous, apex acuminate, (3–)5–7(–9) × (2–)3–6.5 cm; palmatinerved, actinodromous, margin entire; adaxial leaf side green, with sparse septate trichomes, 0.6–1 mm long, with acroscopic orientation; abaxial leaf side pale green with dense septate trichomes, especially along the veins. Flowers axillary, solitary or placed in a racemose inflorescence, flower pedicels (6–)8–11(–12) × 0.5–0.8 mm, with dense septate trichomes. Bract lanceolate, 0.2–0.35 × 0.15–0.3 cm. Perianth geniculate, 1.2–1.5 cm long, white-yellowish, becoming purple toward the limb, veins brown-orange, sparse septate trichomes along the veins; utricle spheroid, 0.46–0.6 × 0.37–0.5 cm, internal surface yellow with purple dots at the base, covered with moniliform mucilaginous trichomes; tube bent 60°–90°, basally widened, 5–7 × 2.6–3.2 mm, internal surface glabrous; limb orbicular, slightly cordate at the base, apiculate at the apex, 0.3–0.4 × 0.7–1.0 cm, purplish red area covered by dark-purple setose hairs or protuberances, in lateral view cup-shaped; throat reniform, dark-purple, glabrous, smooth; syrinx infundibuliform, eccentric, 1.4–1.6 mm long, 1–1.3 mm wide at base and 1 mm wide at apex. Gynostemium coroniform, stipitate, 1.5–1.8 mm × 1.3–1.6 mm; stipe 0.5–0.7 mm long, stigmatic lobes 5, 0.7–0.9 mm long, anthers 5, 0.7–0.8 mm long. Ovary inferior, lanceolate, 0.7–0.9 × 0.15 mm, with sparse to dense septate trichomes. Fruit a capsule, spheroid, 1.3–1.5 × 1.1–1.3 cm, brown-yellow, green-purple when young, with septicidal dehiscence at the apex. Seeds deltoid, upper part black, lower part yellow-brown, 4–4.5 × 4–5 mm, with granular surface. Figures 7E–H, 8.

Phenology—This species is known to flower from September to December and has been collected in fruit in September.

Distribution and Habitat—*Aristolochia tuitensis* is distributed along the Pacific Coast of the state of Jalisco, from the municipalities of Cabo Corrientes to Puerto Vallarta (Fig. 3). This species grows in open secondary vegetation and in pine-oak forest, on flat areas and steep hills, from 200–700 m altitude.

Etymology—The species epithet refers to the locality of "El Tuito" were the type specimen was collected.

Additional Specimens Examined—MEXICO. Jalisco: Mun. Cabo Corrientes, Carretera Chamela-Puerto Vallarta, El Tuito, 780 m, 24 Nov 1976, *R. Hernández M. 3021* (ENCB, MEXU); pasando el puente del Rio Las Juntas, cerca del Tuito, 450 m, 21 Sept 1976, *A. Delgado S. & R. Hernández 216* (CHAPA, MEXU,); 6 km al N del Tuito, 580 m, 28 Dec 1976, *L. M. Villarreal de Puga & E. Estrada Faudón 9659* (IBUG); steep rocky valley of Rio Las Juntas, 10–13 km southeast of El Tuito, 250–330 m, 14–16 Dec 1970, oak forest, *R. McVaugh 25459* (MICH); El Tuito, 700 m, 10 Oct 1982, *R. Hernández M. & E. Lott 9148* (MEXU); Puerto Vallarta, 200 m, 26 Sept 1992, *R. Ramírez Delgadillo & Steve Rogers 2919 b* (IBUG).

Key to the *Aristolochia buntingii* complex

1. Adaxial leaf side bullate, abaxial leaf side reticulate; throat ovate ..... *Aristolochia buntingii*
1. Adaxial leaf side smooth, abaxial leaf side not reticulate; throat reniform or deltate to elliptic 2
   2. Utricle ellipsoid to irregularly ellipsoid, tube basally straight or widened towards the limb 3
      3. Limb widely ovate and surface with a short vermiform appendage; growing in Nayarit ..... *A. savannoidea*
      3. Limb elliptic and surface with pectinate-fimbriate annulus at the throat, margin smooth on the inner face; growing on Tres Marias Island ..... *A. tresmariae*
   2. Utricle obovoid to ellipsoid or spheroid, tube shape basally widened 4
      4. Leaf auriculiform-ovate: limb in lateral view flat ..... *A. pacifica*
      4. Leaf orbicular to cordiform; limb in lateral view cup-shaped 5
         5. Leaf apex obtuse, rarely apiculate; limb ovate to cordiform, acuminate at the apex ..... *A. nahua*
         5. Leaf apex acuminate; limb orbicular, apiculate at the apex ..... *A. tuitensis*

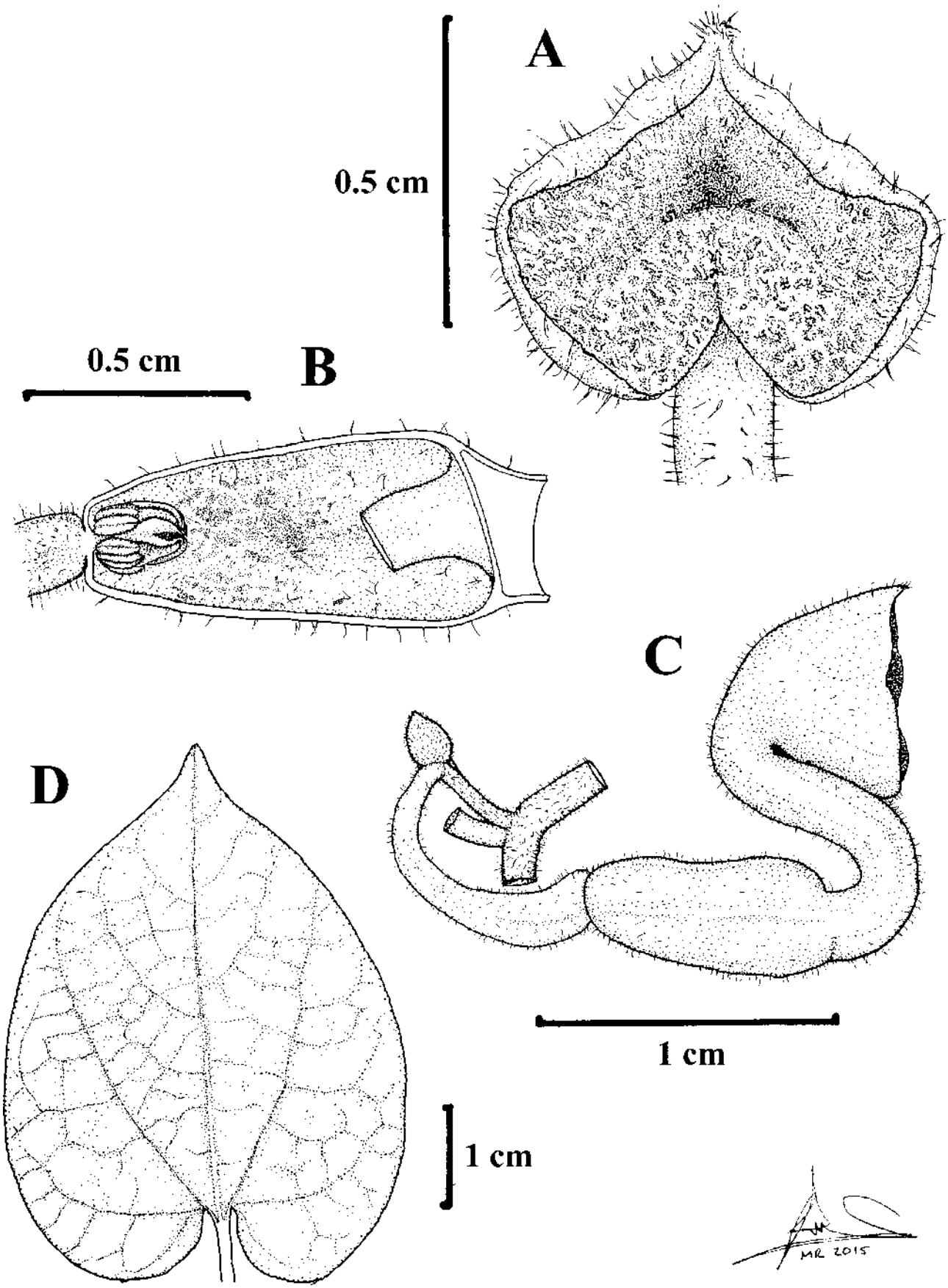

FIG. 6. *Aristolochia savannoidea*. A. Front view of the limb. B. Interior view of the utricle, which is composed of the gynostemium and the syrinx. C. Perianth, inferior ovary, bract and peduncle. D. Leaf blade. Based on *A. Paizanni Guillén 219, 220, 221*; Drawing by José Manuel Ramírez Amezcua.

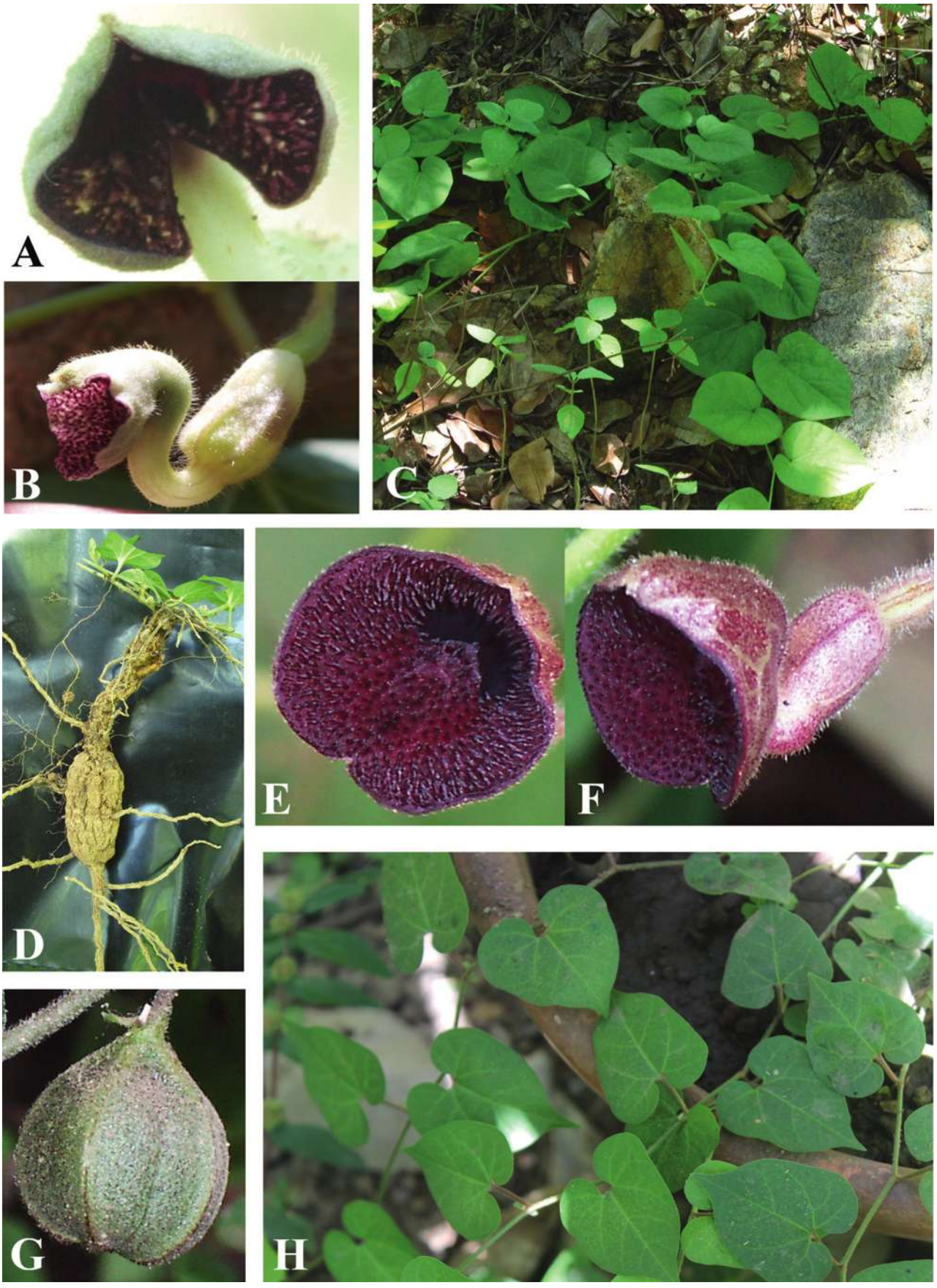

FIG. 7. General morphology of *Aristolochia savannoidea*. A. Front view of the limb. B. Lateral view of the limb. C. Habit. D. Subterranean organ. General morphology of *A. tuitensis*. E. Front view of the limb. F. Lateral view of the limb. G. Fruit. H. Habit. Photographs by A. Paizanni Guillén: A–B, from the type locality; by J. M. Ramírez Amezcua: C–D, from the type locality; and by J. F. Santana Michel: E–H, from the type locality.

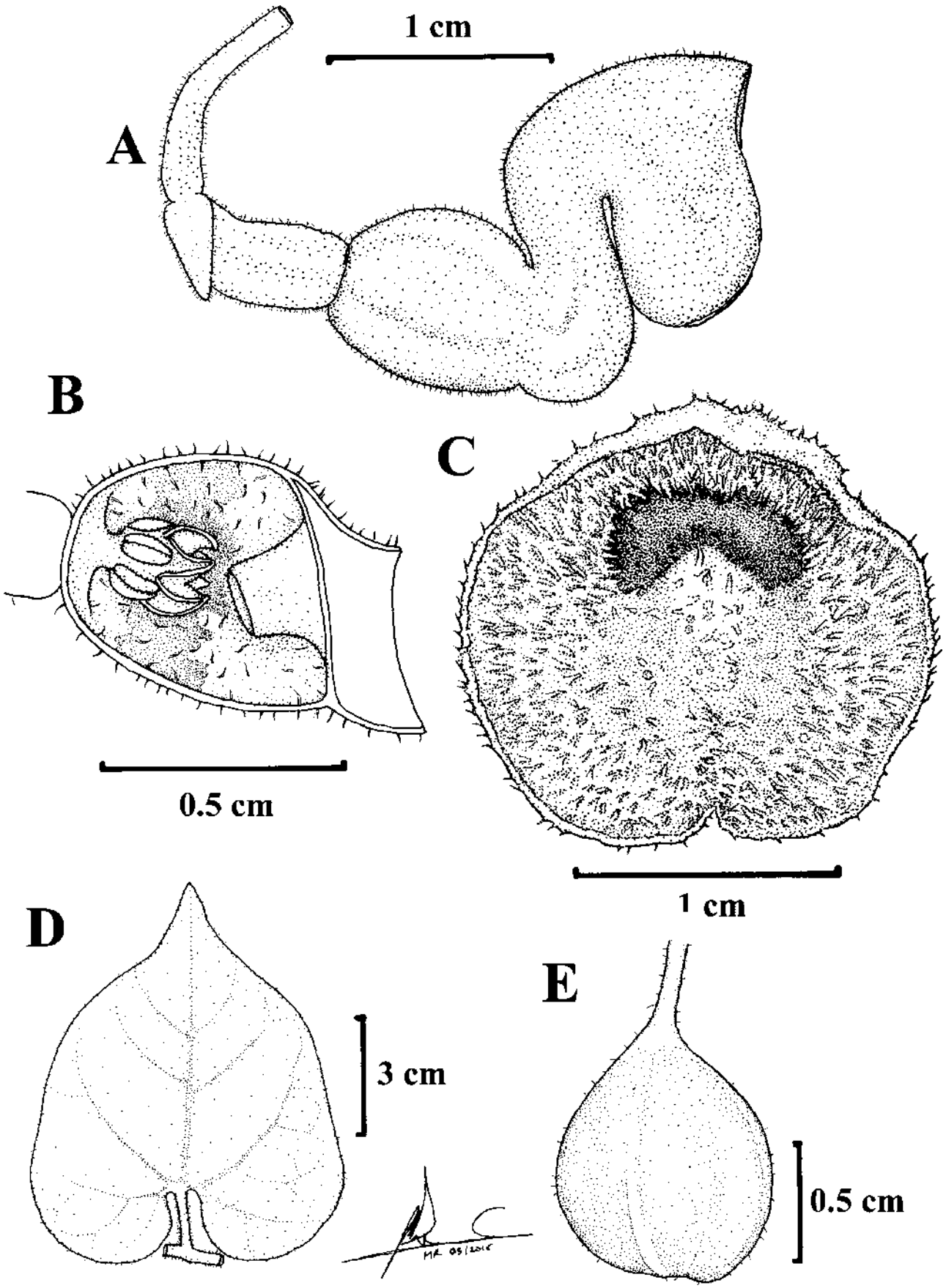

FIG. 8. *Aristolochia tuitensis.* A. Perianth, inferior ovary, bract and pedicel. B. Interior view of the utricle, which is composed of the gynostemium and the syrinx. C. Front view of the limb. D. Leaf blade. E. Fruit. Based on *F. J. Santana-Michel & J. M. Michel Fuentes 11444*; drawing by José Manuel Ramírez Amezcua.

TABLE 1. Morphological characteristics of the *Aristolochia buntingii* complex.

Species	*A. buntingii*	*A. nahua*	*A. pacifica*	*A. savannoidea*	*A. tuitensis*	*A. tresmariae* (according to Pfeifer 1970)
Leaf shape	Ovate	Orbicular to cordiform	Auriculiform-ovate	Elliptic to cordiform rarely auriculiform	Cordiform	Ovate–cordate
Leaf base	Cordate	Cordate	Cordate	Cordate with oblique lobes	Cordate	Cordate
Leaf apex	Obtuse, slightly acute	Obtuse, rarely apiculate	Acuminate	Acuminate and rarely obtuse	Acuminate	Obtuse
Leaf size	(4.0–)5.0–6.5(–8.0) × (3.2–)4.5–5.5(–6.8) cm	(2.0–)2.8–4.5(–7.5) × (1.7–)3.3–4.6(–7.4) cm	(3.5–)4–9 × 3–6.5 cm	(2.3–)4.5–6.0(–8.6) × (2.3–)3.5–5.0(–7.2) cm	(3–)5–7(–9) × (2–)3–6.5 cm	4–7 × 3.5–6 cm
Flower pedicel and peduncle size	9–15 × 0.4–0.6 mm	5–10 × 0.5–0.8 mm	5–10 × 0.5–0.7 mm	8–25 × 0.2–0.7 mm	(6–)8–11(–12) × 0.5–0.8 mm	1.0–1.5 mm long
Bract shape	Lanceolate	Ovate	Cordiform	Ovate	Lanceolate	Ovate
Perianth size	(1.1–)1.5–2 cm long	1.3–1.9(–2.3) cm long	2–2.6 cm long	(1.2–)1.8–2.3 cm long	1.2–1.5 cm long	1.5 cm long
Utricle shape	Obloid	Obovoid to ellipsoid	Spheroid	Irregularly ellipsoid	Spheroid	Ellipsoid
Utricle size	(0.37–)0.4–0.5 × (0.2–)0.3–0.5 cm	0.5–0.6 × (0.2–)0.3–0.5 cm	0.65–0.74 × 0.5–0.55 cm	(0.4–)0.7–0.9 × (0.2–)0.3–0.4 cm	0.46–0.6 × 0.37–0.5 cm	0.5 × 0.3 cm
Tube curvature	70°–90°	90°–120°	90°	30°–50°	60°–90°	120°–140°
Tube shape	Basally straight, widened toward the limb	Basally widened	Basally widened	Basally straight	Basally widened	Widened toward the limb
Tube size	3–4(–9) × 1–2 mm	3.5–5(–6) × 3–5 mm	1.0–1.5 × 3–3.5 mm	(2.5–)6–9 × 1.2–2 mm	5–7 × 2.6–3.2 mm	5 × 3 mm
Tube internal surface	White uniseriate trichomes	Glabrous	Glabrous	Glabrous	Glabrous	Not seen
Limb shape	Obovate	Ovate to cordiform	Cordiform	Widely ovate	Orbicular	Elliptic
Limb base	Slightly cordate	Cordate	Cordate	Deeply or slightly cordate	Slightly cordate	Cordate
Limb apex	Apiculate	Acuminate	Obuse or slightly apiculate	Acuminate	Apiculate	Acute
Limb size	0.7–2 × (0.5–)0.7–1.25 cm	0.5–0.7(–1) × 0.6–1cm	1.3–1.7 × 1.2–1.6 cm	0.45–0.60(–1) × (0.5–)0.65–0.9 cm	0.3–0.4 × 0.7–1.0 cm	0.7 × 0 6 cm
Limb lateral view	Cup-shaped	Cup-shaped	Flat	Cup-shaped	Cup-shaped	Flat
Limb surface	Fimbriate trichomes	Papillose protuberances	Tuberculate reticulate protuberances, sparse white trichomes deciduous	Short vermiform appendage	Setose hairs or protuberances	Pectinate–fimbriate annulus at the throat–limb margin, smooth on the inner face
Limb color	Dark- purple	Yellowish area, covered by purplish red protuberances	Yellowish area, covered by dark purple protuberances	Yellowish area, covered by dark-purple appendage	Purplish red area, covered by dark-purple protuberances	Green and reddish purple
Throat shape	Ovate	Reniform	Reniform	Reniform to deltate	Reniform	Elliptic
Throat surface	White trichomes	Glabrous, minutely granular	White trichomes	Glabrous, minutely granular	Glabrous, smooth	Glabrous
Fruit shape	Mature fruit not seen	Spheroid	Spheroid	Not seen	Spheroid	Botuliform
Fruit color	Purple when young	Brown-yellow, green when young	Brown-yellow, purple when young	Not seen	Brown-yellow, green-purple when young	Unknown

Discussion

Aristolochia subsection *Pentandrae* is especially diverse in the state of Jalisco where 15 species are now known, making up 32% of the total species diversity of the subsection. Five species occur in each of the neighboring states, Colima and Nayarit. These three states together form a biodiversity hotspot of *Pentandrae* (51% of the known species), and according to Morrone (2005) this area is the core of the biogeographical province of the Mexican Pacific Coast. The principal vegetation types characterizing the region are deciduous tropical forest and tropical arid scrub communities (Rzedowski 1978). In addition, in the northern Mexican Pacific Coast, savannoid vegetation and palm forests are more prominent (Morrone 2005).

The Mexican Pacific Coast province spans from the Piaxtla river (state of Sinaloa) in the north to the state of Chiapas in the south (Sarukhán et al. 2008). The main mountain chains flanking the region are the Sierra Madre Occidental in the north, the Trans-Mexican Volcanic Belt in the central-east part, and the Sierra Madre del Sur in the south. The predominant climate is sub-humid warm (74% of the entire area of the Mexican Pacific Coast) with summer rainfall and precipitation ranging from 500–1,500 mm per year (Sarukhán et al. 2008). These climatic characteristics, together with the geology and topography, have been thought to be the main factors contributing to the floristic richness of the area (Ramamoorthy et al. 1998). The species newly described here occur in an area that is not only well known for the species richness of the pentandrous *Aristolochia* species, but also for other flowering plant lineages. One example is the genus *Cosmos* Cav. (Asteraceae), which has been shown to be particularly diverse with a high number of endemics in the state of Jalisco. Additional flowering plant lineages with a considerable number of endemic species to this region are the genera *Pinus* L., *Quercus* L., and *Salvia* L., among others (Vargas-Amado et al. 2013; González-Gallegos et al. 2014).

The four new species had previously been identified as *Aristolochia buntingii*, but are morphologically distinct from it. Moreover, their geographic distributions differ from that of *A. buntingii* s. s., which is only known from two localities in the municipality of Arteaga in Michoacán (Fig. 3). The identification of closely related species, which might have diverged relatively recently, is often difficult (Carolina Granados Mendoza, unpublished data). Within *Pentandrae*, vegetative characters such as leaf shape might be prone to parallel evolution as an adaptation of recurring abiotic factors caused by high niche fragmentation. As a result of vegetative similarity, herbarium material is commonly misidentified or new species remain unrecognized. Reproductive and vegetative organs are often indispensable for untangling species complexes such as the *A. buntingii* complex. However, flowers of herbarium specimens potentially provide limited diagnostic characters that occasionally cannot even be restored upon rehydration of the material. Details such as ornamentation, tridimensionality of the limb, the indument inside the perianth and its texture, and bending of the flower are often difficult to observe. Additionally, rehydration is considered as destructive sampling by many herbaria and thus often not allowed. As a consequence, it is essential to be able to observe living plants and dissect the flowers in the field in order to study this plant group. Therefore, given the difficulty of distinguishing species of *Pentandrae*, additional new species are expected in unexplored areas throughout Mexico.

The only revision treating pentandrous species in detail was published by Pfeifer (1970). In this treatment we can see the close affinity of *A. buntingii* and *A. tresmariae*. These species share the following characteristics: procumbent habit, small flowers (2–3(–5) cm long), and leaves ranging from 2–9 cm long (Table 1). However, the four new species described here are different from *A. buntingii* and *A. tresmariae* by the acuminate leaf apex, the limb shape which may be cordiform, ovate, widely ovate, or orbicular, and the reniform or deltate throat.

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Literature Cited

Calzada, J. I., F. G. Flores, and V. O. Téllez. 1997. *Aristolochia davilae* (Aristolochiaceae), a new species from Nayarit, Mexico. *Contributions from the University of Michigan Herbarium* 21: 143–146.

González, F. and D. W. Stevenson. 2000. Perianth development and systematics of *Aristolochia*. *Flora* 195: 370–391.

González, F., H. E. Esquivel, G. A. Murcia, and N. Pabón-Mora. 2010. *Aristolochia pentandra* (Aristolochiaceae) in Colombia: Biogeographic implications and proposed synapomorphies between the pentandrous species of *Aristolochia* and its South American sister group. *Revista de la Academia Colombiana de Ciencias* 34: 467–478.

González, F., S. T. Wagner, K. Salomo, L. Symmank, M.-S. Samain, S. Isnard, N. P. Rowe, C. Neinhuis, and S. Wanke. 2014. Present trans-Pacific disjunct distribution of *Aristolochia* subgenus *Isotrema* (Aristolochiaceae) was shaped by dispersal, vicariance and extinction. *Journal of Biogeography* 41: 380–391.

González-Gallegos, J. G., G. Vargas-Amado, E. De Castro-Arce, A. Castro-Castro, and M. E. Mendoza-López. 2014. Diversidad y distribución de Lamiaceae en el Occidente de México. Pp. 115–151 *In* González-Gallegos, J. G. *Sistemática de Salvia sección Membranaceae (Lamiaceae) y diversidad de Lamiaceae en el occidente de México.* PhD thesis. Guadalajara, Mexico: Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara.

Harris, J. G. and M. W. Harris. 2006. *Plant identification terminology, an illustrated glossary.* Ed 2. Spring Lake, Utah: Spring Lake Publishing.

INEGI. 2014. http://www.inegi.org.mx/geo/contenidos/datosrelieve/continental/Descarga.aspx (2014/08/01).

Kelly, L. M. 2000. Aristolochiaceae. *Flora del Valle de Tehuacán-Cuicatlán* 29: 1–8.

Moreno, P. N. 1984. *Glosario botánico ilustrado*. Xalapa: Instituto Nacional de Investigaciones sobre Recursos Bióticos.

Morrone, J. J. 2005. Toward a synthesis of Mexican Biogeography. *Revista Mexicana de Biodiversidad* 76: 207–252.

Neinhuis, C., S. Wanke, K. W. Hilu, K. Müller, and T. Borsch. 2005. Phylogeny of Aristolochiaceae based on parsimony, likelihood, and Bayesian analyses of *trnL-trnF* sequence. *Plant Systematics and Evolution* 250: 7–26.

Ohi-Toma, T., T. Sugawara, H. Murata, S. Wanke, C. Neinhuis, and J. Murata. 2006. Molecular phylogeny of *Aristolochia sensu lato* (Aristolochiaceae) based on sequences of *rbcL, matK*, and *phyA* genes, with special references to differentiation of chromosome numbers. *Systematic Botany* 31: 481–492.

Ortega Ortiz, J. F. and R. V. Ortega Ortiz. 1995. Contribución al estudio de Aristolochiaceae de México, I. Una especie nueva de *Aristolochia* de Puebla, México. *Acta Botánica Mexicana* 32: 39–45.

QGIS Development Team. 2014. QGIS 2.6 Brighton. Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org. Accessed 18 January 2015.

Pfeifer, H. W. 1966. Revision of the north and central American *Hexandrous* species of *Aristolochia* (Aristolochiaceae). *Annals of the Missouri Botanical Garden* 53: 115–196.

Pfeifer, H. W. 1970. *A revision of the pentandrous species of Aristolochia*. Connecticut: The University of Connecticut Publication Series.

Ramamoorthy, T. P., R. Bye, A. Lot, and J. Fa. 1998. *Diversidad biológica de México: Orígenes y distribución*. México. D. F.: Instituto de Biología Universidad Nacional Autónoma de México.

Rzedowski, J. 1978. *Vegetación de México. México*. D. F.: Limusa.

Santana-Michel, F. J. 1995. Una especie nueva de *Aristolochia* (subsección *Pentandrae*) del estado de Jalisco, México. *Ibugana* 3: 87–90.

Santana-Michel, F. J. and S. Lemus Juárez. 1996. *Aristolochia occidentalis* (Aristolochiaceae), una especie nueva de la subsección *Pentandrae* de Colima y Jalisco, México. *Acta Botánica Mexicana* 36: 53–57.

Santana-Michel, F. J. 2002. Una especie nueva de *Aristolochia* L., subsección *Pentandrae* (Aristolochiaceae) del estado de Colima, México. *Acta Botánica Mexicana* 58: 63–67.

Santana-Michel, F. J. 2007. Una especie nueva de *Aristolochia* L., subsección *Pentandrae* (Aristolochiaceae) de la reserva de la biosfera Sierra de Manantlán, Jalisco, México. *Acta Botánica Mexicana* 79: 81–87.

Santana-Michel, F. J. and J. A. Solís-Magallanes. 2007. *Aristolochia emiliae* (Aristolochiaceae: subsección *Pentandrae*), una especie nueva de la costa de Jalisco, México. *Acta Botánica Mexicana* 82: 7–13.

Santana-Michel, F. J. and L. Guzmán-Hernández. 2014. *Aristolochia rzedowskiana* (Aristolochiaceae), una especie nueva para la subsección *Pentandrae* del estado de Jalisco, México. *Acta Botánica Mexicana* 106: 1–7.

Santana-Michel, F. J. and R. Cuevas Guzmán. 2014. Diversidad y distribución de *Aristolochia* (Aristolochiaceae) en el estado de Colima, México. *Ibugana* 5: 95–132.

Sarukhán, J., J. Soberón, G. Halffter, and J. Llorente-Bousquets. 2008. La Perspectiva Biogeográfica y Ecosistémica. Pp. 31–35 in *Capital Natural de México* Vol. 1, México: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Vargas-Amado, G., A. Castro-Castro, M. Harker, J. L. Villaseñor, E. Ortiz, and A. Rodríguez. 2013. Geographic distribution and richness of the genus Cosmos (Asteraceae: Coreopsidae). *Revista Mexicana de Biodiversidad* 84: 536–555.

Wanke, S., F. González, and C. Neinhuis. 2006. Systematics of pipevines: Combining morphological and fast-evolving molecular characters to investigate the relationships within subfamily Aristolochioideae (Aristolochiaceae). *International Journal of Plant Sciences* 167: 1215–1227.