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Gymnopodium toledense (Polygonaceae), a new species from Belize resolved by morphology and distance analyses of molecular data

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Abstract: *Gymnopodium floribundum* Rolfe (Polygonaceae) is a shrub or a small tree inhabiting the tropical dry forests of Mesoamerica. A detailed examination of herbarium specimens for the taxonomic treatment of the genus for *Flora Mesoamericana* allowed us to find a gathering from Toledo, Belize that showed a different set of morphological characters from those of *G. floribundum*. We then carried out a morphological and molecular study to test if the Toledo plant was a different taxon. Morphological characters such as glandular trichomes on leaves, inflorescences and flowers, as well as longer inflorescences and perianth segments, are unique for the Toledo plant. The delimitation between it and *G. floribundum* was also supported by molecular characters obtained from ITS and LFY nuclear markers. The Toledo plant is described here as a new species, *G. toledense* Ancona & Ortiz-Díaz, based on diagnostic characters of morphology and the ITS and LFY markers. It is endemic to southern Belize, in the biogeographic region of Eastern Central America, and is assessed according to IUCN categories and criteria as Critically Endangered. A morphological diagnosis and description, key, illustration and distribution map for *G. toledense* are included.

Key words: Belize, Central America, endemism, *Flora Mesoamericana*, *Gymnopodium*, ITS, LFY, new species, Polygonaceae

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Introduction

Gymnopodium floribundum Rolfe is an important component of the seasonal forests and savannahs of southern Mexico, Guatemala and Belize from sea level up to 1000 m (Miranda 1952; Flores & Espejel 1994; Goodwin & al. 2013). Along its distribution area, *G. floribundum* exhibits variation in shape, size and degree of pubescence of leaf blades, inflorescences, flowers and fruits, and hence

two varieties have been described. However, Ortiz-Díaz (1994; unpublished data) recognized only one highly polymorphic species. Phylogenetic studies in the subfamily *Eriogonoideae* confirm the monophyly of *Gymnopodium* Rolfe and place it as a sister group of the tribe *Eriogoneae* (Burke & al. 2010; Burke & Sánchez 2011).

During the review of herbarium specimens for the taxonomic treatment of *Gymnopodium* for the *Flora Mesoamericana* project, a population from Toledo in the

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south of Belize, represented by a gathering made in 1997, showed a set of characters in leaf, inflorescence, flower and fruit different from those described for *G. floribundum*; however, only three specimens of the same population were available. Due to the limited collections, we wanted to look for new characters to support the morphological ones, so we carried out a molecular study using the nuclear markers *ITS1*, *5.8S* and *ITS2* as well as *lfy* (exon2 and intron 2). The *ITS1*, *5.8S* and *ITS2* markers have been used in the last two decades as excellent barcodes for several groups of angiosperms because of their short length and easy amplification (Hoot & Taylor 2001; Lee & al. 2002; Kress & al. 2005; Cowan & al. 2006; Pang & al. 2011; CPBOL 2011; Liu & al. 2014; Michel & al. 2016; Nithaniyal & Parani 2016). Moreover, the *lfy* marker has also been used in phylogenetic studies and species delimitation (Hoot & Taylor 2001), particularly in *Polygonaceae* (Schuster & al. 2011; Sánchez & Kron 2011). This work describes *G. toledense* Ancona & Ortiz-Díaz as a new species using an integrative approach to include diagnostic characters of morphology and multiple DNA loci.

Material and methods

Morphological studies — The present study was based on a morphological and biometrical analyses carried out on material collected by two of the authors and on material preserved in the herbaria BM, CHIP, CICY, HEM, MEXU, MO and UADY (herbarium codes according to Thiers 2018+). All the studied material was compared and measured to the nearest 0.1 mm using the Absolute Digimatic Mitutoyo digital calliper.

Plant selection for molecular studies — Based on the distribution and morphological variation, 29 individuals representing eight populations of *Gymnopodium* were collected by the first author in Mexico. Two further samples of *Gymnopodium* from Belize were obtained from herbarium collections (in MO) to complete the sampling. Unfortunately, we could not find any individuals of *G. toledense* during our field trip in 2016 to re-locate the Toledo population because the landscape there has been drastically transformed since 1997. *Coccoloba uvifera* (L.) L. and *Podopterus mexicanus* Bonpl. were selected as outgroups according to Burke & al. (2010). All Mexican specimens were deposited in the herbarium UADY. For further details see Table 1. Foliar tissue from new leaves was collected from three or four individuals at each population and maintained in silica gel to transport it to the Biodiversity and Ecophysiology Laboratory of the Universidad Autónoma de Yucatán. Then, in the lab, we proceeded to extract and isolated DNA from each sample.

DNA isolation, amplification, sequencing — DNA was purified from 50 mg of foliar tissue of three or four individu-

als of each Mexican population and from each herbarium specimen from Belizean populations (Table 1) using the DNeasy Plant mini Kit (QIAGEN). Genomic DNA was amplified for *ITS1*, *5.8S* and *ITS2*, using *ITS5* (GGAAGTAAAAGTCGTAACAAGG) and *ITS4* (TCCTCCGCTTATTGATATGC) primers from White & al. (1990); and *lfy* (exon2 and intron 2) using *lfy2i1* (CCTGCCGACATANTGGCGCATCTTGGGCTT) and *lfy23* (TGCAAGGGTAAGAAGAACGGCCTTGA) primers from Sánchez & Kron (2011). All amplifications were conducted in a 10 µl reaction using the standardized protocol of Sánchez & Kron (2009). Amplified fragments were visualized in 0.8 % agarose gels. Bands with the expected size for the amplicons were purified with the Pure Link® Quick Gel PCR Purification Combo Kit for Sanger sequencing in MacroGen Inc., South Korea. Homology of the sequences was tested using the sequence from Ancona & Ortiz 186 in BLAST using the module megaBLAST search (Morgulis & al. 2008) from NCBI and confirmed with the retrieval of sequences of the same region from *Gymnopodium floribundum* and other *Polygonaceae* species mostly of the genera *Ruprechtia* C. A. Mey. and *Triplaris* Loefl.

Sequences for *ITS1*, *5.8S* and *ITS2* did not include ambiguous nucleotide calls, suggesting that each individual is homozygous for this locus. All *lfy* (exon2 and intron 2) sequences did include ambiguous nucleotide calls for several sites, suggesting that each individual is heterozygous for this locus. Alleles per individual were inferred using DNAsp v.6 (Rozas & al. 2017).

Phylogenetic analyses — The nucleotide data matrix for *ITS1*, *5.8S* and *ITS2* contained 32 sequences, as did that for *lfy* (exon2 and intron 2). For each region, the alignment was obtained using ClustalW algorithm (Thompson & al. 1994), as implemented in MEGA v 6.0 (Tamura & al. 2013), and then visually checked.

To delimit this new species, we employed the phylogenetic species concept, which defines a species as: “the smallest aggregation of (sexual) populations or (asexual) lineages diagnosable by a unique combination of character states” (Wheeler & Platnick 2000). The distance approach was used to infer if *Gymnopodium floribundum* and *G. toledense* aggregate as unique or separate entities (monophyletic groups) in the neighbour-joining trees obtained for each one of the regions. The neighbour-joining trees was computed by the maximum composite likelihood method (Tamura & al. 2004), and clustering of the branches were tested using 500 bootstrap replications (Felsenstein 1985), as implemented in MEGA v 6.0 (Tamura & al. 2013). For the final analyses, all positions containing gaps and missing data were excluded.

Distribution map — The distribution map of *Gymnopodium floribundum* and *G. toledense* was made using the SimpleMappr program (Shorthouse 2010) and geographic coordinates of herbarium specimens databased in Tropicos (<http://www.tropicos.org>).

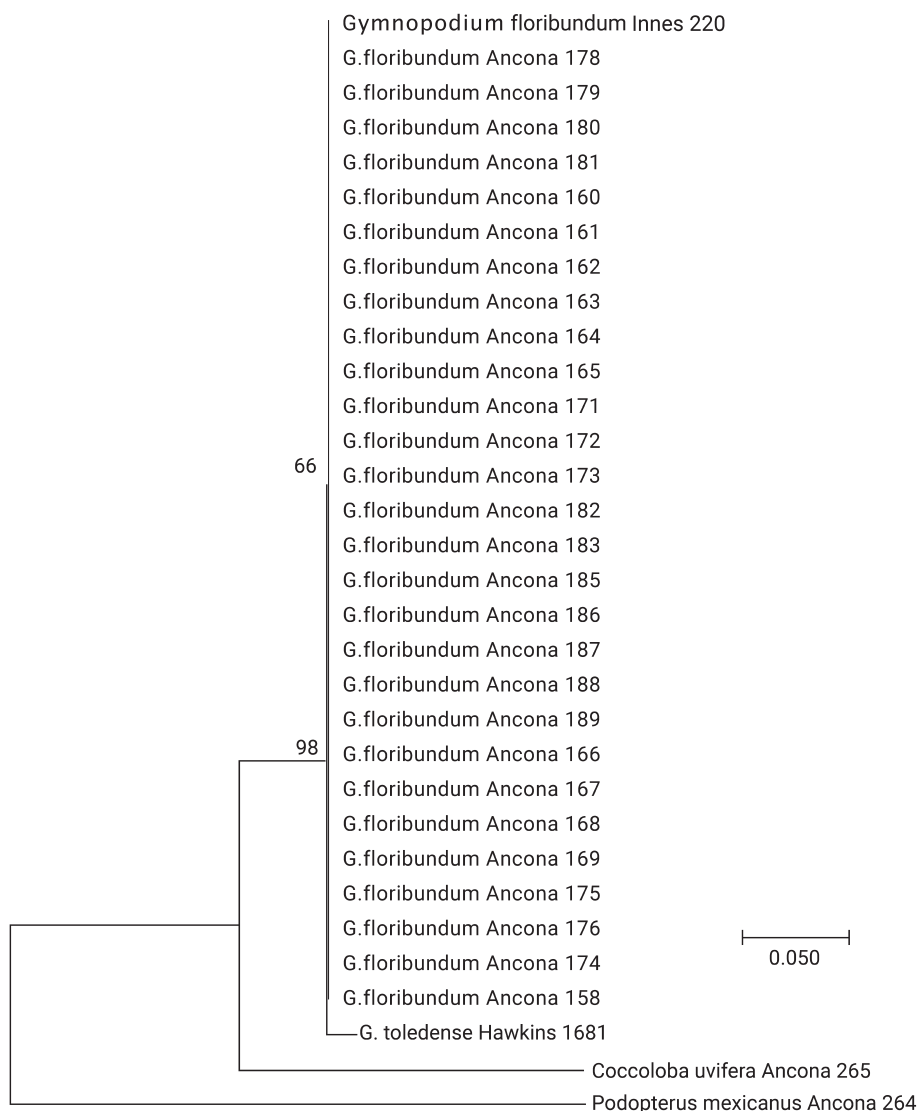


Fig. 1. Neighbour-joining optimal tree using *ITS1*, *5.8S* and *ITS2* sequences; percentage of 500 replicates that subtended nodes are shown next to branches; collector and number refer to specimen voucher column in Table 1.

Results and Discussion

The diagnostic morphological characters between *Gymnopodium floribundum* and *G. toledense* are shown in Table 2. *Gymnopodium toledense* differs from *G. floribundum* by, e.g., its sparse or dense glandular trichomes on the leaves, inflorescences and flowers, and its longer inflorescences and perianth segments. All the *G. floribundum* specimens examined lack the basal gland on the trichomes, and their racemes and perianth segments are shorter than those of *G. toledense* (see also Ortiz-Díaz 1994).

The total length of the *ITS1*, *5.8S* and *ITS2* sequence was 745 sites including gaps and for the *lfy* sequence 562 sites including gaps. The sequence from Ancona & Ortiz 186 obtained from the BLAST search for *ITS1*, *5.8S* and

ITS2 has a 98 % identity to the sequence of the same region from *Gymnopodium floribundum* (GB GQ206251). The *lfy* (exon2 and intron 2) sequence from Ancona & Ortiz 186 has a 92 % identity to sequences of the same region from *G. floribundum* (GB HQ693138.1), and included sequences of the same region from *Coccoloba swartzii* Meisn. (GB EF442787.1) and *Gilmania luteola* (Coville) Coville (GB EF438069.1).

The neighbour-joining trees built with molecular data show *Gymnopodium toledense* separated from *G. floribundum* (Fig. 1 & 2). The sum of the branch lengths was 0.60421319 for the *ITS1*, *5.8S* and *ITS2* and 4.68968630 for the *lfy* (exon2 and intron 2) trees. The bootstrap support values for the nodes grouping *G. toledense* and *G. floribundum* were 98 % for *ITS1*, *5.8S* and *ITS2* and 97 % for *lfy* (exon2 and intron 2). The bootstrap support values for the nodes connecting the *G. floribundum* terminal branches were 66 % for *ITS1*, *5.8S* and *ITS2* and 38 % for *lfy* (exon2 and intron 2). The molecular data of *ITS1*, *5.8S* and *ITS2* and *lfy* (exon2 and intron 2) markers provided numerous substitutions to delimit both species.

The neighbour-joining trees showed *G. toledense* as the sister species of *G. floribundum* (Fig. 1 & 2). The *ITS1*, *5.8S* and *ITS2* and *lfy* (exon2 and intron 2) markers have been successfully used to infer relationships between species of *Ruprechtia* and *Triplaris* (Sánchez & Kron 2011) and of *Fallopia* Adans., *Muehlenbeckia* Meisn. and *Reynoutria* Houtt. (Schuster & al. 2011).

The morphological and molecular characters allowed us to clearly define the boundaries between *Gymnopodium floribundum* and *G. toledense*. Such evidence supports the proposal of *G. toledense* as a new species. Moreover, both phylogenetic trees built with the *ITS1*, *5.8S* and *ITS2* and *lfy* (exon2 and intron 2) markers suggest that *G. floribundum* is a polymorphic taxon. Further studies including morphometry and chloroplast markers

could provide new evidence to confirm this hypothesis.

Taxonomic treatment

Gymnopodium toledense Ancona & Ortiz-Díaz, **sp. nov.** – Fig. 3.

Holotype: Belize, Toledo, Las Sierritas, 20 km W of Big Creek Settlement, ridge and W slopes of Cerrito in Las Sierritas hills, 16°31'45"N, 88°36'05"W, 160–213 m, ridge-top vegetation of mixed hardwood species growing on thin soils over exposed limestone, vegetation severely damaged by recurrent fires, 6 Dec 1997, *T. Hawkins 1681* (MO [barcode MO-321695 accession no. 04950838]; isotypes: BM [barcode BM000565699], MEXU [catalogue no. 898235]).

Morphological diagnosis — *Gymnopodium toledense* differs from *G. floribundum* by bearing trichomes with basal glands on the petioles, leaf blades, inflorescence rachis, pedicels and ovary, prominent veins on the abaxial surface of the leaf blade, and the basal segment of the pedicel hidden by the ochreole (see also Table 2).

Morphological description — *Shrubs* often scrambling, 2–4 m tall; *bark* grey to dark brown, fissured; *young branches* divaricate, flexuous, grey to pale brown; *internodes* 2–3 cm long; *ochrea* deciduous, annular, c. 1 mm long, sparsely pubescent, trichomes with basal glands. *Leaves* alternate, arising from ochrea, fasciculate (2 or 3 together) on small vegetative shoots (brachyblasts), simple; *petiole* 1.5–2 × c. 1 mm, canaliculate, densely pubescent, trichomes dark brown, 0.3–0.5(–7) mm long with basal glands; *leaf blade* obovate to obpyriform, 5–7 × 3–4 cm, chartaceous, base obtuse, margin entire, apex obtuse to slightly emarginate; veins prominent abaxially; abaxial surface of leaf blade densely pubescent, trichomes 0.3–0.5 mm long with basal glands, adaxial surface glabrous except puberulent on midvein. *Inflorescence* terminal; *racemes* single or paired, on brachyblasts, 12–20 cm long; *rachis* 15–18 cm long, densely pilose, trichomes 0.3–0.5(–0.7) mm long with light yel-

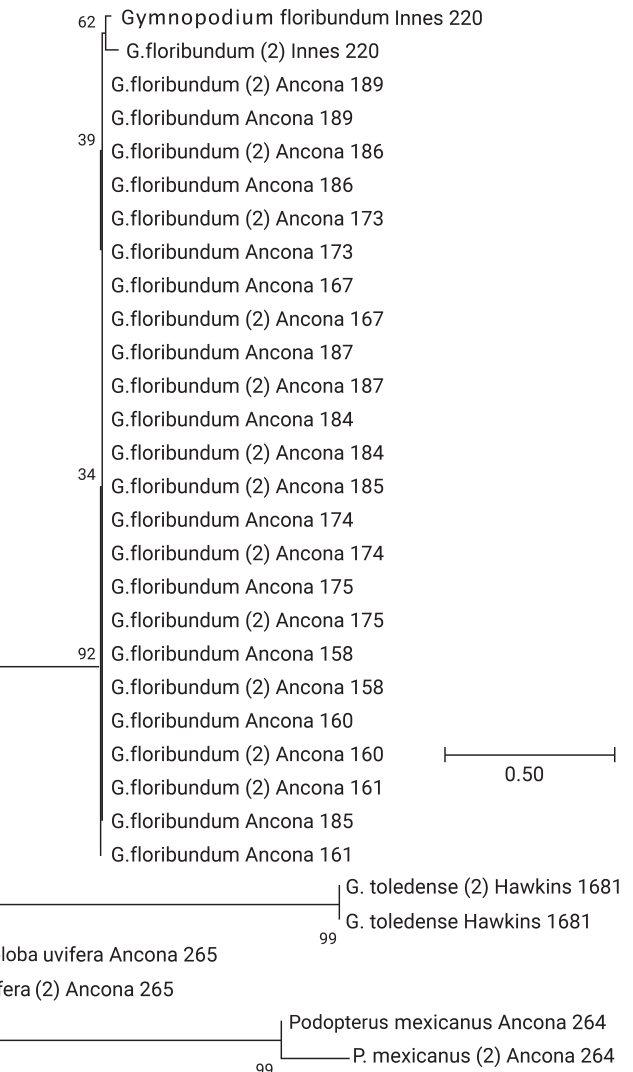


Fig. 2. Neighbour-joining optimal tree using *lfy* (exon2 and intron 2) sequences; percentage of 500 replicates that subtended nodes are shown next to branches; collector and number refer to specimen voucher column in Table 1; “(2)” indicates second allele.

low basal glands; *flowers* in fascicles of 2–4(–6); *ochreoles* lanceolate, 1–2 mm long, membranous, sparsely to densely pubescent, trichomes with basal glands; *pedicels* articulated below middle, basal segment 0.5–1 mm long, hidden by ochreole, distal segment 5–6.5(–8) mm long, densely pubescent, trichomes 0.3–0.5(–8) mm long with basal glands. *Flowers* hermaphrodite; *perianth segments* 6, 3 outer and 3 inner; *outer segments* greenish yellowish, ovate-orbicular to cordate, 8.5–9(–9.5) × 6–6.5(–7) mm, papery, sparsely to densely pubescent when young, glabrous to pubescent when mature, persistent and accrescent in fruit, reticulate; *inner segments* subulate-lanceolate, smaller than outer ones, (5–)5.5–6 × 1.5–1.8(–2) mm, papery, glabrous when young to scarcely pubescent when mature, apex long acuminate; *stamens* 6, filiform, outer 3 inserted into a basal disk, inner 3 arising opposite ovary grooves; *filaments* c. 2 mm long; *anthers* versatile, suborbicular, 0.5–0.7 mm long, bilocular, dehiscence longitudinal. *Ovary* superior, sessile, trigonous, compressed, to 1

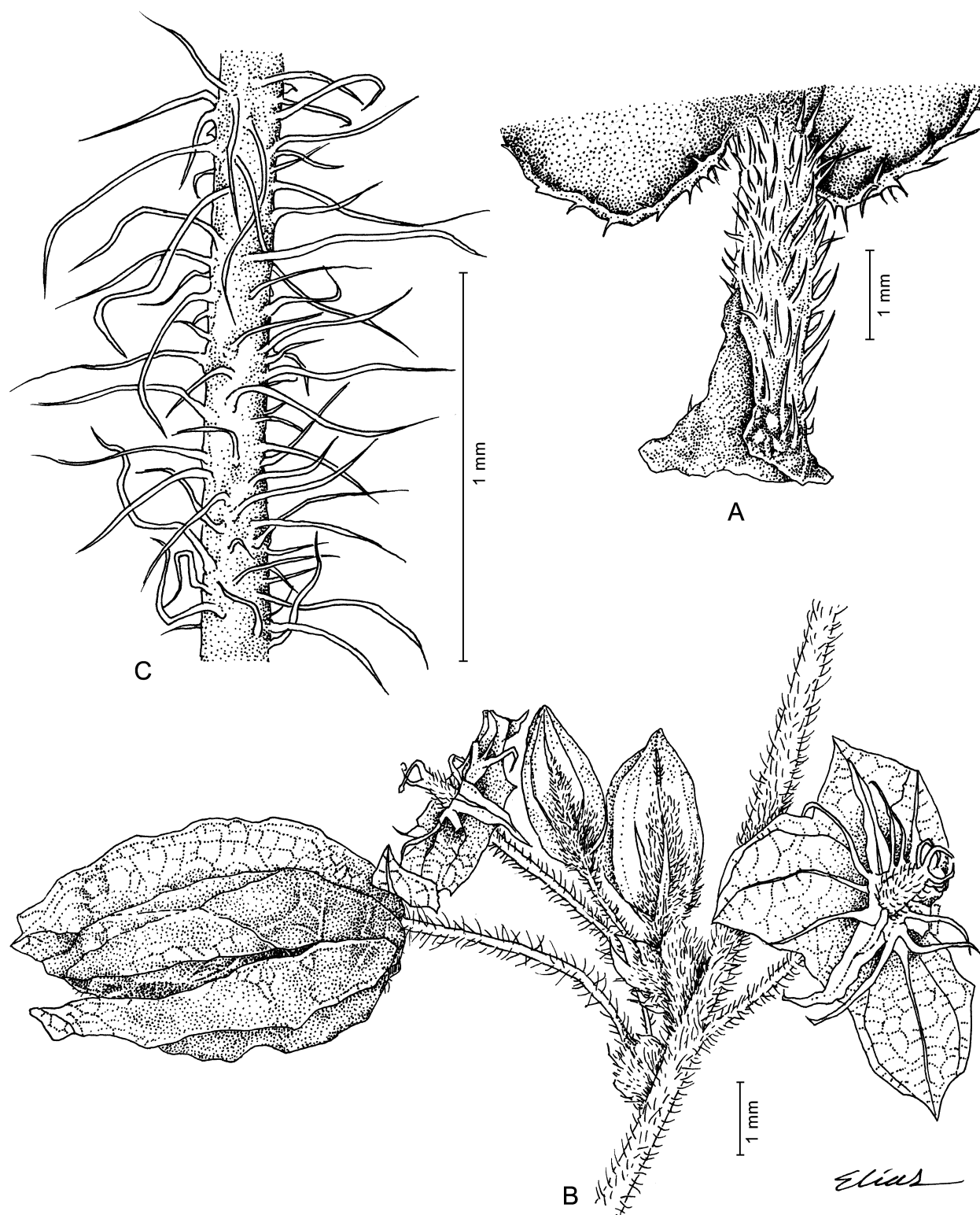


Fig. 3. *Gymnopodium toledense* – A: petiole and basal portion of leaf blade; B: young and mature flowers, showing rachis, outer and inner perianth segments and pubescent ovary; C: detail of pedicel, showing trichomes with basal glands. – Drawn by Jesús Elías García López based on the holotype.

× c. 0.5 mm, unilocular, densely pubescent at vertices, trichomes with basal glands; styles 3, filiform, 1.5–1.7 mm long; stigmas 3, capitate. Fruit an achene, light brown, lustrous, trigonous, 5(–5.5) × 2(–2.5) mm, smooth, included in perianth segments; seed 1.

Distribution and ecology — *Gymnopodium toledense* is so far known as an endemic species of the seasonal forests of southern Belize (Fig. 4), in the biogeographic region of Eastern Central America. It could possibly be found also in Guatemala and Honduras.

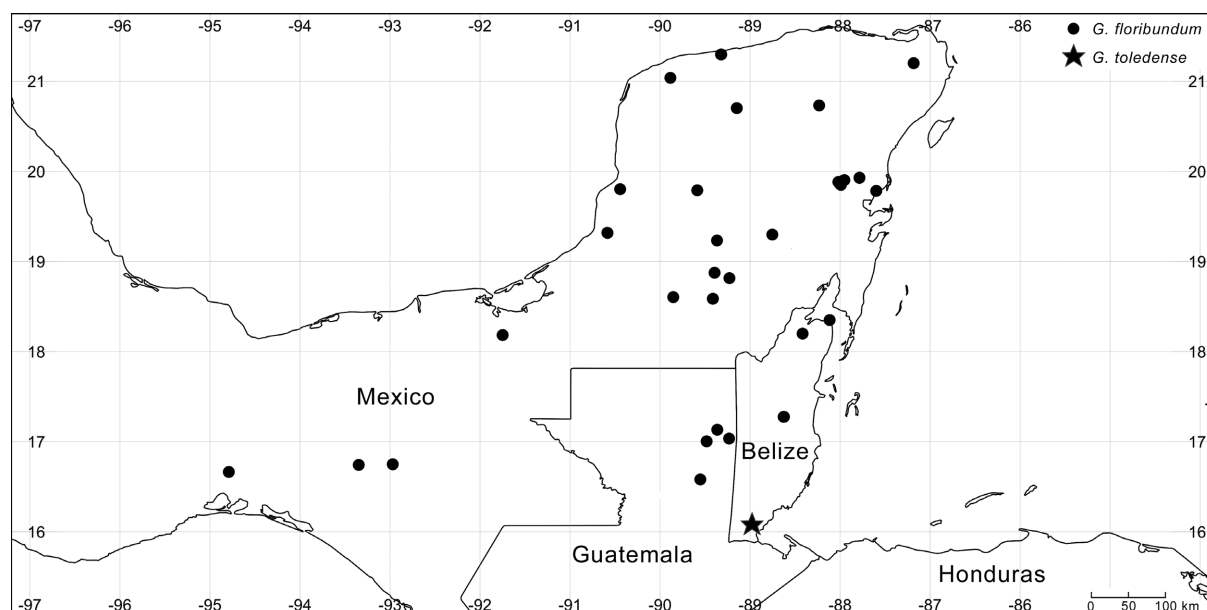


Fig. 4. Distribution map of *Gymnopodium floribundum* (●) and *G. toledense* (★).

Conservation status — *Gymnopodium toledense* is currently known only of the population last recorded in December 1997 at Las Sierritas in the Toledo district of southern Belize. Our efforts to find the population during our field work in 2016 were not successful owing to the landscape having undergone a drastic transformation since 1997, and indeed the field notes of the type gathering included “vegetation severely damaged by recurrent fires”. Therefore, the new species meets the following IUCN (2012) criteria for the category Critically Endangered (CR): B1 = Extent of Occurrence estimated to be less than 100 km²; a = severely fragmented or known to exist at only a single location; and b(iii) = continuing decline, observed, inferred or projected, in area, extent and/or quality of habitat; i.e. CR B1ab(iii). Further exploration is needed through the seasonal forests of southern Belize and adjacent areas of Guatemala and Honduras to assess the frequency and status of any extant subpopulations.

Etymology — The specific epithet refers to the area where this species was collected, Toledo district, Belize.

Key to the species of *Gymnopodium*

1. Leaves, inflorescence rachises and pedicels glabrous or covered with sparse to dense simple trichomes without basal glands; veins not prominent on abaxial surface of leaf blade; pedicel basal segment not hidden by ochreole; outer perianth segments 6.5–8 × 5–6.5(–7) mm; inner perianth segments (4–)4.5(–5) × 1–1.5 mm; fruit 4–4.5(–5) × 1.5(–2) mm *G. floribundum*
- Leaves, inflorescence rachises and pedicels covered with sparse to dense simple trichomes with basal

glands; veins prominent on abaxial surface of leaf blade; pedicel basal segment hidden by ochreole; outer perianth segments 8.5–9(–9.5) × 6–6.5(–7) mm; inner perianth segments (5–)5.5–6 × 1.5–1.8 mm; fruit 5(–5.5) × 2(–2.5) mm *G. toledense*

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References

- Burke J. M. & Sánchez A. 2011: Revised subfamily for *Polygonaceae*, with a tribal classification for *Eriogonoideae*. – *Brittonia* **63**: 510–520.
- Burke J. M., Sánchez A., Kron K. A. & Luckow M. 2010: Placing the woody tropical genera of *Polygonaceae*: a hypothesis of character evolution and phylogeny. – *Amer. J. Bot.* **97**: 1377–1390.
- Cowan R. S., Chase M. W., Kress W. J. & Savolainen V. 2006: 300,000 species to identify: problems, progress, and prospects in DNA barcoding of land plants. – *Taxon* **55**: 611–616.
- CPBOL 2011: Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should

- be incorporated into the core barcode for seed plants. – *Proc. Natl. Acad. Sci. U.S.A.* **108**: 19641–19646.
- Felsenstein J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. – *Evolution* **39**: 783–791.
- Flores J. & Espejel I. 1994: Tipos de vegetación de la península de Yucatán. – *Etnoflora Yucatanense* **3**. – Mérida: Universidad Autónoma de Yucatán.
- Goodwin Z. A., Lopez G. N., Stuart N., Bridgewater S. G., Haston E. M., Cameron I. D., Michelakis D., Ratter J. A., Furley P. A., Kay E. & Whiteford C. 2013: A checklist of the vascular plants of the lowland savannas of Belize, Central America. – *Phytotaxa* **101**: 1–119.
- Hoot S. B. & Taylor W. C. 2001: The utility of nuclear ITS, a LEAFY homolog intron, and chloroplast *atpB-rbcL* spacer region data in phylogenetic analyses and species delimitation in *Isoetes*. – *Amer. Fern J.* **91**: 166–177.
- IUCN 2012: IUCN Red List categories and criteria: version 3.1, ed. 2. – Gland & Cambridge: IUCN.
- Kress W. J., Wurdack K. J., Zimmer E. A., Weigt L. A. & Janzen D. H. 2005: Use of DNA barcodes to identify flowering plants. – *Proc. Natl. Acad. Sci. U.S.A.* **102**: 8369–8374.
- Lee J., Baldwin B. G. & Gottlieb L. D. 2002: Phylogeny of *Stephanomeria* and related genera (*Compositae*–*Lactuceae*) based on analysis of 18S–26S nuclear rDNA ITS and ETS sequences. – *Amer. J. Bot.* **89**: 160–168.
- Liu J.-X., Shi L.-C., Han J.-P., Li G., Lu H., Hou J.-Y., Zhou X.-T., Meng F.-Y. & Downie S. R. 2014: Identification of species in the angiosperm family *Apiaceae* using DNA barcodes. – *Molec. Ecol. Res.* **14**: 1231–1238.
- Michel C. I., Meyer R. S., Taveras Y. & Molina J. 2016: The nuclear internal transcribed spacer (ITS2) as a practical plant DNA barcode for herbal medicines. – *J. Appl. Res. Med. Aromat. Pl.* **3**: 94–100.
- Miranda F. 1952: La vegetación de Chiapas. – México: Ediciones del Gobierno de Chiapas.
- Morgulis A., Coulouris G., Raytselis Y., Madden T. L., Agarwala R. & Schäffer A. A. 2008: Database indexing for production MegaBLAST searches. – *Bioinformatics* **24**: 1757–1764.
- Nithaniyal S. & Parani M. 2016: Evaluation of chloroplast and nuclear DNA barcodes for species identification in *Terminalia* L. – *Biochem. Syst. Ecol.* **68**: 223–229.
- Ortiz-Díaz J. J. 1994: *Polygonaceae*. – *Etnoflora Yucatanense* **10**. – Mérida: Universidad Autónoma de Yucatán.
- Pang X.-H., Song J.-Y., Zhu Y.-J., Xu H.-X., Huang L.-F. & Chen S.-L. 2011: Applying plant DNA barcodes for *Rosaceae* species identification. – *Cladistics* **27**: 165–170.
- Rozas J., Ferrer-Mata A., Sánchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E., & Sánchez-Gracia A. 2017: DnaSP 6: DNA sequence polymorphism analysis of large data sets. – *Molec. Biol. Evol.* **34**: 3299–3302.
- Sánchez A. & Kron K. A. 2009: Phylogenetic relationships of *Afrobrunnichia* Hutch. & Dalziel (*Polygonaceae*) based on three chloroplast genes and ITS. – *Taxon* **58**: 781–792.
- Sánchez A. & Kron K. A. 2011: Phylogenetic relationships of *Triplaris* and *Ruprechtia*: re-delimitation of the recognized genera and two new genera for Tribe *Triplarideae* (*Polygonaceae*). – *Syst. Bot.* **36**: 702–710.
- Schuster T. M., Wilson K. L. & Kron K. A. 2011: Phylogenetic relationships of *Muehlenbeckia*, *Fallopia*, and *Reynoutria* (*Polygonaceae*) investigated with chloroplast and nuclear sequence data. – *Int. J. Pl. Sci.* **172**: 1053–1066.
- Shorthouse D. P. 2010: SimpleMappr, an online tool to produce publication-quality point maps. – Retrieved from <http://www.simplemappr.net> [accessed 12 Jun 2017].
- Tamura K., Nei M. & Kumar S. 2004: Prospects for inferring very large phylogenies by using the neighbour-joining method. – *Proc. Natl. Acad. Sci. U.S.A.* **101**: 11030–11035.
- Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013: MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. – *Molec. Biol. Evol.* **30**: 2725–2729.
- Thiers B. 2018+ [continuously updated]: Index herbariorum. A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. – Published at <http://sweetgum.nybg.org/science/ih/> [accessed 11 Nov 2018].
- Thompson J. D., Higgins D. G. & Gibson T. J. 1994: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. – *Nucl. Acids Res.* **22**: 4673–4680.
- Wheeler Q. D. & Platnick N. I. 2000: A defense of the phylogenetic species concept (sensu Wheeler and Plarnick). – Pp. 185–197 in: Wheeler Q. D. & Meier R. (ed.), *Species concept and phylogenetic theory: a debate*. – New York: Columbia University Press.
- White T. J., Bruns T., Lee S. & Taylor J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – Pp. 315–322 in: Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J. (ed.), *PCR protocols: a guide to methods and applications*. – San Diego: Academic Press.

Table 1. Taxa, localities, specimen vouchers, herbaria and GenBank accession numbers for the nuclear markers *ITS1*, 5.8S and *ITS2* and *lfy* (exon2 and intron 2).

Taxon	Country	State	Locality	Latitude, longitude	Specimen voucher	Herb.	ITS	LFY
<i>Gymnopodium floribundum</i>	Mexico	Yucatán	Dzemul	21°17'53.9"N, 89°19'25.5"W	Ancona & Ortiz 158	UADY	MK098142	MK097205, MK097206
<i>G. floribundum</i>	Mexico	Yucatán	Dzemul	21°17'53.9"N, 89°19'25.5"W	Ancona & Ortiz 160	UADY	MK098143	MK097207, MK097208
<i>G. floribundum</i>	Mexico	Yucatán	Dzemul	21°17'53.9"N, 89°19'25.5"W	Ancona & Ortiz 161	UADY	MK098144	MK097209, MK097210
<i>G. floribundum</i>	Mexico	Yucatán	Huhi	20°42'11.8"N, 89°08'54.8"W	Ancona & Ortiz 162	UADY	MK098145	
<i>G. floribundum</i>	Mexico	Yucatán	Huhi	20°42'11.8"N, 89°08'54.8"W	Ancona & Ortiz 163	UADY	MK098146	
<i>G. floribundum</i>	Mexico	Yucatán	Huhi	20°42'11.8"N, 89°08'54.8"W	Ancona & Ortiz 164	UADY	MK098147	
<i>G. floribundum</i>	Mexico	Yucatán	Huhi	20°42'11.8"N, 89°08'54.8"W	Ancona & Ortiz 165	UADY	MK098148	
<i>G. floribundum</i>	Mexico	Quintana Roo	Vigía Chico	19°55'54.9"N, 87°47'03.6"W	Ancona & Camara 166	UADY	MK098149	
<i>G. floribundum</i>	Mexico	Quintana Roo	Vigía Chico	19°55'54.9"N, 87°47'03.6"W	Ancona & Camara 167	UADY	MK098150	MK097211, MK097212
<i>G. floribundum</i>	Mexico	Quintana Roo	Vigía Chico	19°55'54.9"N, 87°47'03.6"W	Ancona & Camara 168	UADY	MK098151	
<i>G. floribundum</i>	Mexico	Quintana Roo	Vigía Chico	19°55'54.9"N, 87°47'03.6"W	Ancona & Camara 169	UADY	MK098152	
<i>G. floribundum</i>	Mexico	Campeche	Calakmul	18°52'31.6"N, 89°23'42.6"W	Ancona & Ortiz 171	UADY	MK098153	
<i>G. floribundum</i>	Mexico	Campeche	Calakmul	18°52'31.6"N, 89°23'42.6"W	Ancona & Ortiz 172	UADY	MK098154	
<i>G. floribundum</i>	Mexico	Campeche	Calakmul	18°52'31.6"N, 89°23'42.6"W	Ancona & Ortiz 173	UADY	MK098155	MK097213, MK097214
<i>G. floribundum</i>	Mexico	Campeche	Xtampac	19°47'26.2"N, 89°35'09.2"W	Ancona & Camara 174	UADY	MK098156	MK097215, MK097216
<i>G. floribundum</i>	Mexico	Campeche	Xtampac	19°47'26.2"N, 89°35'09.2"W	Ancona & Camara 175	UADY	MK098157	MK097217, MK097218
<i>G. floribundum</i>	Mexico	Campeche	Xtampac	19°47'26.2"N, 89°35'09.2"W	Ancona & Camara 176	UADY	MK098158	
<i>G. floribundum</i>	Mexico	Chiapas	El Chorreadero	16°45'04.6"N, 92°58'12.6"W	Ancona & Ortiz 178	UADY	MK098159	
<i>G. floribundum</i>	Mexico	Chiapas	El Chorreadero	16°45'04.6"N, 92°58'12.6"W	Ancona & Ortiz 179	UADY	MK098160	
<i>G. floribundum</i>	Mexico	Chiapas	El Chorreadero	16°45'04.6"N, 92°58'12.6"W	Ancona & Ortiz 180	UADY	MK098161	
<i>G. floribundum</i>	Mexico	Chiapas	El Chorreadero	16°45'04.6"N, 92°58'12.6"W	Ancona & Ortiz 181	UADY	MK098162	
<i>G. floribundum</i>	Mexico	Chiapas	Ocozocuaula	16°44'33.9"N, 93°20'49.3"W	Ancona & Hernández 182	UADY	MK098163	
<i>G. floribundum</i>	Mexico	Chiapas	Ocozocuaula	16°44'33.9"N, 93°20'49.3"W	Ancona & Hernández 183	UADY	MK098164	
<i>G. floribundum</i>	Mexico	Chiapas	Ocozocuaula	16°44'33.9"N, 93°20'49.3"W	Ancona & Hernández 184	UADY		MK097219, MK097220
<i>G. floribundum</i>	Mexico	Chiapas	Ocozocuaula	16°44'33.9"N, 93°20'49.3"W	Ancona & Hernández 185	UADY	MK098165	MK097221, MK097222
<i>G. floribundum</i>	Mexico	Oaxaca	Las Anonas	16°39'50.1"N, 94°47'27.5"W	Ancona & Ortiz 186	UADY	MK098166	MK097223, MK097224
<i>G. floribundum</i>	Mexico	Oaxaca	Las Anonas	16°39'50.1"N, 94°47'27.5"W	Ancona & Ortiz 187	UADY	MK098167	MK097225, MK097226
<i>G. floribundum</i>	Mexico	Oaxaca	Las Anonas	16°39'50.1"N, 94°47'27.5"W	Ancona & Ortiz 188	UADY	MK098168	
<i>G. floribundum</i>	Mexico	Oaxaca	Las Anonas	16°39'50.1"N, 94°47'27.5"W	Ancona & Ortiz 189	UADY	MK098169	MK097227, MK097228
<i>G. floribundum</i>	Belize	Cayo	Belmopan	17°16'35.3"N, 88°37'20.9"W	R. R. Innes 220	MO	MK098170	MK097229, MK097230
<i>Gymnopodium toledense</i>	Belize	Toledo	Toledo	16°05'25.8"N, 88°58'43.2"W	T. Hawkins 1681	MO	MK098171	MK097231, MK097232
<i>Podopterus mexicanus</i>	Mexico	Yucatán	Dzibilchaltun	21°05'29.9"N, 89°35'44.2"W	Ancona & Ortiz 264	UADY	MK098172	MK097233, MK097234
<i>Coccoloba uvifera</i>	Mexico	Yucatán	Merida	20°52'00.2"N, 89°37'22.7"W	Ancona & Ortiz 265	UADY	MK098173	MK097235, MK097236

Table 2. Morphological comparison between *Gymnopodium toledense* and *G. floribundum*.

Character	<i>Gymnopodium toledense</i>	<i>Gymnopodium floribundum</i>
Ochrea indumentum	densely pubescent, trichomes with basal glands	glabrous to densely pubescent, trichomes without basal glands
Petiole indumentum	densely pubescent, trichomes with basal glands	glabrous to densely pubescent, trichomes without basal glands
Leaf blade texture	chartaceous	papery to coriaceous
Leaf blade indumentum	abaxially densely pubescent, trichomes with basal glands, adaxially glabrous except puberulent on midvein	abaxially densely pubescent, trichomes without basal glands, adaxially glabrous
Leaf blade apex	obtuse to slightly emarginate	acute to rounded
Leaf blade veins on abaxial surface	prominent	not prominent
Raceme length [cm]	12–20	(3–)5–10(–12)
Pedicel basal segment length [mm]	0.5–1, hidden by ochreole	1.5–2, exserted from ochreole
Outer perianth segments length × width [mm]	8.5–9(–9.5) × 6–6.5(–7)	6.5–8 × 5–6.5(–7)
Inner perianth segments length × width [mm]	(5–)5.5–6 × 1.5–1.8(–2)	(4–)4.5(–5) × 1–1.5
Ovary indumentum	densely pubescent at vertices, trichomes with basal glands	glabrous
Fruit length × width [mm]	5(–5.5) × 2(–2.5)	4–4.5(–5) × 1.5(–2)

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