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# *Dictyanthus reflexiflorus* (Apocynaceae: Asclepiadoideae) a new species from Mexico

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## Abstract

A new species of *Dictyanthus* from the state of Michoacan, Mexico, is described and illustrated *Dictyanthus reflexiflorus sp. nov.* It grows in tropical deciduous forests located in the municipality of Tacámbaro. A phylogenetic analysis based on molecular data (plastid regions *trn*L-*trn*F and *rps* 16) was conducted to explore the relationship of the new species. Results place the new species in *Dictyanthus*. The morphological characters that support this relationship are the presence of uncinate hairs and a reticulate corolla. The species has unique characteristics within the genus, such as a reflexed corolla and the tube 10-lobed closely pressed to the side of the gynostegium. A key to distinguish *Dictyanthus reflexiflorus* from congeners in Mexico is included.

**Key words:** Asclepiadoideae, endemic, Gonolobinae, Michoacán, phylogeny

## Introduction

*Dictyanthus* Decaisne (1844: 604) belongs to the subtribe Gonolobinae (Apocynaceae, Asclepiadoideae) and contains 18 species distributed in Central America, from northern Mexico to Nicaragua, with 87% of its species endemic to Mexico (Stevens 2001; Stevens 2009; Endress *et al.* 2019; González-Martínez *et al.* 2019; Alvarado-Cárdenas *et al.* 2020; Alvarado-Cárdenas *et al.* 2022). The genus was considered a subgenus of *Matelea* Aublet (1775: 277) due to the similar flowers and a mixed indumentum of long straight, short, and glandular trichomes (Woodson 1941; Stevens 1988; Krings *et al.* 2008; Endress *et al.* 2014).

Recent phylogenetic analyses recovered *Matelea s.l.* as paraphyletic, favoring the reinstatement of the *Dictyanthus* clade as a genus (McDonnell *et al.* 2018; González-Martínez *et al.* 2019). *Dictyanthus* can be distinguished from the rest of the subtribe by a palmately 5-lobed gynostegial corona, confluent with the corolla lobes, uncinate trichomes on the vegetative parts, and follicles with a ridge at the base and prickly projections (González-Martínez 2019; Stevens 1988; Stevens 2000; Stevens 2001; Stevens 2009). The corolla in *Dictyanthus* is shallowly or deeply campanulate to urceolate sacciform (Stevens 1988; Stevens 2009).

During fieldwork in the state of Michoacán, Mexico, we collected specimens that could not be assigned to any known species of *Dictyanthus*. We conducted phylogenetic analyses to determine the relationships of this new species and we describe it as *Dictyanthus reflexiflorus*. We also provide a key to the species of *Dictyanthus* found in Mexico.

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## Materials and methods

### Extraction, amplification, and sequencing of DNA

Total genomic DNA of *Dictyanthus reflexiflorus* was extracted from leaves using the CTAB method (Martínez-González *et al.* 2017). The DNA was quantified with a Nanodrop 2000c (Thermo, USA). We prepared dilutions at 20 ng. Three molecular markers were used: the *rps* 16 intron, was amplified using the primers rpsF and rpsR (Oxelman *et al.* 1997); the *trn*L intron, and the *trn*L-*trn*F spacer, both markers amplified with the primers C, D, E, F (Taberlet *et al.* 1991). The PCR reaction mixture was prepared in a final volume of 15μL containing buffer of the enzyme 1x Taq DNA polymerase, 0.8mM deoxynucleoside triphosphate (0.2mM of each one), 100 ng DNA, 20 pmol of each primer and 2 units of GoTaq DNA (Promega, USA). The amplification of each region was undertaken through an initial denaturing cycle at 94°C for four minutes followed by thirty-five cycles of denaturing at 94°C for forty-five seconds, annealing for one minute at the specific temperature of each gene, and extension at 72°C for five minutes. All PCR reactions were conducted in a Peltier Thermal Cycler PTC-200 (BIORAD, México). The amplifications were verified through electrophoresis in a 1.5% agarose gel prepared with 1× TAE buffer (Tris-Acetate-EDTA) and run at 87V $cm^{-3}$ for one hour. The gel was stained with GelRed (Biotium, USA), and the bands were visualized in an Infinity 3000 transilluminator (Vilber Lourmat, Germany). The amplified products were purified with the ExoSAP Purification kit (Affymetrix, USA), following the manufacturer's instructions. These products were sequenced using an Applied Biosystems model 3730XL (Applied BioSystems, USA) at the Biology Institute of the Universidad Nacional Autónoma de México.

### Sequence assembly

The forward and reverse sequences of each of the three regions were edited and assembled using BioEdit version 7.0.5 (Hall 1999) to generate a consensus sequence. The sequences were aligned separately using MAFFT v. 7 (Katoh & Standley 2013) with the FFT–NS–i iterative refinement method, and the rest of the parameters selected by default. Subsequently, the alignments were manually edited using PhyDE v. 0.9971 (Müller *et al.* 2010).

### Phylogenetic analysis

We downloaded sequences which had been used in previous phylogenetic studies of Gonolobinae (Liede-Schumann *et al.* 2005; Krings *et al.* 2008; Mangelsdorff *et al.* 2016). Taxa were chosen from the species with data from the molecular markers that we sequenced. The complete list of GenBank accession numbers for the species included is presented in Appendix 1.

For the substitution model of each marker, we used Model Finder (Kalyaanamoorthy *et al.* 2017) employing default parameters and chosen under the Akaike information criteria (AIC). Maximum Likelihood Analysis (ML) was performed in IQ-TREE web server (Trifinopoulos *et al.* 2016) with ultrafast bootstrapping (Hoang *et al.* 2018) of 1000 replicates and the rest of parameters selected by default. The consensus tree and bootstrap percentages (BS) were viewed and edited in Fig Tree v. 1.4.3 (Rambaut 2016). Bayesian Inference (BI) was performed using Mr. Bayes v. 3.2.6 (Ronquist *et al.* 2012) with two independent runs and four Markov chains Monte Carlo (MCMC) each. Each chain ran for 10 million generations and was sampled every 1,000 generations. The convergence of the chains was checked using Tracer v. 1.7.1 (Rambaut *et al.* 2018), and according to the performance of the chains, 25% of the trees were eliminated as burn-in. The remaining trees were used to build a consensus tree, which was viewed using FigTree v. 1.4.3 (Rambaut 2016). The aligned sequences and resulting trees are available in Dryad (Martínez-Ambriz *et al.* 2023).

### Morphological observations

Material of the new species was collected during two trips to the state of Michoacán (municipality of Tacámbaro) in September 2015 and September 2016. Specimens were deposited in the herbaria FCME, IEB, and MEXU (acronyms according to Thiers, 2021). Additionally, flowers were fixed in 70% alcohol for detailed observations with a dissecting microscope. This material was compared with all known species of *Dictyanthus* (Stevens 1988; Stevens 1999; Stevens 2000; González-Martínez *et al.* 2019; Alvarado-Cárdenas *et al.* 2022), from specimens in the herbaria ENCB, FCME,

MEXU, and XAL. Those species found in Mexico were included in a key with the new species. IUCN criteria (IUCN 2022) were used to assess the conservation status of the new species.

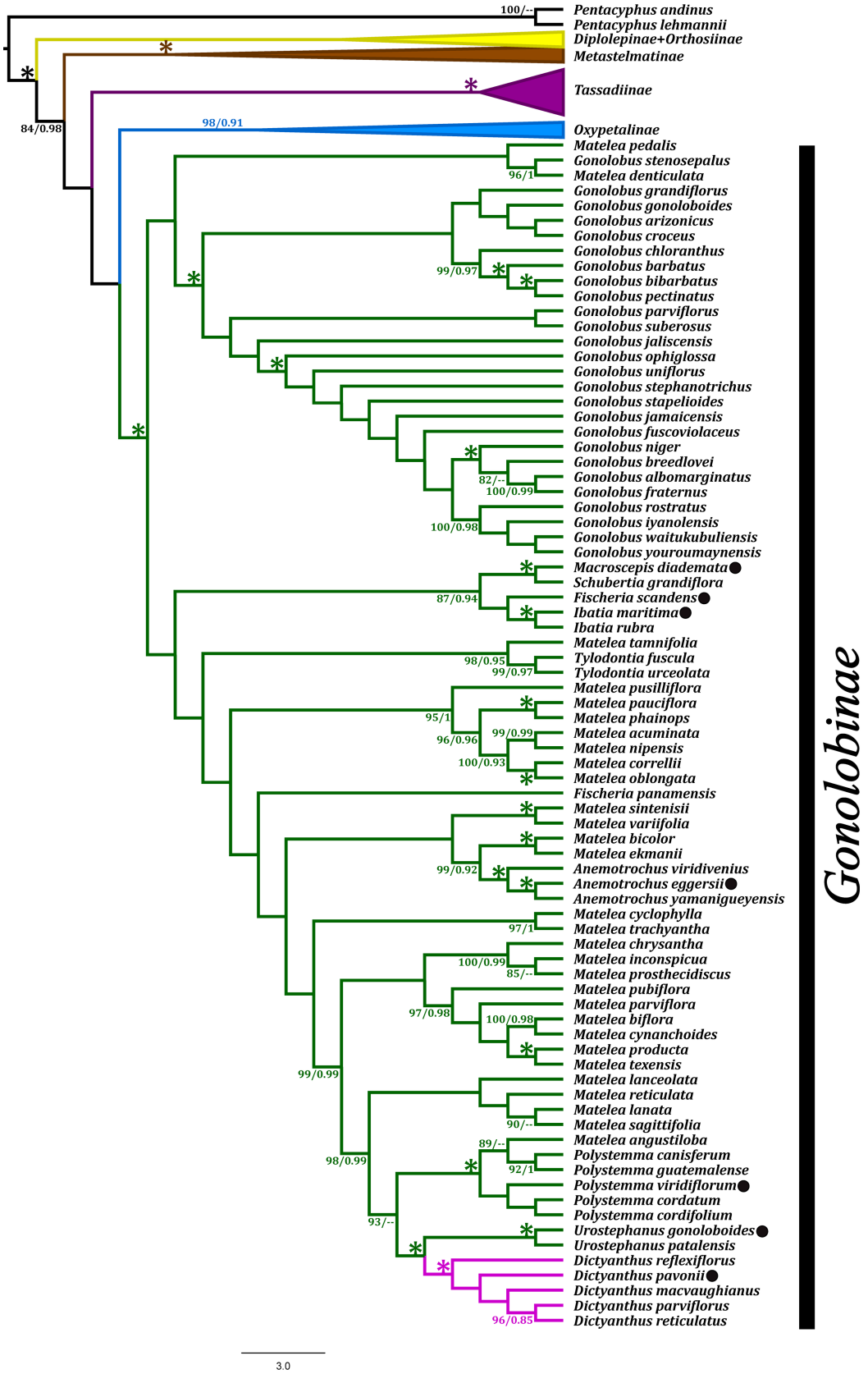


**FIGURE 1.** Tree derived from Bayesian Inference, showing the position of *Dictyanthus reflexiflorus* in Gonolobinae. Numbers on branches indicate Maximum Likelihood bootstrap support (BS) / Posterior Probability (PP). Positions where BS = 100 and PP = 1.0 are indicated by asterisks. Only BS ≥ 80 or PP ≥ 0.8 are indicated. Type species included in the analysis are indicated by a circle. To save space the clades of *Diplolepinae+Orthosinae*, *Metastelmatinae*, *Oxypetalinae* and *Tassadiinae* were collapsed.

## Results

A summary of the data from the aligned sequences is presented in Table 1. The marker *rps*16 was the most variable (16.4% variable sites), followed by *trn*L-*trn*F (9.3%).

**TABLE 1.** Summary of statistics for the analyzed markers used to run ML and BI analyses.

| Region | *rps16* | *trnL-trnF* |
|---|---|---|
| No. of taxa | 172 | 172 |
| Aligned length (bp) | 947 | 1067 |
| Conserved characters | 662 | 829 |
| Variable characters | 155 | 99 |
| Substitution model | TVM+F+I+G4 | TVM+F+G4 |

ML and BI analyses recovered Gonolobinae as monophyletic (BS = 100; PP = 1), which is consistent with previous studies. Both BI and ML analyses, recovered the four species of *Dictyanthus* as monophyletic. The new species, *Dictyanthus reflexiflorus,* is sister (BS = 100, PP = 1; Fig. 1) to these four congeners included here: *D. macvaughianus* (Stevens) Stevens (2000: 243)*, D. parviflorus* Hemsley (1882: 329)*, D. pavonii* Decaisne (1844: 605) (the type of *Dictyanthus*) and *D. reticulatus* (Turcz.) Bentham & Hooker f. ex Hemsley (1882: 329). The position of the new species was the same in the trees from BI and ML analyses. *Dictyanthus* was recovered as sister to a clade (BS = 100; PP = 1), composed of *Urostephanus gonoloboides* Robinson & Greenman (1895: 159–160) (the type of *Urostephanus* Robinson & Greenman (1895: 159)) and *U. patalensis* (Donn. Sm.) González-Martínez, Lozada-Pérez & Alvarado (2024: 13). Both clades are deeply nested within *Matalea*.

## Taxonomy

***Dictyanthus reflexiflorus*** Lozada-Pérez, E.B. Cortez & Martínez-Ambriz, *sp.nov.* (Figs. 2, 3).

Type :—MEXICO. Michoacán: Municipio Tacámbaro. Camino de Chipícuaro a Las Ánimas, 19º09’07”N, 101º23’00”W, 1170 m, 12 Sept 2016, *L. Lozada-Pérez, E. Martínez-Ambriz & L. Pérez-García 3835* (holotype: FCME!; isotypes: IEB!, MEXU!).

*Dictyanthus reflexiflorus* shares a climbing habit with *D. altatensis* (Brandegee) Stevens (2000: 243) but differs from it by the reflexed corolla 10-lobed at base and inflorescence with peduncles 3–4 mm long (vs. corolla rotate, without lobes at the base and inflorescence with peduncles 4–8 cm long).

*Herbaceous climber* to 2.5 m long, with a woody base, latex clear. *Stems* and branches cylindrical, violet to purple, slightly corky, lignified at the base and glabrescent with age; young stems with evenly distributed mixed indumentum composed for multicellular and unicellular trichomes, multicellular ones can be hooked or straight, 0.75–1.25 mm long, yellowish, unicellular trichomes are glandular or eglandular, less than 0.1 mm long, grayish. *Leaves* opposite, without stipules, petioles 1–3 cm long, with indumentum similar to the stem; lamina membranaceous, 2.0–7.0 × 1.0–3.5 cm, ovate, 5 or 6 secondary veins, adaxial surface sparsely pubescent with scattered uncinate hairs, slightly denser on the midvein near the base, abaxial surface densely puberulent with short uncinate trichomes and scattered glandular trichomes, veins with scattered long uncinate trichomes; base cordate, apex acuminate, base cordate; colleters 4–6, at the adaxial junction of petiole and lamina. *Inflorescence* extra-axillary, one per node, umbelliform, with 3–5 flowers; peduncle 3–4 mm long; bracts 3.0–4.0 × 0.3–0.5 mm, narrowly lanceolate; pedicels 9.0–11.0 mm long. *Flowers* pentamerous. *Calix* green, lanceolate, 4.5–5.0 × 1.5–2.0 mm, pubescent with widely scattered uncinate trichomes on the outside and shorter trichomes on the margin; colleters 1 per sinus. *Corolla* reflexed, pale to greenish yellow with dark reticulation, adaxially densely hirsute, abaxially densely hirsute with short uncinate trichomes and scattered glandular trichomes, tube 3.0–3.5 mm long, formed by erect slightly 10-lobed closely pressed to the side of the gynostegium and reaching to just below the corona, lobes ovate-lanceolate, 4.5–5.0 × 4.0–4.3 mm; gynostegial corona black, 5-lobed, lobes alternating with anthers, ca. 1.5 × 0.5 mm, each lobe 2-keeled, each keel parallel to

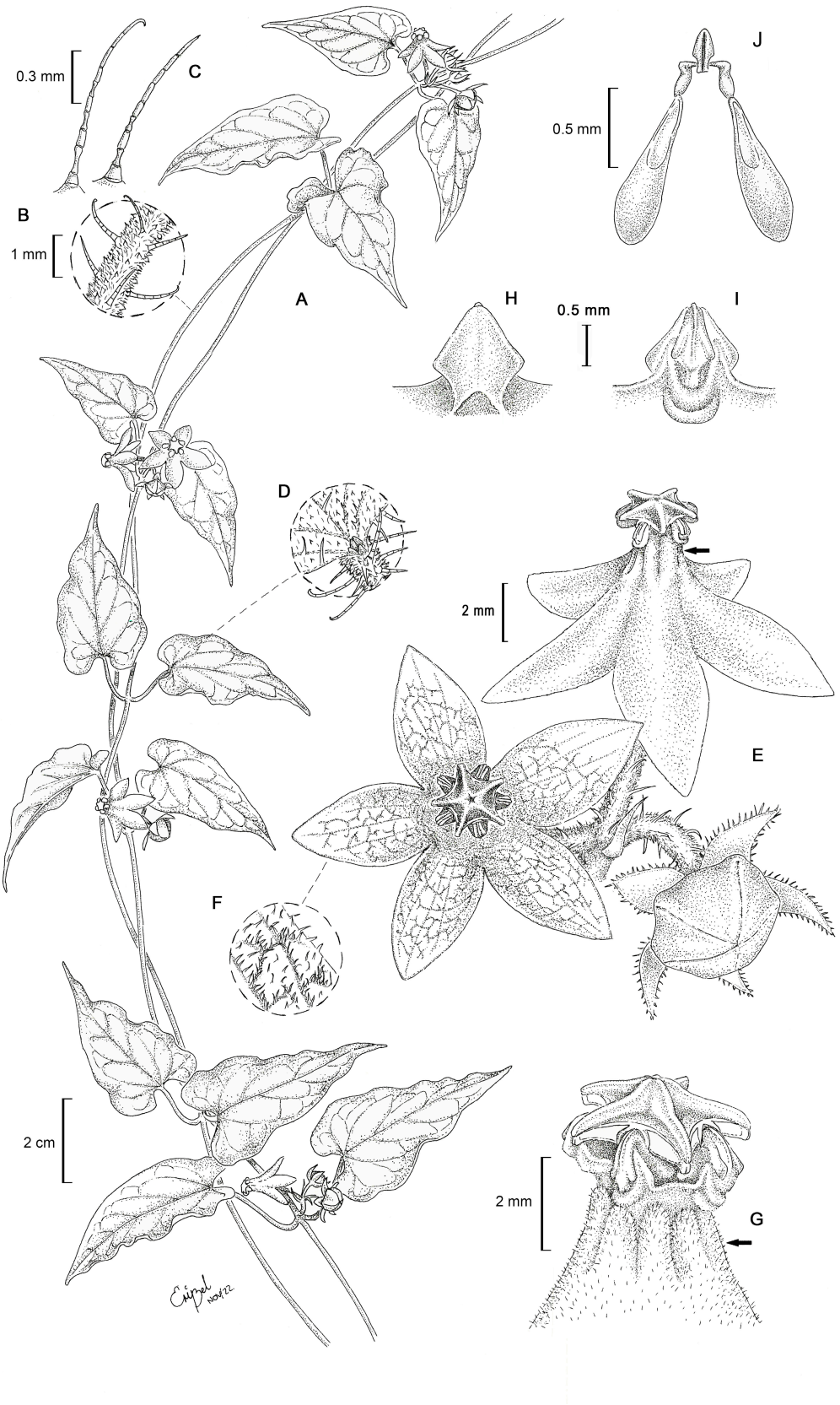


**FIGURE 2.** *Dictyanthus reflexiflorus*. A. Plant with inflorescences. B. Close-up of branch showing mixed indumentum. C. Close-up of thichomes. D. Colleters at base of leaf-blade. E. Inflorescence. F. Close-up of the indument of the abaxial surface of the corolla. G. Gynostegium with style-head. H. Gynostegial corona lobe in posterior view. I. Gynostegial corona lobe in frontal view. J. Pollinarium. Arrows showing the corolla lobes. Illustrations by Ericka B. Cortez, based on Lozada-Pérez *et al.* 3835.

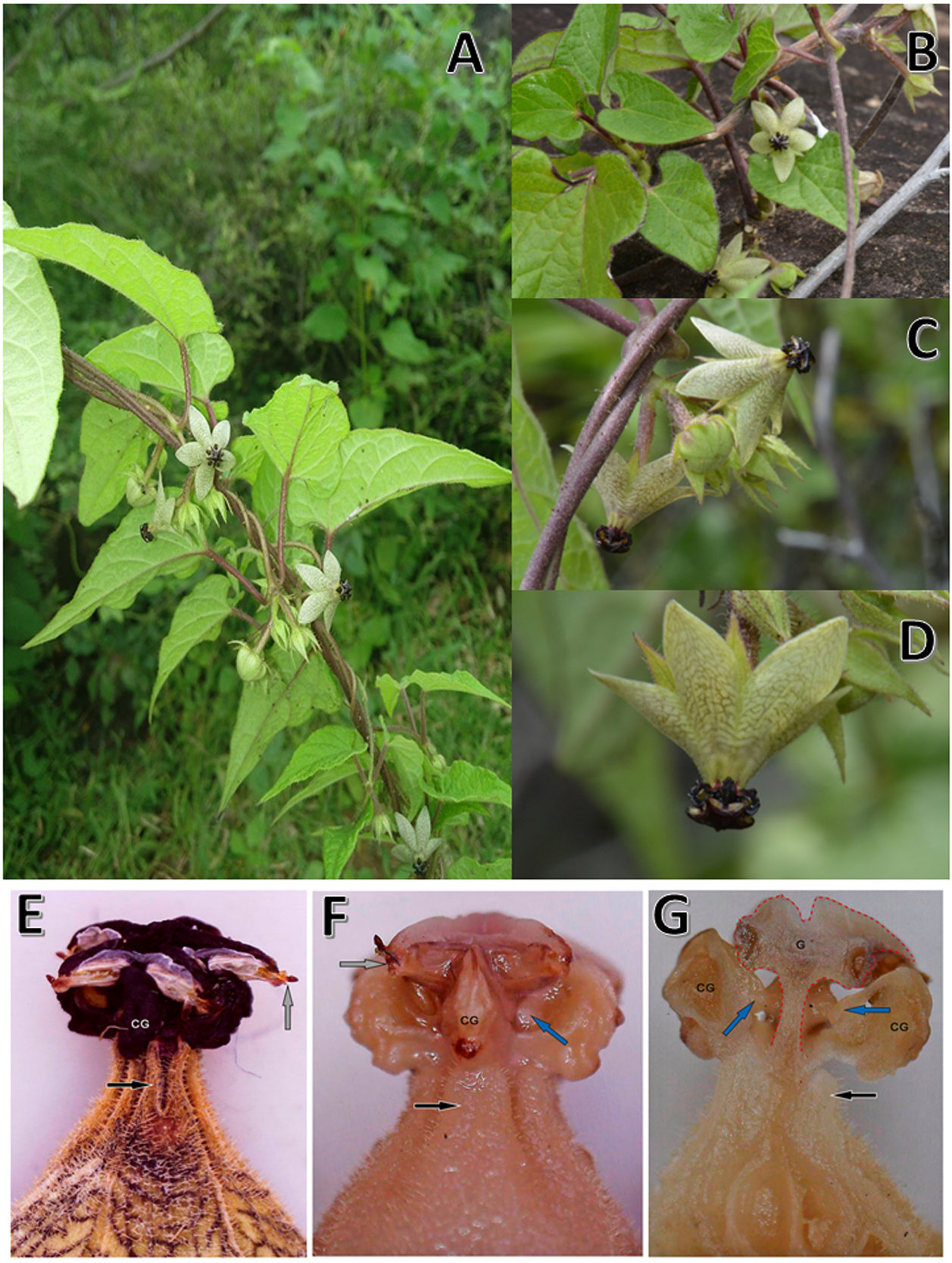


**FIGURE 3.** *Dictyanthus reflexiflorus*. A-C. Plant and inflorescences with mature flowers. D. Side view of corolla and gynostegium. E. Dried flower. F. Flower fixed in alcohol. G. Vertical section of corona and gynostegium. (GC: Gynostegial corona, G: Style head, black arrows = tube lobes, gray arrows = corpuscle and blue arrows = keels). Photos by María de la Luz Perez-Garcia and Lucio Lozada-Pérez.

the adjacent keel; gynostegium, ca. 4 mm diam., deeply pentagonal, convex above; pollinarium 1.1–1.4 mm long, corpusculum dark brown, slightly sagittate, ca. 0.2 × 0.07 mm, caudicles 0.17–0.2 mm long, pollinia elliptic, 0.8–1.0 mm long, apically excavated. *Follicles* unknown.

**Distribution and Habitat:**—*Dictyanthus reflexiflorus* is distributed in a small area of central Michoacán (Fig. 4), in the Balsas Basin province (Morrone *et al.* 2017), in tropical deciduous forest, from 1170–1185 m.

**Phenology:**—Flowering has been recorded in September.

**Etymology:**—The epithet *reflexiflorus* refers to the reflexed corolla, a distinctive feature that is unique in *Dictyanthus*.

**IUCN conservation assessment:**—Information about *Dictyanthus reflexiflorus* is scarce, with only one population known, although the surrounding area was explored, ca. 1 km$^2$. For this reason, we suggest that it be assigned the category of Data Deficient (DD) (IUCN 2022) until a thorough evaluation is conducted in the adjacent hills and ravines. The type locality is not part of a natural protected area, and we have not carried out an extensive vegetation survey of the area.

**Taxonomic notes:**—*Dictyanthus reflexiflorus* is very distinctive due to its reflexed corolla, with a tube 10-lobed closely pressed to the side of the gynostegium (Fig. 3 E, G). All other species have a rotate or campanulate corolla, which is smooth towards its base inside (Stevens 1988, 1999, 2000; González-Martínez *et al.* 2019; Alvarado-Cárdenas *et al.* 2022). Similarly, the very reduced size of the gynostegial corona, which is limited to the base of the limb, is different from that in other species where the corona is almost completely adnate to the corolla. This unusual structure is contrasted by the star-shaped style-head with an unusually dark.

The new species is endemic to the state of Michoacán and known only from two collections from the same locality. The climbing habit is shared with *D. altatensis*, but in this species the corolla is rotate, and the tube is not 10-lobed at the base.

**Additional specimens examined:**—MEXICO. Michoacán: Tacámbaro, 2.19 km camino de Chipícuaro a Las Ánimas, 19º09’06”N, 101º23’00”W, 1185 m, 3 Sept 2015, *L. Pérez G. et al. 163* (IEB).

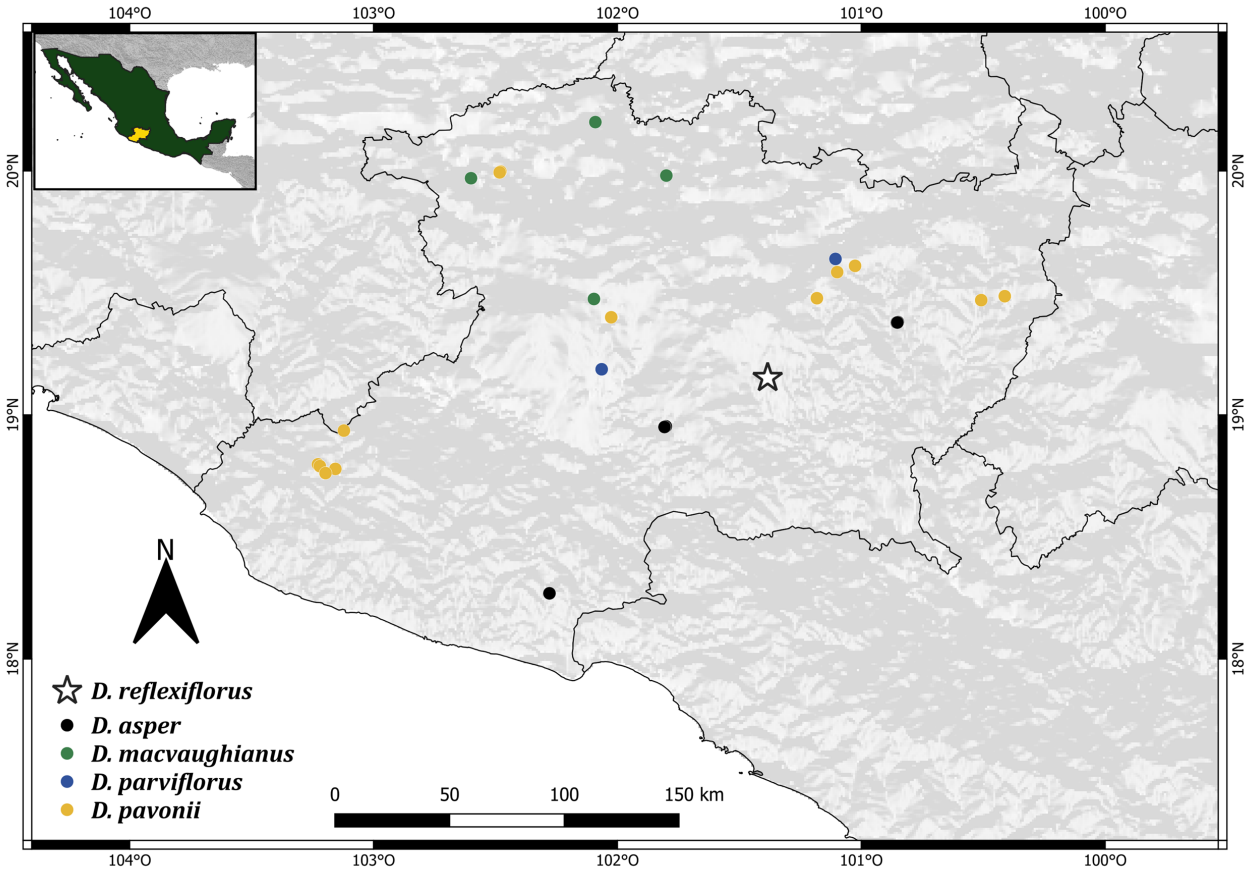


**FIGURE 4.** Distribution of *Dictyanthus reflexiflorus* and their congeners in Michoacan, Mexico.

## Discussion

Our result show that the Gonolobinae is monophyletic, agreeing with previous studies (Liede-Schumann *et al.* 2005; Krings *et al.* 2008; Mangelsdorff *et al.* 2016; McDonnell *et al.* 2018). Within the Gonolobinae, several other genera were also recovered and strongly supported (Mangelsdorff *et al.* 2016; McDonnell *et al.* 2018; González-Martínez *et al.* 2024), including the relationship between the two subclades of *Gonolobus* (McDonnell *et al.* 2018).

The four species of *Dictyanthus* included in our analysis formed a strongly supported clade together with our new species. Although *D. reflexiflorus* not show some diagnostic taxonomic characters from *Dictyanthus*, like the palmately 5-lobed gynostegial corona (Gonzalez-Martínez 2019), shared the uncinate trichomes on the vegetative parts and the reticulation of the inner surface corolla. These morphological evidence with the molecular and distribution data allow us to locate these species like a new member of *Dictyanthus*. The uncommon floral morphology in the new species first made us think in a new genus of Gonolobinae. However, the available data do not support this hypothesis and include this species like a new member of *Matelea s.l.* seems impractical to us. The latest classification of Apocynaceae (Endress *et al.* 2014) did not recognize *Dictyanthus* as an independent genus. But the most recent phylogenetic analysis shows *Matelea s.l.* like a paraphyletic genus with a lack of discrete morphological variation (Morillo 2015; McDonell *et al.* 2018; González-Martínez 2019) and support the monophyly of *Dictyanthus* (Krings *et al.* 2008; McDonnell *et al.* 2018; González-Martínez 2019; González-Martínez *et al.* 2024). Here, we follow the proposal of *Dictyanthus* as an independent genus, which allow a better understanding of the group. Our results highlight the high diversity in *Dictyanthus* and distinguishes Gonolobinae as a source of botanical novelties.

### Identification key for *Dictyanthus* in Mexico (modified from González-Martínez 2019)

1. Corolla tube adaxially with parallel vertical lines, occasionally with perpendicular lines ..... 2
1. Corolla tube adaxially with concentric lines or without perpendicular lines or lacking a distinctive pattern ..... 7
2. Pedicels less than 6 mm long; corolla lobes less than 7 mm long ..... 3
2. Pedicels more than 6 mm long; corolla lobes more than 7 mm long ..... 6
3. Inflorescence with 2 flowers; corona lobes oblong or linear-spathulate, more than half the length of the corolla tube ..... 4
3. Inflorescence with more than 2 flowers; corona lobes rhombic or triangular, less than half the length of the corolla tube ..... 5
4. Leaf (3.1–)3.6–5.6 × 2–4.3(–5) cm; petioles 1.6–4.6 cm; corolla green; corona lobes oblong with rounded or bilobed apex ..... *D. stevensii*
4. Leaf 10.7–13 × 7.1–8.8 cm; petioles 4.7–8.1 cm; corolla white-pink; corona lobes linear-spathulate with elliptical apex ..... *D. lautus*
5. Plant a climber; pedicels 1.5–3.5 mm long; calyx lobes 3–5 mm long; corolla brownish-greenish or whitish; corona-lobes rhombic ..... *D. sepicola*
5. Plant erect; pedicels 4–5 mm long; calyx lobes 5–9 mm long; corolla grayish-white; corona lobes triangular ..... *D. tuberosus*
6. Corolla whitish-yellow; peduncle and pedicels with generally uncinate trichomes; corona with a thin band of short trichomes around and inside the lobes, lobes 6–10 mm long ..... *D. pavonii*
6. Corolla yellowish-green; peduncle and pedicels with straight trichomes; corona glabrous around and inside lobes, lobes 11–25 mm long ..... *D. macvaughianus*
7. Corolla glabrous or almost so within; corona lobes basally connate forming a disc ..... 8
7. Corolla densely pubescent within, at least on the limb; corona lobes sometimes connate, but without forming a disc ..... 9
8. Sepals (3.5–)5–10 × (1–)2.4 mm; corolla rotate, white or green with reticulated venation; lobes 3.5–8 mm long; corona lobes vesicular lobes ..... *D. asper*
8. Sepals 10–17 × 4–6.5 mm; corolla campanulate, pale yellow with concentric venation, lobes 8–13 mm long; corona lobes subulate ..... *D. hamatus*
9. Corona lobes oblong with spherical and roughened apex ..... 10
9. Corona lobes not as above ..... 12
10. Sepals more than 5 mm long; corona lobes as long as corolla tube ..... 11
10. Sepals less than 5 mm long; corona lobes longer than corolla tube ..... *D. suffruticosus*
11. Corolla lobes revolute, limbus and lobes slightly reflexed; grey-purple corolla in fresh with reticulated venation ..... *D. yucatanensis*
11. Corolla lobes not revolute, limbus and lobes patent or slightly ascending; yellowish-green corolla when fresh without obvious venation ..... *D. aeneus*
12. Corona lobes triangular or keeled, less than 2 mm long ..... 13
12. Corona lobes linear or linear-spathulate, more than 4.5 mm long ..... 15
13. Plant erect; leaf 1.3–3.4 cm long; pedicel 3–5 mm long ..... *D. parviflorus*
13. Plant a climber; leaf 2–8.7 cm long; pedicel (6–)9–28 mm long ..... 14
14. Corolla rotate, adaxially without lobes at the base; inflorescences with peduncles 4–6 cm long ..... *D. altatensis*
14. Corolla reflexed, adaxially 10-lobed at base; inflorescences with peduncles 3–4 mm long ..... *D. reflexiflorus*
15. Limb of the corolla with concentric venation ..... 16
15. Limb of the corolla with irregular concentric-reticulate venation ..... 17

16. Corolla tube with concentric venation; corona lobes linear lobes ..... *D. tigrinus*
16. Corolla tube with parallel vertical venation; corona lobes linear-spatulate ..... *D. eximus*
17. Corona lobes 5–8 mm long; style-head concave above ..... *D. reticulatus*
17. Corona lobes 8–11 mm long; style-head apiculate above ..... *D. ceratopetalus*


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**APPENDIX 1.** Sequences from GenBank used in this study, including taxa and GenBank accession numbers. Accession numbers that include the *trn*L intron and the *trn*L-*trn*F spacer are indicated with an asterisk.

| Species | *rps*16 | *trn*L intron | *trn*L-*trn*F spacer |
|---|---|---|---|
| ***Pentacyphinae*** | | | |
| *Pentacyphus andinus* | AJ699335 | AJ492151 | AJ492150 |
| *Pentacyphus lehmannii* | AJ704928 | AJ290888 | AJ290889 |
| *Anemotrochus eggersii* | HF547187 | HF547131* | |
| ***Diplolepinae*** | | | |
| *Diplolepis boerhaviifolia* | AJ699331 | AJ428608 | AJ428607 |
| *Diplolepis geminiflora* | AJ699332 | AJ410183 | AJ410182 |
| *Diplolepis hieronymi* | AJ699333 | AJ410213 | AJ410212 |
| *Diplolepis menziesii* | AJ699276 | AJ699275 | AJ699273 |
| *Diplolepis nummulariifolia* | AJ699334 | AJ290851 | AJ290852 |
| ***Orthosiinae*** | | | |
| *Jobinia formosa* | AJ699346 | AJ428641 | AJ428640 |
| *Jobinia hatschbachii* | HE611842 | HE611802 | HE611763 |
| *Jobinia umbellata* | AJ704319 | AJ704318 | AJ704316 |
| *Monsanima morrenioides* | AJ699348 | AJ428686 | AJ428685 |
| *Orthosia ellemannii* | AJ699350 | HE611812 | HE611773 |
| *Orthosia kunthii* | HE611857 | HE611813 | HE611774 |
| *Orthosia scoparia* | HE611862 | AY163703* | |
| *Scyphostelma beckii* | AJ704304 | AJ704306 | AJ704307 |
| *Scyphostelma bifidum* | HE611873 | HE611829 | HE611790 |
| *Scyphostelma ecuadorense* | HE611874 | HE611830 | HE611791 |
| *Scyphostelma harlingii* | AJ704311 | AJ704310 | AJ704308 |
| *Scyphostelma microphyllum* | AJ699347 | HE611831 | HE611792 |
| ***Metastelmatinae*** | | | |
| *Barjonia chloraeifolia* | AJ704463 | AY163667* | |
| *Barjonia glazioui* | JN574479 | JN574618* | |
| *Barjonia laxa* | JN574481 | JN574620* | |
| *Blepharodon ampliflorum* | JN574482 | JN574620* | |
| *Blepharodon bicuspidatum* | JN574483 | JN574621* | |
| *Blepharodon glaucescens* | AJ699292 | AJ699291 | AJ699289 |
| *Blepharodon grandiflorum* | AJ699337 | AJ290838 | AJ290837 |
| *Blepharodon lineare* | AJ704466 | AY163668* | |
| *Blepharodon mucronatum* | AJ699338 | AJ290839 | AJ290840 |
| *Blepharodon pictum* | AJ704467 | AY163669* | |
| *Ditassa banksii* | AJ704473 | AY163674* | |
| *Ditassa burchellii* | AJ699293 | AJ699295 | AJ699296 |

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**APPENDIX 1.** (Continued)

| Species | *rps*16 | *trn*L intron | *trn*L-*trn*F spacer |
|---|---|---|---|
| *Ditassa decussata* | AJ704217 | AJ704219 | AJ704220 |
| *Ditassa ditassoides* | AJ704476 | AY163678* | |
| *Ditassa endoleuca* | HE611840 | HE611801 | HE611762 |
| *Ditassa hispida* | AJ704477 | AJ704480 | AJ704478 |
| *Ditassa micromeria* | AJ704225 | AJ704237 | AJ704248 |
| *Ditassa mucronata* | AJ704279 | AJ704278 | AJ704259 |
| *Ditassa niruri* | AJ699340 | AJ428752 | AJ428751 |
| *Ditassa retusa* | AJ704280 | AJ704282 | AJ704283 |
| *Ditassa tomentosa* | AJ704483 | AJ704486 | AJ704484 |
| *Gonioanthela hilariana* | AJ704488 | AY163688* | |
| *Hemipogon acerosus* | AJ704288 | AJ704290 | AJ704291 |
| *Hemipogon andinum* | AJ704295 | AJ704294 | AJ704292 |
| *Hemipogon hatschbachii* | JN574498 | JN574637* | |
| *Hemipogon sprucei* | AJ704296 | AJ704298 | AJ704299 |
| *Metastelma fiebrigii* | HF547188 | HF547134 | |
| *Metastelma latifolium* | HF547190 | HF547137* | |
| *Metastelma linearifolium* | AJ699341 | AJ428809 | AJ428808 |
| *Metastelma oranense* | HF547197 | HF547145* | |
| *Metastelma palmeri* | HF547199 | HF547148* | |
| *Metastelma schaffneri* | AJ699343 | AJ410216 | AJ410215 |
| *Metastelma schlechtendalii* | HF547207 | HF547156* | |
| *Metastelma tubatum* | HF547216 | HF547164* | |
| *Minaria acerosa* | AJ699285 | AJ699287 | AJ699288 |
| *Minaria cordata* | AJ699300 | AJ699299 | AJ699297 |
| *Minaria decussata* | DQ026707 | AY163677* | |
| *Minaria grazielae* | AJ699339 | AJ410204 | AJ410203 |
| *Nautonia nummularia* | AJ699344 | AJ410228 | AJ410227 |
| *Nephradenia acerosa* | AJ704496 | AY163705 | AY163704 |
| *Nephradenia asparagoides* | AJ704499 | AY163707 | AY163706 |
| *Peplonia asteria* | AJ704303 | AJ704302 | AJ704300 |
| *Petalostelma martianum* | JN574509 | JN574648* | |
| *Petalostelma sarcostemma* | AJ699345 | AJ428788 | AJ428787 |
| ***Tassadiinae*** | | | |
| *Tassadia berteroana* | AJ699336 | AJ428791 | AJ428790 |
| *Tassadia guanchezii* | LN555752 | LN555748* | |
| *Tassadia obovata* | AJ699284 | AY163724 | AY163723 |
| *Tassadia richardiana* | HF547198 | HF547146* | |
| ***Oxypetalinae*** | | | |
| *Araujia angustifolia* | AJ704333 | AJ704332 | AJ704330 |
| *Araujia plumosa* | AJ704334 | AJ704336 | AJ704337 |
| *Araujia sericifera* | AJ699352 | AJ428794 | AJ428793 |
| *Funastrum angustifolium* | AJ699353 | AJ428761 | AJ428760 |
| *Funastrum odoratum* | AJ699356 | AJ290869 | AJ290870 |
| *Morrenia odorata* | AJ704342 | AJ704344 | AJ704345 |
| *Oxypetalum coccineum* | AJ704327 | AJ704326 | AJ704329 |

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**APPENDIX 1.** (Continued)

| Species | *rps*16 | *trn*L intron | *trn*L-*trn*F spacer |
|---|---|---|---|
| *Oxypetalum coeruleum* | AJ704357 | AJ704356 | AJ704354 |
| *Oxypetalum lanatum* | AJ704506 | AJ704508 | AJ704507 |
| *Oxypetalum minarum* | AJ704511 | AY163713* | |
| *Oxypetalum pannosum* | AJ704512 | AJ704514 | AJ704513 |
| *Oxypetalum sublanatum* | AJ704517 | AY163715* | |
| *Oxypetalum warmingii* | AJ704518 | AJ704520 | AJ704519 |
| *Philibertia boliviana* | AJ704230 | AJ704232 | AJ704233 |
| *Philibertia candolleana* | AJ699357 | AJ410177 | AJ410176 |
| *Philibertia discolor* | AJ704526 | AY163700* | |
| *Philibertia fontellae* | AJ699358 | AJ492154 | AJ492153 |
| *Philibertia gilliesii* | AJ699359 | AJ290899 | AJ290898 |
| *Philibertia globiflora* | AJ704238 | AJ704236 | AJ704234 |
| *Philibertia lysimachioides* | DQ026714 | AJ290900 | AJ290901 |
| *Philibertia multiflora* | AJ704246 | AJ704245 | AJ704243 |
| *Philibertia parviflora* | AJ699361 | AJ410225 | AJ410224 |
| *Philibertia vaileae* | AJ699362 | AJ290905 | AJ290904 |
| *Tweedia brunonis* | AJ704256 | AJ704258 | AJ704260 |
| ***Gonolobinae*** | | | |
| *Anemotrochus viridivenius* | LN901514 | LN901496* | |
| *Anemotrochus yamanigueyensis* | LN901526 | LN929899 | LN929898 |
| *Dictyanthus macvaughianus* | MH464890 | MH464896* | |
| *Dictyanthus parviflorus* | EU038871 | EU031617 | |
| *Dictyanthus pavonii* | MH464892 | MH464898* | |
| *Dictyanthus reticulatus* | EU038868 | EU031614 | |
| *Fischeria panamensis* | EU038832 | EU031577 | |
| *Fischeria scandens* | EU038833 | EU031578 | |
| *Gonolobus albomarginatus* | EU038834 | EU031579 | |
| *Gonolobus arizonicus* | EU038835 | EU031580 | |
| *Gonolobus barbatus* | AJ704264 | AJ704263 | AJ704261 |
| *Gonolobus bibarbatus* | EU038836 | EU031581 | |
| *Gonolobus breedlovei* | EU038837 | EU031582 | |
| *Gonolobus chloranthus* | EU038838 | EU031583 | |
| *Gonolobus croceus* | EU038839 | EU031584 | |
| *Gonolobus fraternus* | EU038840 | EU031585 | |
| *Gonolobus fuscoviolaceus* | EU038841 | EU031586 | |
| *Gonolobus gonoloboides* | EU038842 | EU031587 | |
| *Gonolobus grandiflorus* | EU038843 | EU031588 | |
| *Gonolobus iyanolensis* | EU038844 | EU031589 | |
| *Gonolobus jaliscensis* | EU038845 | EU031590 | |
| *Gonolobus jamaicensis* | EU038846 | EU031591 | |
| *Gonolobus niger* | EU038847 | EU031592 | |
| *Gonolobus ophioglossa* | LN901515 | LN901497* | |
| *Gonolobus parviflorus* | DQ026712 | AY163689* | |
| *Gonolobus pectinatus* | EU038848 | EU031593 | |
| *Gonolobus rostratus* | EU038849 | EU031594 | |

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**APPENDIX 1.** (Continued)

| Species | *rps*16 | *trn*L intron | *trn*L-*trn*F spacer |
|---|---|---|---|
| *Gonolobus stapelioides* | EU038850 | EU031595 | |
| *Gonolobus stenosepalus* | EU038851 | EU031596 | |
| *Gonolobus stephanotrichus* | EU038852 | EU031597 | |
| *Gonolobus suberosus* | EU038854 | AJ704276 | AJ704277 |
| *Gonolobus uniflorus* | EU038855 | EU031601 | |
| *Gonolobus waitukubuliensis* | EU038856 | EU031602 | |
| *Gonolobus youroumaynensis* | EU038857 | EU031603 | |
| *Ibatia maritima* | EU038875 | EU031621 | |
| *Ibatia rubra* | EU038885 | EU031632 | |
| *Macroscepis diademata* | AJ704265 | AJ704267 | AJ704268 |
| *Matelea acuminata* | EU038858 | EU031604 | |
| *Matelea angustiloba* | EU038859 | EU031605 | |
| *Matelea bicolor* | EU038860 | LN901498* | |
| *Matelea biflora* | KF539850 | KY582366 | |
| *Matelea chrysantha* | EU038861 | EU031607 | |
| *Matelea correllii* | EU038864 | EU031610 | |
| *Matelea cyclophylla* | EU038865 | AJ704272 | AJ704269 |
| *Matelea cynanchoides* | KY449041 | KY582368 | |
| *Matelea denticulata* | EU038867 | EU031613 | |
| *Matelea ekmanii* | EU038869 | EU031615 | |
| *Matelea inconspicua* | EU038872 | EU031618 | |
| *Matelea lanata* | EU038873 | EU031619 | |
| *Matelea lanceolata* | EU038874 | EU031620 | |
| *Matelea nipensis* | LN901517 | LN901543 | LN901550 |
| *Matelea oblongata* | EU038877 | EU031623 | |
| *Matelea parviflora* | KY449038 | KY582371 | |
| *Matelea pauciflora* | EU038878 | EU031625 | |
| *Matelea pedalis* | AJ704530 | AY163699* | |
| *Matelea phainops* | EU038879 | EU031626 | |
| *Matelea producta* | KY449036 | KY582365 | |
| *Matelea prosthecidiscus* | EU038880 | EU031627 | |
| *Matelea pubiflora* | EU038881 | EU031628 | |
| *Matelea pusilliflora* | EU038882 | EU031629 | |
| *Matelea reticulata* | EU038884 | EU031631 | |
| *Matelea sagittifolia* | KY449037 | KY582372 | |
| *Matelea sintenisii* | EU038886 | EU031633 | |
| *Matelea tamnifolia* | EU038887 | LN901499* | |
| *Matelea texensis* | KY449039 | KY582376 | |
| *Matelea trachyantha* | EU038888 | EU031635 | |
| *Matelea variifolia* | EU038889 | EU031636 | |
| *Polystemma canisferum* | KY449035 | KY582364 | |
| *Polystemma cordatum* | EU038862 | EU031608 | |
| *Polystemma cordifolium* | EU038863 | EU031609 | |
| *Polystemma guatemalense* | KY449043 | KY582353 | |
| *Polystemma viridiflorum* | EU038866 | EU031612 | |

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**APPENDIX 1.** (Continued)

| Species | *rps*16 | *trn*L intron | *trn*L-*trn*F spacer |
|---|---|---|---|
| *Schubertia grandiflora* | AJ699364 | AJ428827 | AJ428826 |
| *Tylodontia fuscula* | LN901520 | LN901544 | LN901551 |
| *Tylodontia urceolata* | LN901521 | LN901545 | LN901552 |
| *Urostephanus gonoloboides* | EU038870 | EU031616 | |
| *Urostephanus patalensis* | MH464894 | | |

**APPENDIX 2.** Information (taxon, collector, and coll. number (herbarium)) for specimens used in the distribution map.

***Dictyanthus asper* (Mill.) W.D. Stevens:** *J. Rzedowski 36889a* (ENCB), *J.C. Soto N. 982* (MEXU, MO, TEX), *J.C. Soto N. 3491* (MEXU, MO); ***Dictyanthus macvaughianus* (W.D. Stevens) W.D. Stevens:** *J.N. Labat 321* (P), *J.N. Labat 1749* (P), *R. McVaugh 24934* (MICH), *E. Pérez C. & E. García L.* 1359 (MEXU); ***Dictyanthus parviflorus* Hemsl.:** *R.L. Oliver et al. 865* (MO), *G. Arsene s.n.* (G); ***Dictyanthus pavonii* Decne.:** *C.P. Cowan 5686* (MO, TEX), *H.G. Galeotti 1394* (MO), *G.B. Hinton 13275* (LL, MO), *G.B. Hinton 15061* (LL, MO), *A.L. Mirón M. & G. Cornejo T. 369* (MEXU, MO), *J.C. Soto N. et al. 9393* (MEXU).