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Two new species of *Iresine* (*Amaranthaceae*: *Gomphrenoideae*) from Mexico supported by morphological and molecular characters

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Abstract: Two well-defined new species of *Iresine* from Mexico are described based on character data covering vegetative and floral morphology, pollen, and sequences of plastid *matK-trnK*, *trnL-F* and *rpl16* as well as nuclear ITS. We provide morphological and molecular descriptions, as well as a discussion on diagnostic characters and taxonomic affinities. Both species are distributed in cloud forests; *I. borschii* is known only from two collections in Veracruz, whereas *I. sousae* has been collected several times in Oaxaca and Chiapas. Both species are illustrated from herbarium specimens to facilitate their recognition. New field collections and observations are needed to improve our knowledge on the habitat and conservation status of these new species.

Key words: *Amaranthaceae*, *Caryophyllales*, cloud forest, endemism, *Flora Mesoamericana*, *Gomphrenoideae*, *Iresine*, Mexico, molecular diagnosis, neotropics, new species, pollen morphology

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Introduction

The neotropical genus *Iresine* P. Browne comprises c. 45 species and ranges from the S United States and the Caribbean to S South America. In Mexico it is the largest genus of the *Amaranthaceae* (subfamily *Gomphrenoideae*, Müller & Borsch 2005a; Sánchez del Pino & al. 2009) with more than half of all species being endemic. *Iresine* is variable in habit including big trees, shrubs, lianas and annual herbs, mostly dioecious, but some species also are gynodioecious (Zumaya & al. 2013). The androecium consists of a short tube (usually less than 25 % of total stamen length) with five individual stamens and five obvious

elongate androecial tube appendages not homologous to stamens (but previously called “pseudostaminodia”) arising between the filaments, probably related to animal pollination (Vridaghs & al. 2014). The pollen is pantoporate with moderately vaulted mesoporia and numerous evenly spread microspines (*Iresine* type pollen, Borsch 1998). As part of on-going research on phylogenetic relationships of the genus (Borsch & al., unpubl. data), and revisionary work in the context of monographing *Iresine* in Mexico (Zumaya 2008) and developing a treatment for *Flora Mesoamericana* by the senior author (T.B.), we found two further very clearly delimited hitherto unrecognized taxa based on both morphological and molecular evidence. In

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the course of this work, we also recently described *I. rzedowskii* Zumaya & al., which is a widespread liana in the Mexican highlands, and *I. valdesii* Zumaya & al., which is endemic to the Tehuacán-Cuicatlán area (Zumaya & al. 2013). Considering that *Iresine* is a complicated genus with several lineages of morphologically closely allied dioecious taxa that are still poorly understood, we regard the use of sequence data to have a high importance for understanding species. As a consequence, sequence data are particularly important for diagnosing and describing species (González-Gutiérrez & al. 2013). This paper therefore aims to describe two new species using an integrative approach to including morphological and molecular diagnostic characters from multiple loci.

Material and methods

Plant material — In the course of extensive taxonomic and phylogenetic study, around 4000 specimens from ASU, B, CHAPA, CIIDIR, ENCB, F, FCME, GH, IBUG, IEB, K, M, MEXU, NY, OAX, RSA-POM, SERO, UC, US and XAL were examined. The respective specimens included in this study as belonging to the new species described are listed here. In the course of the study we visited the localities provided by the collectors of the specimens, trying to relocate the plants for additional collections and field observations, but this was not successful.

Palynology — Pollen grains were sampled from herbarium specimens. Air dried grains were placed on silicon plates (Plano GmbH, Marburg, Germany) mounted on aluminium stubs and examined under cold field emission in a scanning electron microscope (HITACHI SU8010). Because there was enough material available, grains of *I. sousae* were immersed in dimethoxypropane for three days and then critical-point dried before placing them on silicon plates.

DNA isolation, amplification, sequencing — Total DNA was isolated from herbarium specimens using a triple CTAB extraction method (Borsch & al. 2003). The *trnK-matK* region in *I. sousae* was amplified and sequenced in two fragments using the primer pairs *trnK-Fbry* plus *ACmatK-1400R* and *ACmatK490F* plus *trnK2R* following the protocol described in Borsch & al. (2011). For *I. borschii* the upstream fragment had to be divided into two further halves because of stronger DNA degradation using the additional internal primers *ACmatK100F* (Müller & Borsch 2005a) and *ACmatK200R* (Müller & Borsch 2005b) and substituting *ACmatK1400R* by *ACmatK1401R*.

The *trnL-F* region was amplified and sequenced following the protocol described in Sánchez del Pino & al. (2009) but generally using the primers *trnTd* and *trnL460F* (Worberg & al. 2007) as internal sequencing primers. This way the complete intron and spacer could

be covered. The *rpl16* intron was amplified by forward *rpl16F* or *rpl16-1216F* and reverse *rpl16R* or *CARYrps3-112R*. These primers were also used for sequencing plus the internal sequencing primers *rpl16IRE689R* and *GOMrpl16-495F* as long A/T microsatellites required to sequence towards them in both strands. Amplification and sequencing of the nrITS region followed Fuentes-Bazan & al. (2012). The sequences were edited and manually aligned using PhyDE v.0 995 (Müller & al. 2007). Boundaries of the genomic regions studied were annotated using multiple sequence alignment in comparison with completely sequenced and annotated plastid genomes of *Nicotiana tabacum* (Z00044; Shinozaki & al. 1986) and *Spinacia oleracea* (AJ400848; Schmitz-Linneweber & al. 2001). The respective character positions were then determined with reference to each specific sequence because of length variability within the genomic regions.

Distribution map — The map was produced using QGIS Brighton (2.6.1) employing layers from INEGI (2009) and CONABIO (1998).

Results and Discussion

***Iresine borschii* Zumaya & Flores Olv., sp. nov.** — Fig. 1, 4A, C.

Holotype: Mexico, Veracruz, Mpio. Chocamán, 1 km N of Chocamán, gorge of river upstream from Chocamán-Coscomatepec highway, 7 Dec 1981, *Nee 23904* [staminate plant] (XAL; isotype: NY).

Morphological diagnosis (based on staminate plant) — *Iresine borschii* differs from *I. interrupta* Benth. by having stems solid; stems and leaves with conspicuous lanate indumentum with dark brown trichomes c. 1 mm long; bracts shorter than bracteoles, the latter reaching less than ½ length of tepals, glabrous, apex mucronate to apiculate; tepals villous (middle and inner ones), apex mucronulate.

Molecular diagnosis — Nucleotide state “C” in position 195, “A” in pos. 204 and 206 as part of an extended poly-A stretch unique to this species (equally parsimonious alignment would assume a gap between primary sequence pos. 204 and 205), “A” in pos. 557 and “C” in pos. 822 of *rpl16* intron; a simple sequence repeat of “CACATTATG” that extends (template and copy) from pos. 827 to 844 in *matK* coding sequence, and “T” in pos. 1309 and a simple sequence repeat of “CTTATACA” that extends (template and copy) from pos. 78–93 downstream of *matK* stop codon in *trnK* intron; “T” in pos. 555 of *trnL* intron, and “G” in pos. 112 in *trnL-F* spacer; “T” in pos. 57 and a gap of 1 nt between pos. 144 and 145 of ITS1.

Morphological description — *Shrubs* perennial, dioecious. *Stems* erect to scandent (?), solid, striate, lanate with c. 1 mm-long, uniseriate, dark brown trichomes. *Leaves*

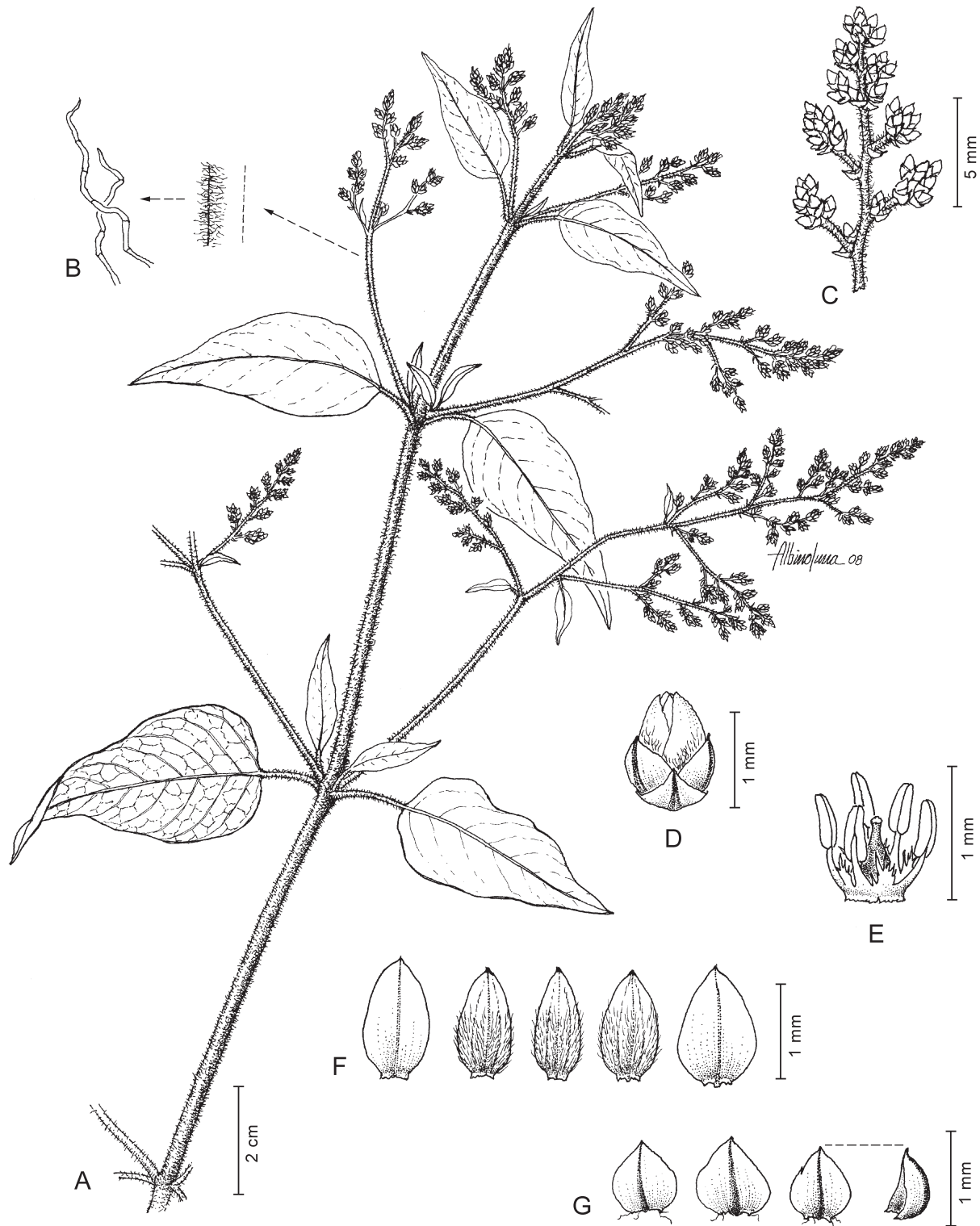


Fig. 1. *Iresine borschii*, staminate plant. – A: branch; B: stem pubescence and trichomes; C: paracladia; D: mature male flower; E: androecial tube (stamens and appendages) and pistillode; F: tepals; G: bract and bracteoles. – From the holotype (Nee 23904, XAL).

opposite; petiole 0.5–2.5 cm long; blade lanceolate to ovate-lanceolate, 1–10 × 0.5–7 cm, membranous, lanate, base rounded, apex acuminate to long acuminate. *Syn-florescences* of staminate plants branched up to 3rd order with a dominant principal axis, 7–15 × 2–5 cm, consisting of 3–27(–33) flowers aggregated into cylindrical

paracladia, these arranged in leafless thyrsoid structures; axis densely tomentose with trichomes 0.9–1.1 mm long; paracladia sessile or subsessile, 0.5–2 cm high, subtended by bract-like scales. *Staminate flowers* with bracts shorter than bracteoles, these reaching less than ½ length of tepals; *bracts* and *bracteoles* dark brown,

ovate to ovate-deltoid, 0.4–1 × 0.6–1 mm, membranous, glabrous, midvein darker, distinct, apex mucronate to apiculate; *tepals* dark brown, ovate to ovate-oblong, outer ones 1.4–2 × 0.6–0.9 mm, middle ones 1.3–1.9 × 0.5–0.6 mm, inner ones 1.3–2 × 0.5–0.6 mm, glabrous to villous (middle and inner ones) abaxially with white, uniseriate, 0.5–1 mm-long trichomes, 1(–3)-veined, vein(s) extending along length of tepal, margin subcoriaceous to membranous, apex mucronulate; *filaments* 0.6–1 mm long, appendages of androecial tube oblong, 0.2–0.6 mm long, fimbriate. *Pistillate flowers* not known. *Pollen* spheroidal, 13–15 µm in diam., with 18–20 apertures; pores 2.4–2.6 µm in diam., all of equal size, 8–12 well-delimited ektexinous bodies, composed of 1–3 (then at base confluent) cone-shaped spinules with acute tips like those on mesoporia, well separated and ± evenly spaced; mesoporia broadly vaulted to almost flat, 1.8–2.3 µm wide, tectum punctate, with round, slightly anulopunctate perforations 60–80 nm in diam., 25–30 perforations per 10 µm², ± evenly spaced, spinules cone-shaped with acute tips, 250–260 nm high, 270–290 nm in diam. at base, with 25–35 spinules per 10 µm², ± evenly spaced.

Molecular description — Sequences from chloroplast genomic regions and nrITS describe the type *Nee 23904* (DNA code AC945) and are available in EMBL/GenBank under accession numbers LT223166 (*rpl16* intron), LT223158 (*trnK* intron including *matK* gene), LT223162 (*trnL-F* region including intron and spacer) and LT223156 (ITS). Further sequences describe the paratype *Ventura 9443* (DNA code AC1049) and are available for the same loci under accession numbers LT223167 (*rpl16*), LT223159 (*matK-trnK*), LT223163 (*trnL-F*) and LT223157 (ITS).

Phenology — The specimens were collected in flower in December and January, but further data are needed.

Distribution and ecology — *Iresine borschii* is poorly known; it has been collected twice in the state of Veracruz, Mexico. The collections of the species were made in December and January in cloud forest at 1300 m and 1350 m above sea level in association with *Alnus acuminata* subsp. *arguta* (Schltdl.) Furlow, *Liquidambar macrophylla* Oerst. and *Platanus mexicana* Moric. Mapping the known localities of *I. borschii* on the layer of cloud forest (CONABIO 1998), the locality of Jilotepec does not show this type of vegetation two decades after collecting the plant there (Fig. 5). It remains to be verified by future field work if the species grows in a transitional vegetation type or if the cloud forest had already been cleared before the assessment by CONABIO (1998).

Conservation status — *Iresine borschii* is known only from two populations in the state of Veracruz in the municipalities of Chocamán and Jilotepec; therefore it meets the criteria B1ab(iii), for the category of Critically Endangered

(CR) according to IUCN (2012). Additional exploration across the cloud forest of Veracruz and surrounding areas is needed to assess the frequency and population status, including the existence of pistillate plants of the species.

Eponymy — *Iresine borschii* is named in honour of Thomas Borsch in recognition of his contributions to the systematics of the *Amaranthaceae* and his support for and collaboration in the study of the Mexican Iresines.

Discussion of molecular characters — Both samples (AC945 and AC1049) have identical sequences in all loci studied. The simple sequence repeat “CACATTATG” in the *matK* CDS is out of frame in our interpretation of a most parsimonious mutational event without involving any additional substitutions between template and copy. Instead of the amino acid sequence ProHisTyr (translated from CCACATTAT) the corresponding sequence is AlaHisTyr (corresponding to the motif “GCACATTAT”).

We follow the concept of González-Gutiérrez & al. (2013) in which the complete sequences constitute the molecular description and selected unique character states the diagnosis. In this case, all molecular diagnostic characters represent synapomorphies for the individuals sequenced for this species in the Iresinoid clade (*Iresine*, *Irenella* Suess. and *Woehleria* Griseb.) sensu Sánchez del-Pino & al. (2009). Being in a transition phase from alpha-taxonomy to species concepts that are fully supported by an evolutionary analysis of species limits (see Borsch & al. 2015), we believe that there will be a continuous increase in the availability of both morphological and molecular data, which will then also be presented in the form of phylogenetic analyses. It may be noted that the morphological data available from so-far published diagnoses and descriptions of species also requires updating and completing before a fully comparative dataset can be made available. Nevertheless, we consider the concept of the species newly described to science in this paper to be so clear that we can safely validate the taxon. This is also of practical importance as regional syntheses (e.g. *Flora Mesoamericana*) and conservation work will need this information in a timely manner. Our considerations also apply to the second species described below.

Taxonomic remarks — The samples of the two known individuals of *Iresine borschii* are recuperated as monophyletic and sister of *I. interrupta* (Borsch & al., unpubl. data). *Iresine borschii* is probably clambering, as is *I. interrupta*; they share ribbed stems, lanceolate to ovate-lanceolate, membranous leaves and ovate-oblong tepals. As diagnosed, *I. borschii* differs from *I. interrupta* by its solid stems, the indumentum and the morphology of bracts, bracteoles and tepals. The pollen morphology is very similar to that of *I. interrupta*, but the latter species has a flatter mesoporia, a higher number of apertures (20–24) and ektexinous bodies (18–20). A detailed assessment throughout the genus will be important to eval-

uate if there are synapomorphies in pollen characters. Because pistillate plants have not been collected, characters in female flowers should be compared to *I. interrupta* in the future.

Additional specimens seen (paratypes) — MEXICO: VERACRUZ: Mpio. Jilotepec, Piedra de Agua, 1300 m, 5 Jan 1974, Ventura 9443 (ENCB, IEB).

Iresine sousae Zumaya, Borsch & Flores Oliv., **sp. nov.** — Fig. 2, 3, 4B, D.

Holotype: Mexico, Chiapas, Mpio. Yajalón, Rancho Carmen, 6 Feb 1984, A. Méndez Ton 7192 (MEXU; isotypes: B, CHIS, MEXU, MO, SERO).

Morphological diagnosis (based on staminate plant) — *Iresine sousae* differs from other species by having stems green to pale brown with small lenticels, velutinous in upper parts with c. 0.2 mm-long simple trichomes; leaves light green, glabrous; bracts distinctly shorter than bracteoles; bracteoles ovate to suborbicular, 0.5–0.8 × 0.6–0.8 mm; flowers small with tepals (of mature flowers) brown-yellowish to hyaline, outer and middle tepals ovate to broadly ovate, inner ones ovate-lanceolate, outer tepals 1.3–1.4 mm long, middle and inner ones slightly shorter, all membranous, glabrous, sometimes slightly 1-veined, apex acute; appendages of androecial tube oblong, 0.3–0.6 mm long, fimbriate. Pollen differs by very flat mesoporia and a higher number (32–43 per 10 µm²) of well-sized (60–80 nm in diam.) perforations compared to spinules (15–22 per 10 µm²).

Molecular diagnosis — Nucleotide state “C” in position 171 of *rpl16* intron; “C” in pos. 144, “A” in pos. 570, “T” in pos. 782 and “G” in pos. 786 of *matK* coding sequence, and “C” in pos. 6 of *trnK* downstream intron part; “T” in pos. 129 and “A” in pos. 185 and 191 of ITS1, and “T” in pos. 107, an additional “G” in pos. 124 (where other species have a gap) and “T” in pos. 179 of ITS2.

Morphological description — *Lianas* dioecious (from label data). *Stems* scandent or clambering, to 5 m tall, light green to light brown with numerous small lenticels, glabrous to velutinous in upper parts with c. 0.2 mm-long, uniseriate trichomes (these also in young leaves and bracts). *Leaves* opposite; petiole 0.5–2 cm long; blade light green, elliptic to oval, 2.5–10.5 × 1–4.5 cm, coriaceous, glabrous or slightly lanulose along veins abaxially when young, base cuneate, narrowly decurrent into petiole, apex acuminate. *Synflorescences* of pistillate and staminate plants similar, branched up to 4th order with a dominant principal axis, 5–32 × 5–18 cm, consisting of 5–17(–23) flowers aggregated into conical heads (paraclydia), these arranged in leafless thyrsoid structures with a dominant principal axis; axis densely tomentose with trichomes 0.1–0.2 mm long; paraclydia sessile or sub-

sessile, 0.1–1 cm high, subtended by brown-yellowish pubescent bracts 0.5–1 × 0.6–1 mm, other inflorescence branches subtended by herbaceous pubescent leaves. *Staminate flowers* with bracts shorter than bracteoles, reaching to c. 25 % length of tepals; *bracts* ovate, 0.5–0.7 × 0.5–0.6 mm, membranous, tomentose, apex acute; *bracteoles* ovate to suborbicular, 0.5–0.8 × 0.6–0.8 mm, reaching to c. 70 % of length of tepals, membranous, glabrous, apex acute; *tepals* (of mature flowers) brown-yellowish to hyaline, outer and middle tepals ovate to broadly ovate, inner ones ovate-lanceolate, outer tepals 1.3–1.4 × 0.8–0.9 mm, middle ones 1.2–1.3 × 0.7–0.7 mm, inner ones 1.2–1.3 × 0.6–0.7 mm, all membranous, glabrous, sometimes slightly 1-veined, vein extending to length of tepal, apex acute; *filaments* 1–1.7 mm long, appendages of androecial tube oblong, 0.3–0.6 mm long, fimbriate. *Pistillate flowers* with bracts shorter than bracteoles, reaching to 50–75 % length of tepals; *bracts* yellowish brown, ovate to deltoid, 0.5–0.7 × 0.4–1 mm, membranous, tomentose, without veins, apex acute; *bracteoles* ovate, 1–1.3 × 0.9–1 mm, hyaline, glabrous, 1-veined, apex acute; *tepals* (of mature flowers) yellowish brown, outer tepals ovate, inner and middle ones narrowly ovate, outer tepals 1.1–1.2 × 0.7–0.9 mm, middle ones 1–1.2 × 0.6–0.7 mm, inner ones 1–1.2 × 0.5–0.6 mm, all membranous, glabrous, 1-veined, vein extending to apex of tepal, apex acute; tepals surrounded by finely undulate white trichomes to 3.8 mm long at maturity at top of a short pedicel; *staminodia* narrowly oblong, appendages of androecial tube oblong, 0.2–0.3 mm long, fimbriate; *ovary* subglobose, 0.5–1 mm long, style conspicuous, stigmas narrowly cylindrical, 0.3–5 mm long. *Seeds* brown-orange or bright red, subglobose, 0.90–1 mm in diam. *Pollen* spheroidal, 13–14 µm in diam., with 22–26 apertures; pores 1.9–2.1 µm in diam., all of ± equal size, 12–16 not so well-delimited ektexinous bodies, well separated and irregularly spaced; mesoporia broadly vaulted to almost flat, 1.8–2.4 µm wide, tectum punctate, with perforations 80–140 nm in diam., round, with 32–43 perforations per 10 µm², ± evenly spaced; spinules cone-shaped with acute tips, 200 nm high, 200–230 nm in diam. at base, with 15–22 spinules per 10 µm², ± evenly spaced.

Molecular description — Sequences from chloroplast genomic regions and nrITS describe the type *A. Méndez Ton 7192* (DNA code AC807) and are available in EMBL/GenBank under accession numbers LT223168 (*rpl16* intron), LT223160 (*trnK* intron including *matK* gene), LT223164 (*trnL-F* region including intron and spacer) and LT223155 (ITS). Further sequences describe the paratype *S. Maya J. 2845* (DNA code AC804) and are available for the same loci under accession numbers LT223169 (*rpl16*), LT223161 (*matK-trnK*), LT223165 (*trnL-F*) and LT223154 (ITS).

Phenology — Flowering and fruiting from January to March.



Fig. 2. Holotype of *Iresine sousae* – A. Méndez Ton 7192 (MEXU).

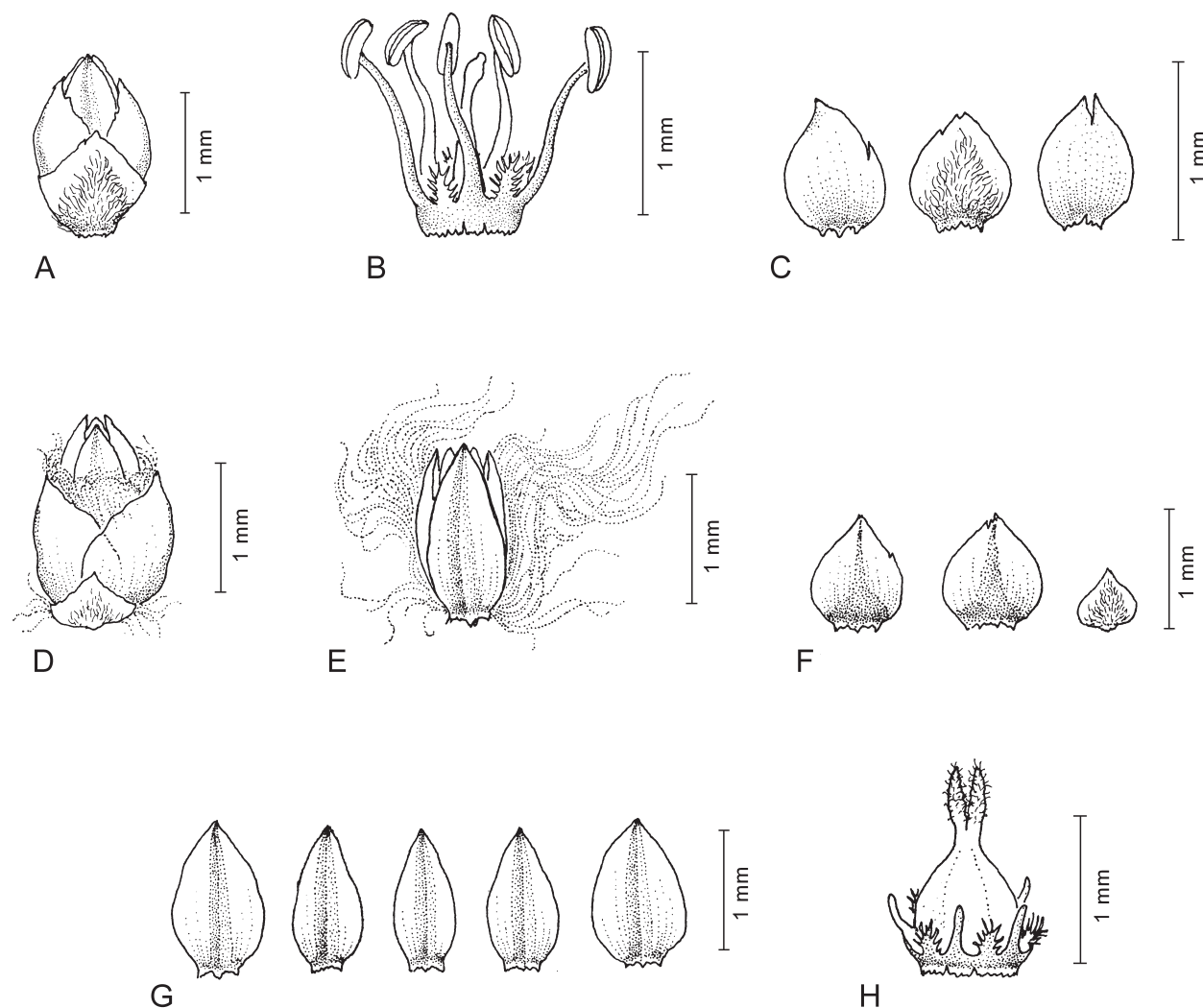


Fig. 3. *Iresine sousae* – A: Male flower with bract and bracteoles; B: androecial tube (stamens and appendages) and pistillode; C: bract and bracteoles; D: female flower with bract and bracteoles; E: female flower at mature stage depicting long trichomes serving dispersal; F: bract and bracteoles; G: tepals; H: gynoecium and reduced androecium. – A–C from A. Méndez Ton 7192 (MEXU); D–H from S. Maya J. 4281 (MEXU).

Distribution and ecology — *Iresine sousae* is endemic to Mexico from Chiapas and Oaxaca (Fig. 5). It grows from 720 m to 1900 m above sea level in cloud forest, close to pine-oak forest, with *Calophyllum* L., *Cecropia* Loebl., *Cedrela* P. Browne, *Liquidambar* L., *Ocotea* Greenm. & C. H. Thoms., *Podocarpus* Pers., *Ulmus* L., etc. More exploration is necessary to better understand the habitat preferences and frequency of this species.

Conservation status — *Iresine sousae* has been collected in Chiapas and Oaxaca. In the state of Chiapas it is known only from one collection in Rancho Carmen, municipality of Yajalón. In Oaxaca *I. sousae* has been collected from two localities in the municipality of San Miguel Chimalapa, and one in the municipality of Santa María Guienagati. None of these areas is protected. According to IUCN (2012), this species meets the criteria B2ab(iii) for the category of Endangered (EN).

Eponymy — The name honours Mario Sousa Sánchez for his work supporting the *Flora Mesoamericana* Project, which provided important specimens from the area, but especially for his vision for the organization of MEXU, being the most important collection for the Mexican flora.

Discussion of molecular characters — Both samples (AC804 and AC807) have identical sequences in *matK-trnK* studied. Sample AC804 differs by a character state “C” in pos. 928 of the *rpl16* intron and a “C” in pos. 424 in the *trnL* intron. The sequence of ITS in pos. 114 of sample AC804 deviates by a “T”. So far, sequences are from staminate plants.

Taxonomic remarks — The two samples of *Iresine sousae* group together in molecular phylogenetic analyses but the closest relatives are less evident (Borsch & al. unpubl. data). There are several synapomorphies in the molecular characters for both individuals indicating a well-isolated

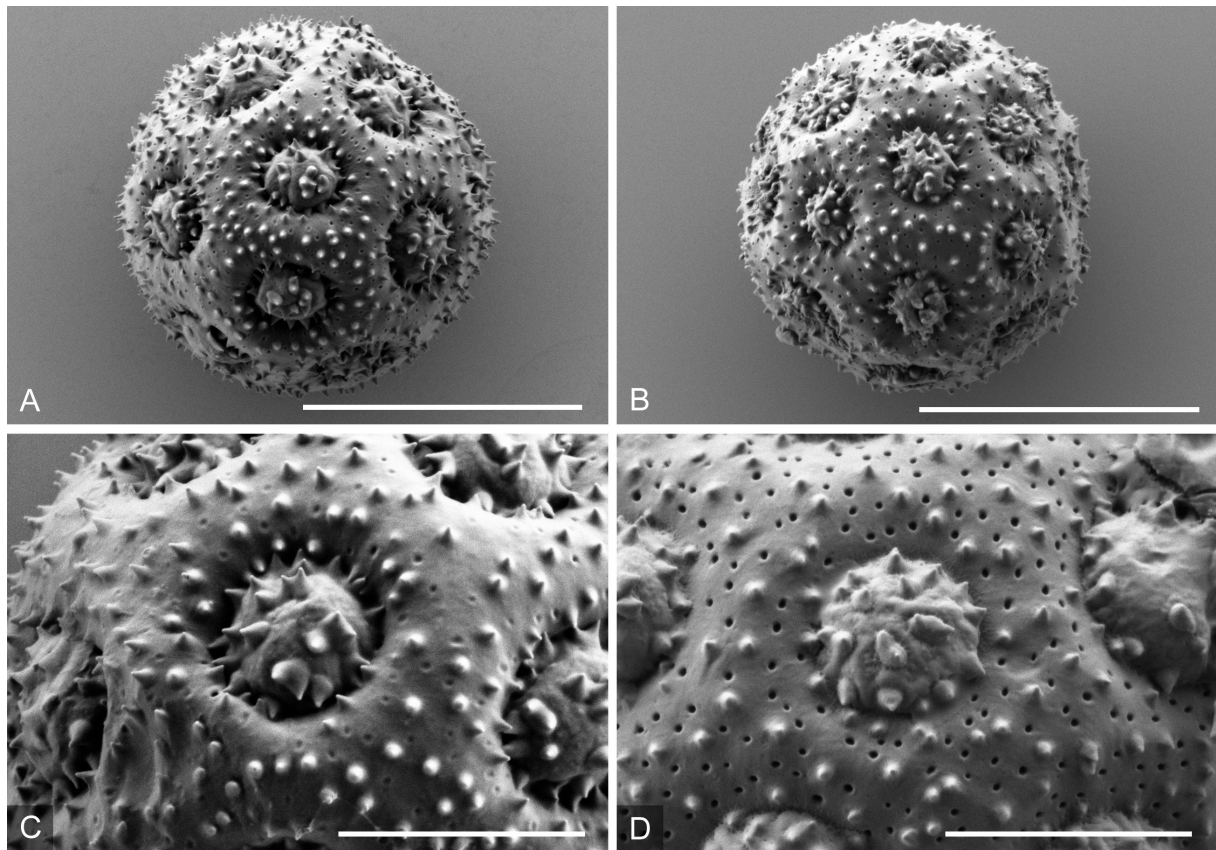


Fig. 4. SEM micrographs of pollen grains and apertures from the type specimens. – A, C: *Iresine borschii*; B, D: *Iresine sousae*. – Scale bars: A, B = 10 μ m; C, D = 4 μ m.

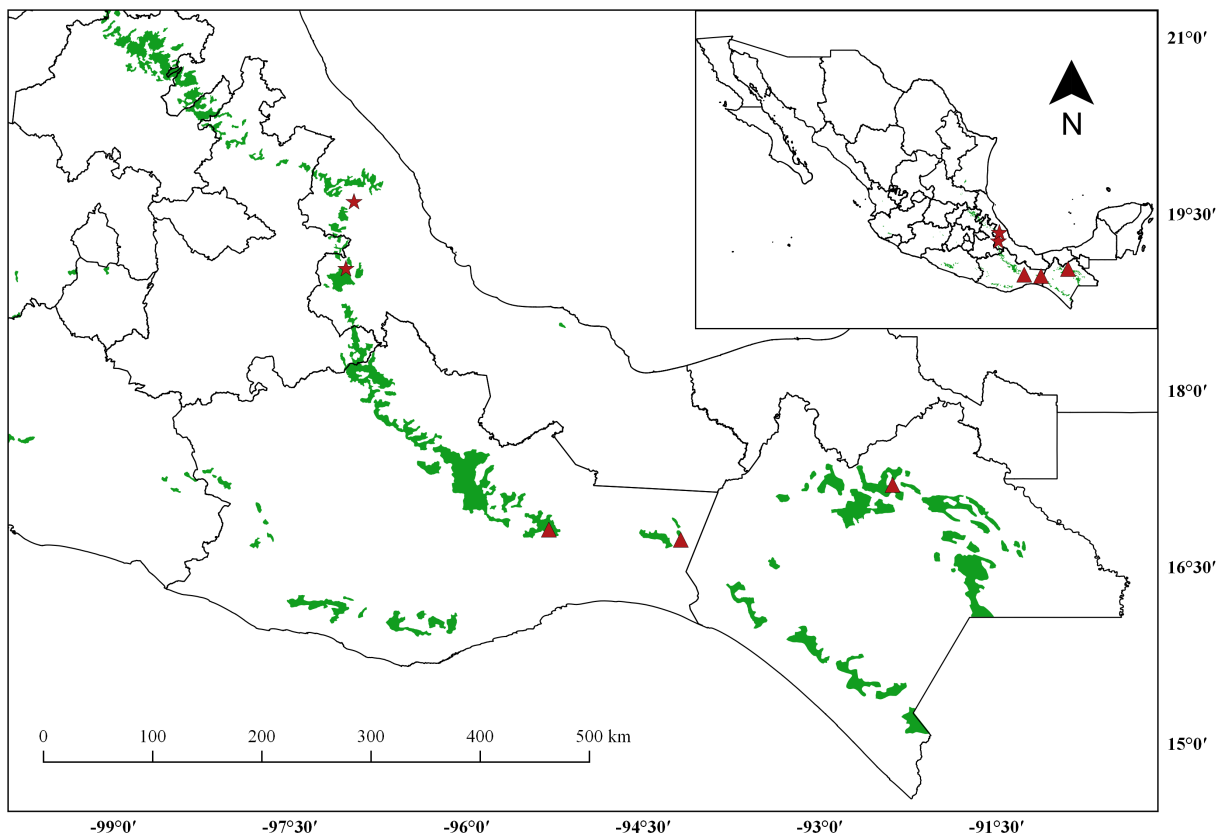


Fig. 5. Distribution of *Iresine borschii* (★) and *I. sousae* (▲). The areas marked in green correspond to cloud forest.

position of this species within one of the major clades of the genus *Iresine*, which is paralleled by several obvious morphological features.

Additional specimens seen (paratypes)—MEXICO: OAXACA: Mpio. Santa María Guienagati, la Cañada, cerca del puente que limita con Pie de Cerro, 1.5 km en línea recta (215°N) de Peña Blanca, 16°49'11.5"N, 95°18'13.6"W, 720 m, 8 Feb 2007, *Velasco 1713* (MEXU, SERO); Mpio. San Miguel Chimalapa, Cerro Sabinal (pico c. 1.5 km al SO del Cerro Guayabitos), c. 3 km en línea recta al NNO de Díaz Ordáz, c. 40 km en línea recta al N de San Pedro Tapanatepec, 16°44'N, 94°11'W, 15 Mar 1987, *S. Maya J. 4281* (CHAPA, MEXU); Mpio. San Miguel Chimalapa, cerro Guayabitos, al NO de Benito Juárez, c. 41 km en línea recta al N de San Pedro Tapanatepec, 16°44'N, 94°11'W, 1800–1900 m, 30 Jan 1986, *S. Maya J. 2845* (CHAPA, MEXU).

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