

## Testing the monophyly of *Heterosperma* (Asteraceae, Coreopsidae)

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### Abstract

The genus *Heterosperma* (Asteraceae, Coreopsidae) currently comprises ten species distributed from Mexico to South America. Molecular phylogenies of the tribe Coreopsidae (including only two species of *Heterosperma*) pointed out that the genus would not be monophyletic. A molecular phylogenetic analysis including seven species of *Heterosperma* and related taxa, using plastid and nuclear sequences, was performed. The results indicated that *Heterosperma* is monophyletic if *Coreopsis cyclocarpa* is included. As a result, a new combination, *Heterosperma mexicanum*, is proposed. Morphological analysis of floral trichomes, pappi and cypselae, is presented. A key to related genera and distribution maps are also provided.

**Keywords:** *Coreopsis cyclocarpa*, *Electranthera*, *Henricksonia*, *Heterosperma*, ITS, *trnL-trnF*

### Introduction

*Heterosperma* Cavanilles (1795–1796: 34) is placed in tribe Coreopsidae of the Asteraceae (Karis & Ryding 1994, Kimball & Crawford 2004, Panero 2007, Crawford *et al.* 2009, Mesfin Tadesse & Crawford 2014). It is comprised of ten species (Cabrera 1978, Ariza Espinar 2000, Hind 2011, Robinson 2006, Allison 2006, Hokche *et al.* 2008, Ariza Espinar & Lizarazu 2015) and distributed from Mexico to South America, including the Caribbean region (McVaugh 1984, Panero 2007). All recognized *Heterosperma* species are characterized by having heteromorphic cypselae (Panero 2007, Crawford *et al.* 2009). Molecular phylogenetic analyses of Coreopsidae using nuclear and plastid sequences (Kimball & Crawford 2004, Mort *et al.* 2008, Crawford *et al.* 2009) pointed out that *Heterosperma* would not be monophyletic because *Heterosperma pinnatum* Cavanilles (1795–1796: 34) appeared closely related to *Coreopsis cyclocarpa* Blake (1914[1913]: 339), while *Heterosperma diversifolium* Kunth (1820: 246) would be related to the monotypic *Henricksonia* Turner (1977: 78). However, only two of the ten species of *Heterosperma* were included in these phylogenies. *Electranthera mutica* (DC.) Mesfin, D.J. Crawford & Pruski (Pruski *et al.* 2015: 7) appears as the sister species to the *Heterosperma*-*Henricksonia*-*Coreopsis cyclocarpa* clade (Kimball & Crawford 2004, Mort *et al.* 2008). Panero (2007) in his treatment of the tribe Coreopsidae included *Coreopsis cyclocarpa* in *Heterosperma* but he did not place *Henricksonia* into the synonymy of the genus.

The aim of the present study is to provide a better phylogenetic hypothesis of *Heterosperma* for generic realignments, including seven of the ten species of the genus and its closely related species, i.e. *Coreopsis cyclocarpa*, *Henricksonia mexicana* Turner (1977: 78), and *Electranthera mutica*. We also analyzed morphological features of the mentioned taxa and discussed them in relation to our phylogenetic results.

### Materials and Methods

#### Morphology

Morphological characters were based on herbarium collections of representatives of the analyzed genera and are summarized in Table 1. Specimens belonging to the following herbaria were examined: COL, NY, SI, TEX and US (acronyms following Thiers 2018). Ray and disc florets were selected and rehydrated with warm soapy water and fixed with FAA. Florets were dehydrated in a graded series of ethanol and critical point-dried (BAL-TEC CPD 030) with liquid CO<sub>2</sub>. Dried samples were partially dissected, mounted on stubs, sputter-coated with gold/palladium (Termo VG

Scientific SC 7620) and observed with a PHILIPS XL30 TMP New Look (Scanning Electronic Microscope, SEM). For the description of the trichomes, the terminology proposed by Ramayya (1962) was used.

**TABLE 1.** Comparisons of morphological characters between *Heterosperma* and related taxa (key characters in bold face).

	<i>Henricksonia</i>	<i>Electranthera mutica</i>	<i>Coreopsis cyclocarpa</i>	<i>Heterosperma</i>
Floral trichomes	<b>moniliform</b>	filiform, conical <b>long glandular</b>	filiform, conical short glandular	filiform, conical, <b>cylindrical</b> short glandular
Ray floret (pappus)	2 (–3) <b>short awns</b>	epappose	epappose	epappose (rarely 2 long awns, retrorsely barbed)
Disc floret (pappus)	<b>4 scales</b>	epappose	epappose	<b>long awns</b> , 2 (–4) aristate, retrorsely barbed or absent ( <i>H. achaetum</i> )
Cypsela	strongly heteromorphic	graded monomorphic [sub-heteromorphic]	graded monomorphic [sub-heteromorphic]	graded monomorphic [sub-heteromorphic] to strongly heteromorphic
Ray cypsela	obcompressed, broadly obovate, mildly winged	obcompressed, broadly ovate or elliptic, winged	obcompressed, orbicular to obovate, winged	obcompressed, ellipsoid or obovate, winged
Disc cypsela	<b>obpyramidal</b> , linear, not winged	obcompressed, narrowly ovate or elliptic, sometimes linear, winged	obcompressed, narrowly orbicular to obovate, winged	obovate to terete, not winged or winged
Anthers (glandular trichomes)	<b>present</b>	absent	absent	absent

### Phylogenetic study

**Taxa selection:**—A total of 13 vouchers were sequenced for this study: ten vouchers of *Heterosperma* (seven species, including the type species *H. pinnatum*), one voucher of *Coreopsis cyclocarpa*, and two vouchers of *Henricksonia mexicana*. We analyzed our species alongside a selection of 37 taxa of Coreopsidae, including *Electranthera mutica*, which is available in Genbank. *Wedelia asperima* Benth (1867: 539) was used to root the trees. The total number of analyzed vouchers was 50. The details of the species included in the analysis, the Genbank numbers and the voucher information of the new sequences are shown in Appendix 1.

**DNA extraction and sequencing:**—Four samples of *Heterosperma* were collected in the field by the first author and dried in silica gel, the remaining six samples were taken from herbarium specimens from COL, TEX, and US. DNA from the field (silica) and herbarium samples was extracted using the modified CTAB (cetyltrimethylammonium bromide) protocol from Doyle & Doyle (1987). Sequences of the nuclear ribosomal ITS and plastid region *trnL-trnF* spacer were PCR amplified. For the *trnL-trnF*, the pairs of primers denoted as c–d and e–f by Taberlet *et al.* (1991) were used; the ITS region was amplified with ITS4 and ITS5 universal primers. The PCR reactions were performed in 25 µl of final volume with 50 ng of template DNA, 0.2 µM of each primer, 25 µM of dNTPs, 5 µM MgCl<sub>2</sub>, buffer 10x, and 1.5 units of Taq polymerase provided by Invitrogen (Brazil). The reaction conditions were: an initial period of denaturation at 94 °C for 5 min., followed by 35 cycles of denaturation at 94°C for 30 s., annealing at 48°C for the *trnL-trnF* and 53°C for ITS during 1 min., extension at 72°C for 1 min. 30 s., and a final extension at 72°C for 6 min. terminated the reactions. PCR products were run out on a 1 % TBE agarose gel and stained with SybrSafe (Invitrogen). Sequencing reactions were performed by MacroGen, Inc. (Seoul, Korea). Edition and manual alignment of the sequences were performed with BioEdit version 5.0.9 (Hall 1999). The matrices can be found in Treebase: <http://purl.org/phylo/treebase/phyloids/study/TB2:S22344>

**Data Analyses:**—Data were analyzed separately and in combination using maximum parsimony (MP), and Bayesian inference (BI) approaches. Parsimony analysis was carried out using TNT version 1.1 (Goloboff *et al.* 2008). The searches involved 1000 replicates, each of which generated a Wagner tree using a random addition sequence of taxa from the data matrix, swapping the initial tree with TBR (tree bisection and reconnection) and retaining a maximum of 10 trees in each replicate. Subsequently, all optimal trees were swapped using TBR, holding a maximum of 10,000

trees. A strict consensus tree was generated from the most parsimonious trees. Branch supports were estimated with Bootstrap (Felsenstein 1985) using a total of 10,000 replicates. Each replicate was analyzed using 10 Wagner trees as a starting point followed by TBR branch swapping, saving only one tree per replicate. Bootstrap values (BS) over 50 % are reported.

The Bayesian analysis was performed with BEAUti and the BEAST Package 1.8.1 (Drummond *et al.* 2012). The appropriate model of nucleotide substitution for each partition was selected using jModelTest 2.1.4 (Darriba *et al.* 2012) following the Akaike Information Criterion (AIC): SYM+ G (ITS), TPM1 $\mu$ f +G (*trnL-trnF*), and GTR + I + G (combined ITS/*trnL-trnF*). The Bayesian analysis was conducted with settings as follow GTR +G and all equal base frequencies for ITS, HKY + G and estimated base frequencies for *trnL-trnF*, and GTR + I + G and estimated base frequencies for the combined matrix. The three matrices were run under four gamma categories, uncorrelated rate variation with lognormal distribution, random starting tree, a Yule process as tree prior, auto-optimization option for all operators and default values for all other settings. Two independent runs of 10 million generations were sampled every 1,000th generation. To identify when the analyses had reached stationarity, we checked the output files for convergence and effective sample size (ESS) > 200 with Tracer v1.6 (Rambaut *et al.* 2013). Based on this convergence diagnostic, the first 2500 sampled tree were discarded as burn-in from each analysis before obtaining a consensus phylogeny. Trees of the two runs were combined with LogCombiner 1.8.4. Posterior probabilities (PP) of branches were calculated from a maximum clade-credibility tree (MCC) obtained with TreeAnnotator 2.4.7. The maximum clade-credibility tree showing posterior probabilities (PP) was edited in FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

## Results

### Phylogenetic Analysis

The *trnL-trnF* alignment included 50 terminals and 831 bp-long, of which 80 (9.6 %) were parsimony informative; the MP analysis resulted in 30 trees of 119 steps, the consensus tree is shown in Fig. 1A. The ITS alignment included 50 terminals and 666 bp-long, of which 268 (40.3%) were parsimony informative, the MP analysis resulted in 30 trees of 1003 steps and the consensus tree is shown in Fig. 1B. For both markers, the separated BI analyses (data not shown) recovered the same supported clades with high values of posterior probabilities (PP), so these PP are indicated in the parsimony consensus trees (Fig. 1A, 1B). In the parsimony analysis as well as in the BI inference, both markers (ITS, *trnL-trnF*) showed all the species of *Heterosperma* joined in a strongly supported clade (BS = 100, PP = 1; BS = 100, PP = 0.92, respectively), which also includes *Coreopsis cyclocarpa*. *Electranthera mutica* appeared as the sister species of this clade while *Henricksonia mexicana* appeared not related to *Heterosperma* (Fig. 1A, 1B). The ITS tree displays more resolution than the *trnL-trnF* one: *Heterosperma* appeared divided in two groups: Group A (BS=96, PP= 0.99) formed by *C. cyclocarpa*, *H. pinnatum*, and *H. achaetum* Blake (1915: 322) and Group B (BS=100, PP= 1) including *H. diversifolium*, *H. nanum* (Nutt.) Sherff (1931: 312), *H. ovale* Blake (1915: 322), *H. ovatifolium* Cavanilles (1802: 204), and *H. tenuisectum* (Griseb.) Cabrera (1978: 404).

The combined matrix included 50 terminals and has 1497 bp-long, of which 348 (23.2 %) were parsimony informative. The MP analysis resulted in seven trees of 1149 steps (data not shown). The strict consensus tree was fully congruent with the tree obtained under Bayesian inference; the MCC tree showing branch lengths is given in Fig. 2. In this figure, again, all species of *Heterosperma* constituted a strongly supported clade that also included *Coreopsis cyclocarpa* (BS= 100, PP=1). *Electranthera mutica* appeared as the sister species (BS=100 %, PP=1) of the *Heterosperma-Coreopsis cyclocarpa* clade while *Henricksonia mexicana* appears not related to this clade.

### Morphological characters

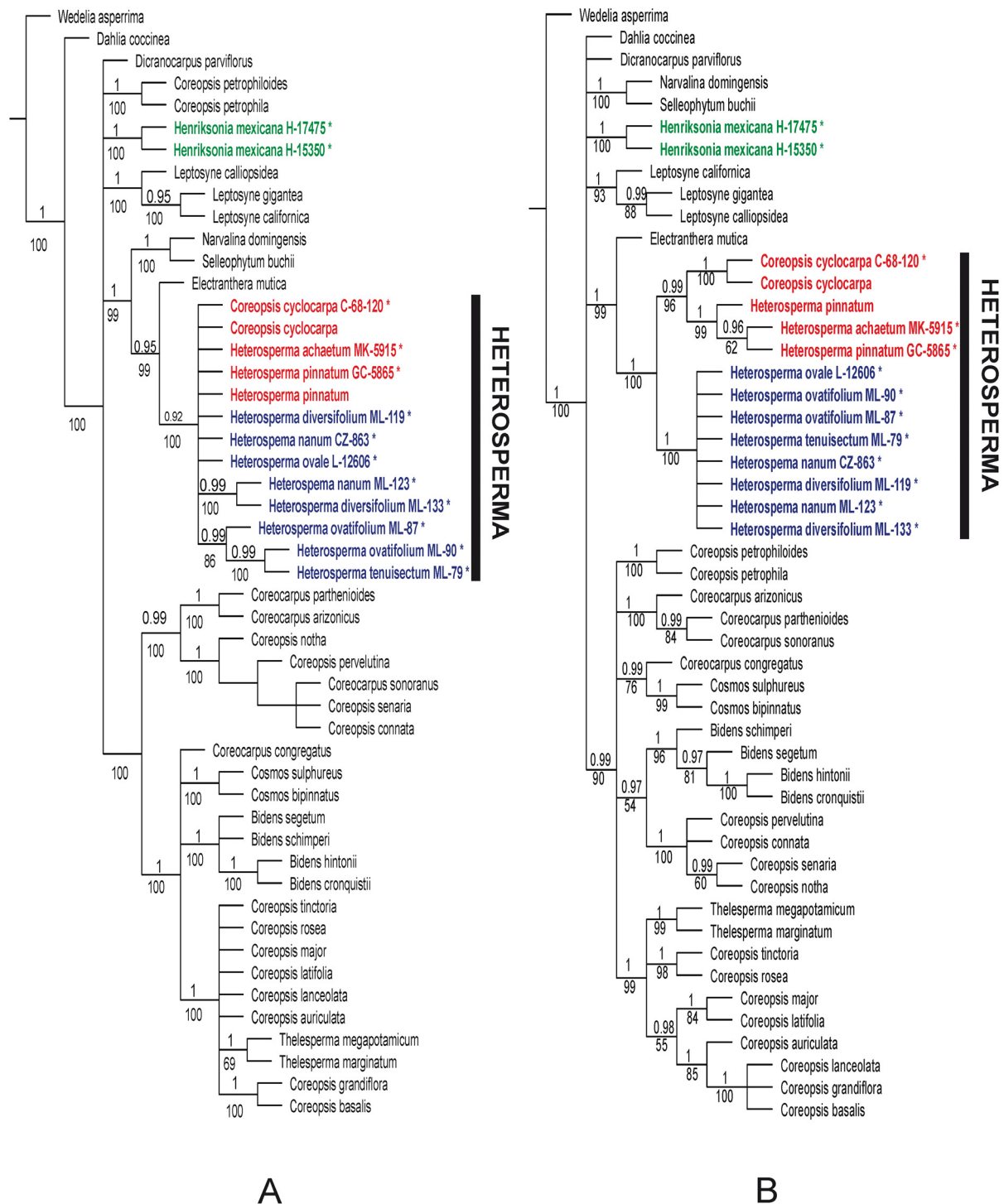
We analyzed the following morphological characters for *Heterosperma* and related taxa.

—*Floral trichomes*: The following trichomes were found on ray and disc corollas:

Moniliform:—uniseriate, 6–10 celled, cells more or less rounded and overlapping, the trichomes resemble beads on a string; present in *Henricksonia* (Fig. 3A).

Filiform:—uniseriate, 4–12 celled, apical cell acute at the apex, all cells with thin walls; present in *Electranthera mutica*, *Heterosperma* (*H. achaetum*, *H. ferreyrii*, *H. ovatifolium*, *H. pinnatum*, and *H. trilobium*), and *Coreopsis cyclocarpa* (Fig. 3C).

Cylindrical:—uniseriate, 8–10 celled, apical cell rounded at the apex, all cells with thin walls; present only in *Heterosperma tenuisectum*.

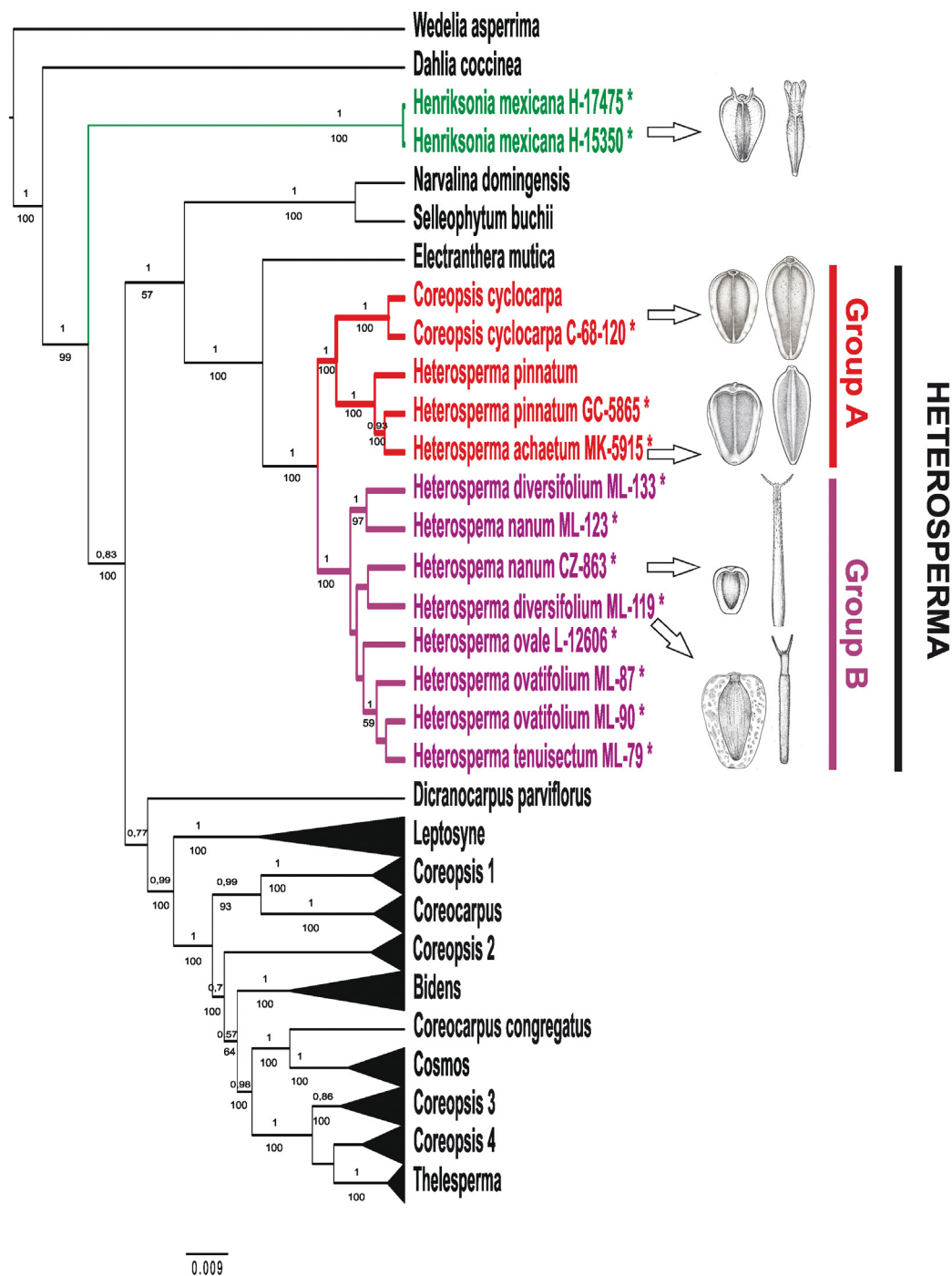


**FIGURE 1.** Parsimony strict consensus tree of: A, *trnL-trnF* and B, ITS. The position of *Heterosperma* is indicated with a black bar. Vouchers sequenced for this study are denoted by asterisks. Number above and below branches indicate Bayesian posterior probabilities (PP) and parsimony bootstrap values (BS), respectively.

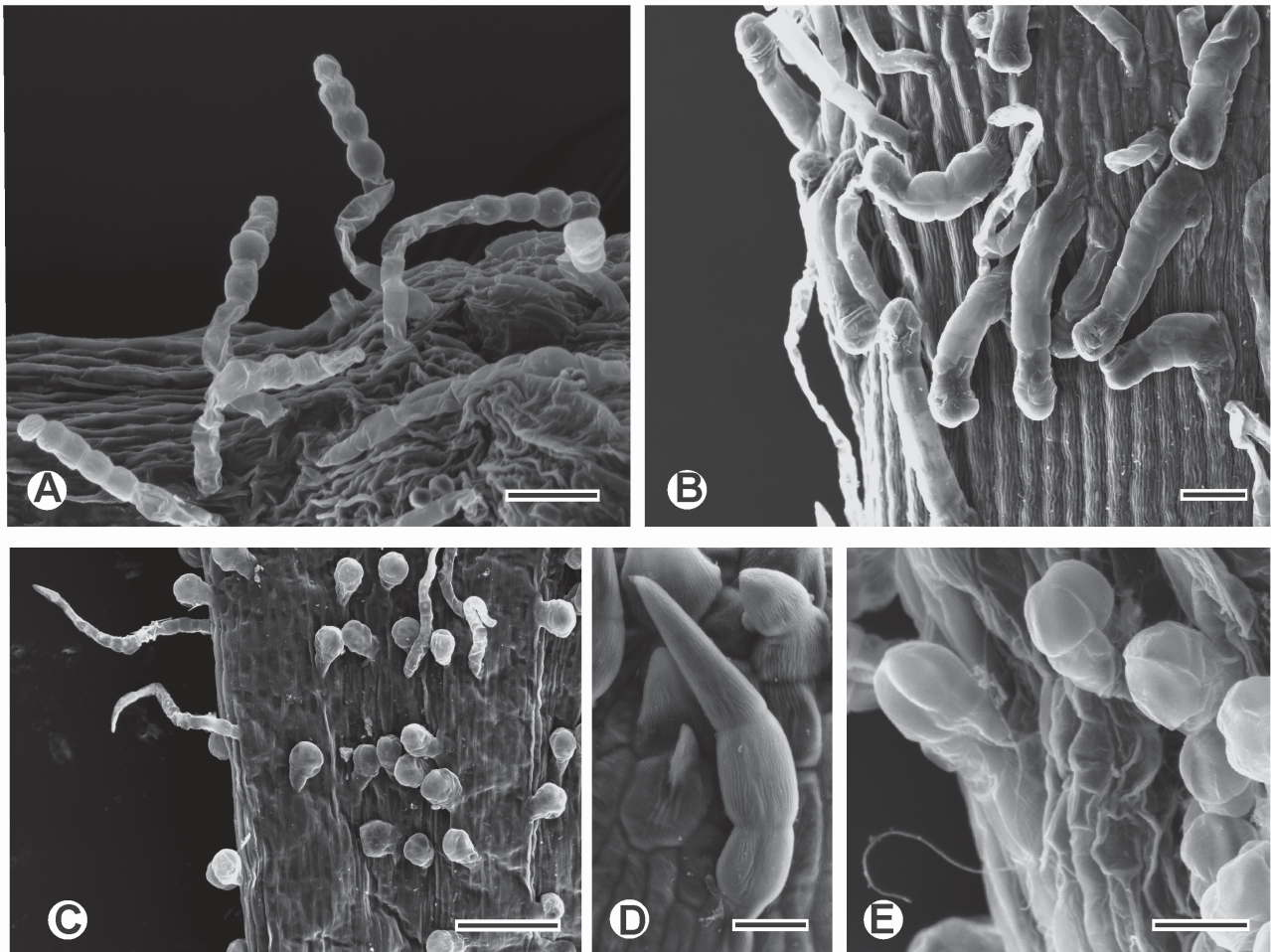
Conical:—uniseriate, 2–3 celled, apical cell acute at the apex, all cells with thick walls; present in *Electranthera mutica*, *Heterosperma pinnatum* and *H. spathulatum* Blake (1915: 323), and *Coreopsis cyclocarpa* (Fig. 3D).

Short glandular trichomes:—foot 1–2 celled; body biseriate with 2–9 cells of thin walls in each row, head 2 celled, the basal cells of the rows are usually short and different in size, towards the apex the cells are longer and similar each other; present in *Heterosperma* (*H. achaetum*, *H. ferreyrii*, *H. ovatifolium*, *H. pinnatum*, and *H. spathulatum*) and *Coreopsis cyclocarpa* (Fig. 3C, E).





**FIGURE 2.** Maximum clade-credibility tree of combined matrix (ITS + *trnL-trnF*). Vouchers sequenced for this study are denoted by asterisks. Number above and below branches indicate Bayesian posterior probabilities (PP) and parsimony bootstrap values (BS), respectively. The shapes of the cypselae are shown for representatives of the different groups, indicated by arrows.



**FIGURE 3.** Floral trichomes (SEM; A–E). *Henricksonia mexicana*, A. Disc florets with moniliiform trichomes. *Electranthera mutica*, B. Disc florets with long glandular trichomes. *Coreopsis cyclocarpa*, C. Ray florets with short glandular trichomes and filiiform trichomes. D. Disc florets with conical trichomes. *Heterosperma sphaatulatum*, E. Ray florets with short glandular trichomes. [A. from Henrickson 15350. B. from Cronquist 10404. C–D. from Carman 68–120. E. from Sodiro 480]. Scale bars: A, B = 50  $\mu$ m; C = 100  $\mu$ m; D–E = 25  $\mu$ m

Long glandular trichomes:—foot 1 or 2 celled, body biseriate elongated with 3–4 long cells in each row, head 1–2 celled sharply to slightly demarcated; present in *Electranthera mutica* (Fig. 3B).

—Pappi

Squamous:—membranous with the margins jagged and the outer lower half surface covered with hairs; present on disc cypselae of *Henricksonia*.

Aristate:—a) Short awns or bristles: these are firm and short, cylindric, glabrous or covered with few hairs, present on ray cypselae of *Henricksonia*, b) Long awns or bristles: these are firm and long, flat, covered with long hairs, downwardly (retroscely) directed; present on disc cypselae in *Heterosperma* (except *H. achaeum*) and on ray cypselae of *H. trilobium* Blake (1915: 324) and *H. ovale*.

—Cypselae. All cypselae are heteromorphic, varying in *Heterosperma* from graded monomorphic (sub-heteromorphic) to strongly heteromorphic. *Henricksonia* is distinguished by having strongly heteromorphic cypselae, disc cypselae obpyramidal, linear, not winged, (vs. obovate to terete, winged or not in *Heterosperma*). The cypselae of *Electranthera mutica* and *Coreopsis cyclocarpa* are graded monomorphic (sub-heteromorphic), epappose, and obcompressed (Fig 1). All morphological features are summarized in Table 1.

## Discussion

The phylogeny presented here showed that *Heterosperma* is monophyletic if *Coreopsis cyclocarpa* is included in it. This result corroborates the relationship proposed by Kimball & Crawford (2004) and Mort *et al.* (2008) between *Heterosperma pinnatum*–*Coreopsis cyclocarpa* in their phylogenies of Coreopsideae, and the inclusion of *Coreopsis cyclocarpa* in *Heterosperma* suggested by Panero (2007).

The monotypic genus *Henricksonia* appeared included in *Heterosperma* in the Coreopsideae phylogenies mentioned above, being this position of *Henricksonia* unexpected by the authors. Here, using two new vouchers of *Henricksonia mexicana*, the inclusion of *Henricksonia* in *Heterosperma* was rejected (according to a personal communication of D. Crawford, some kind of error could have occurred with the sequence of *Henricksonia* used in their Coreopsideae phylogenies and submitted to Genbank). This rejection is also in accord with morphological evidence, as was reported by Panero (2007), since *Henricksonia* clearly differs from *Heterosperma* in its ray cypselae with 2(–3) short awns (vs. epappose cypselae or cypselae with 2 long awns in *Heterosperma*), disc cypselae with 4 scales (vs. without scales or with 2(–4) long awns), corolla with moniliform trichomes (vs. filiform, conical, cylindric trichomes), and anthers with glandular trichomes (vs. trichomes absent) (Table 1).

Within *Heterosperma*, *Coreopsis cyclocarpa* appears related to the clade *Heterosperma pinnatum*–*H. achaetum* of Group A. This relationship is strongly supported by three molecular synapomorphies of the ITS alignment. In concordance with this, the three taxa share the narrowly filiform to linear leaf segments,  $\leq 2$  mm (vs. lanceolate to ovate,  $\geq 4$  mm in Group B) and Northern distribution, i.e. *H. pinnatum* occurs from Southern U.S. to North Colombia, *H. achaetum* is restricted to Colombia, and *C. cyclocarpa* is restricted to Mexico (Fig. 4). In addition, *Coreopsis cyclocarpa* also shares with all *Heterosperma* species its floral trichomes and resembles to *H. achaetum* in having graded monomorphic (sub-heteromorphic), and epappose cypselae (Table 1).

The Group B of *Heterosperma* includes four South American and one Caribbean species (*H. ovale*). This clade is strongly supported by seven molecular synapomorphies of the ITS alignment. The species of Group B have predominantly strongly heteromorphic cypselae and simple to bipinnatisect leaves with broad blades or lanceolate to ovate segments, respectively.

*Electranthera mutica*, the type species of *Electranthera* Mesfin, D.J. Crawford, & Pruski (Pruski *et al.* 2015: 4) appears as the sister species of the clade *Heterosperma*–*Coreopsis cyclocarpa* corroborating the results of the Coreopsideae phylogenies of Kimball & Crawford (2004) and Mort *et al.* (2008). *Electranthera* comprises three Mexican species (*E. mutica*, *E. cuneifolia* (Greenm.) Mesfin, Crawford, & Pruski (Pruski *et al.* 2015: 5) and *E. parvifolia* (S.F. Blake) Mesfin, Crawford, & Pruski (Pruski *et al.* 2015: 15). *Electranthera mutica* shares with *Heterosperma* such features as that of the corolla, with conical and filiform trichomes, and ray cypselae with wings but mainly differs from it in having flat receptacle (vs. convex), 5–40 capitula (vs. 3–5 or 1), corolla with long glandular trichomes (vs. short glandular trichomes) (Table 1), and ray cypselae that are 5–13 mm long (vs. 1.5–4 mm long).

## Key to *Heterosperma* and related genera

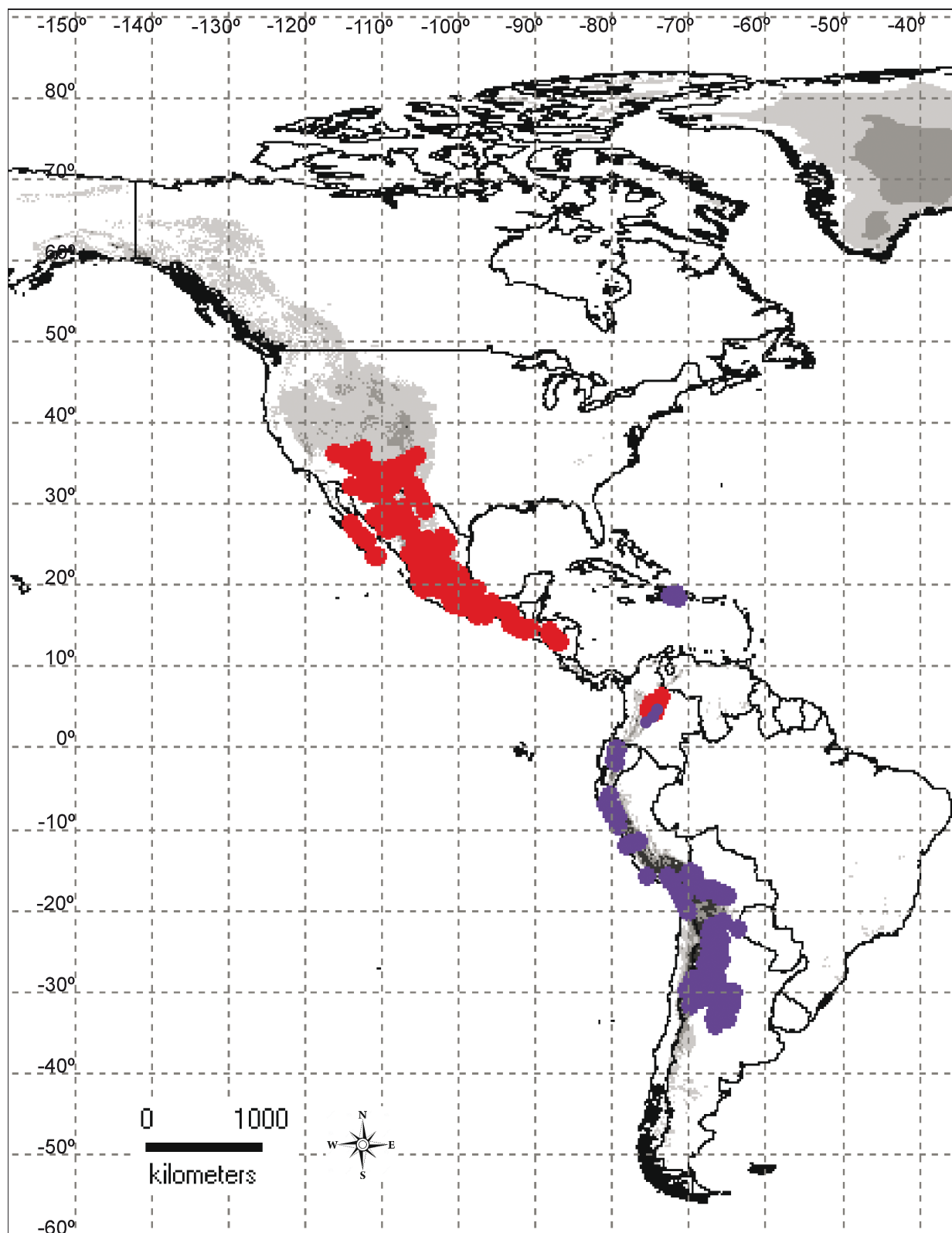
1. Disc cypselae with 4 scales, not winged; ray cypselae with 2(–3) short awns; anthers with glandular trichomes; corolla with moniliform trichomes and without glandular trichomes ..... *Henricksonia*
- Disc cypselae with 2(–4) long awns or epappose, usually winged; ray cypselae with 2 long awns or epappose; anthers without glandular trichomes; corolla with filiform, conical, cylindrical, and glandular trichomes ..... 2.
2. Receptacle flat; capitula 5–40, cypselae graded monomorphic (sub-heteromorphic); ray cypselae 5–13 mm long; disc cypselae epappose; corolla with long glandular trichomes; pollen 31.7–32  $\mu\text{m}$  diam ..... *Electranthera mutica*
- Receptacle convex; capitula 3–5 or 1, cypselae graded monomorphic (sub-heteromorphic) to strongly heteromorphic; ray cypselae 1.5–4 mm long; disc cypselae epappose or with 2(–4) long awns; corollas with short glandular trichomes; pollen 18.5–23.3  $\mu\text{m}$  diam ..... *Heterosperma* (including *Coreopsis cyclocarpa*)

## Taxonomic Treatment

***Heterosperma*** Cavanilles (1794: 34).

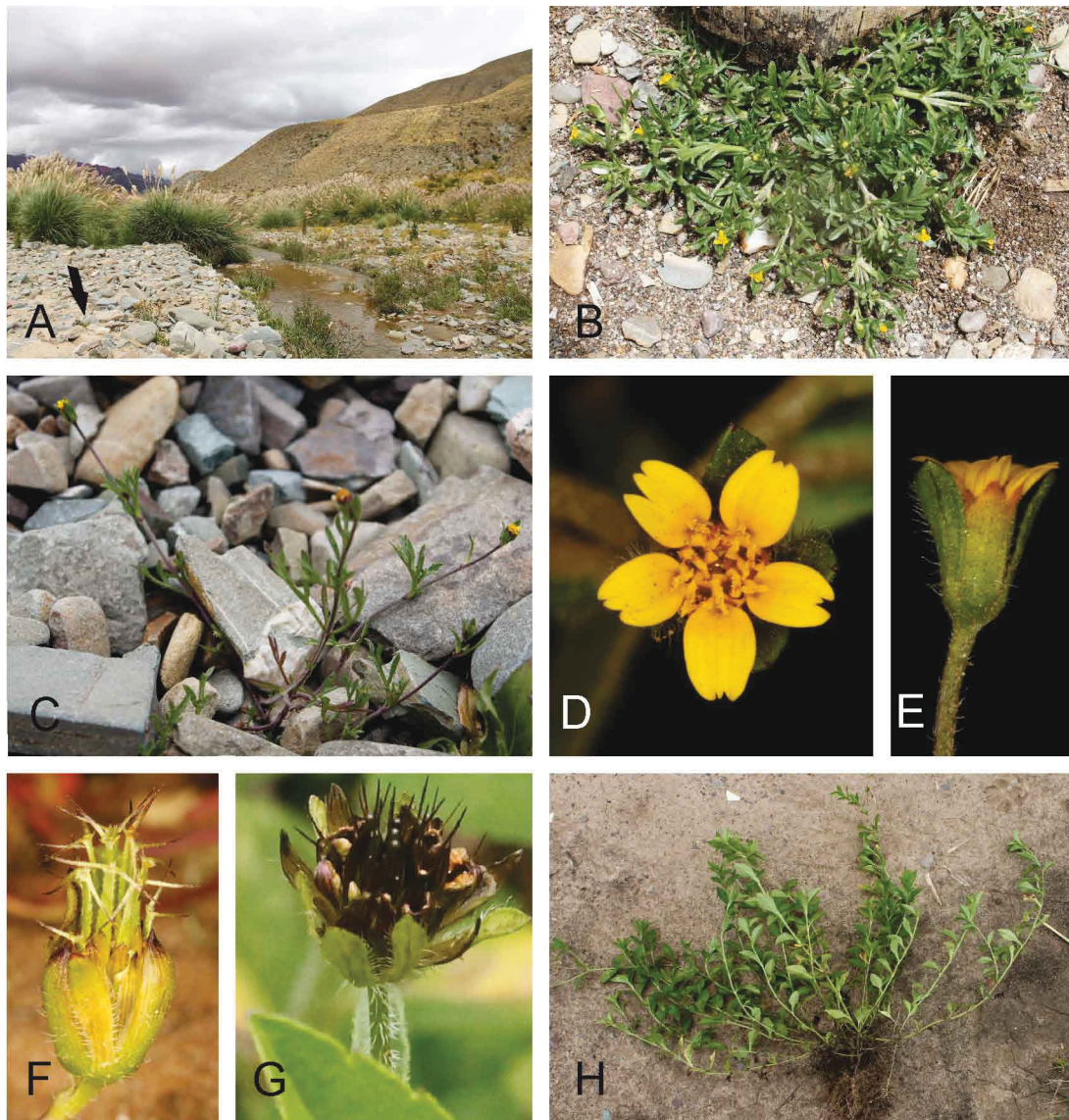
TYPE SPECIES:—*Heterosperma pinnatum* Cav.

**Geographic distribution and habitat:**—W North America to NW South America and Caribbean (Fig. 4); wooded grassland, roadside ditches, and open mountain slopes: 650–4500 m (Fig. 5).



**FIGURE 4.** Geographic distribution of *Heterosperma* Group A (red circles) and Group B (violet circles).





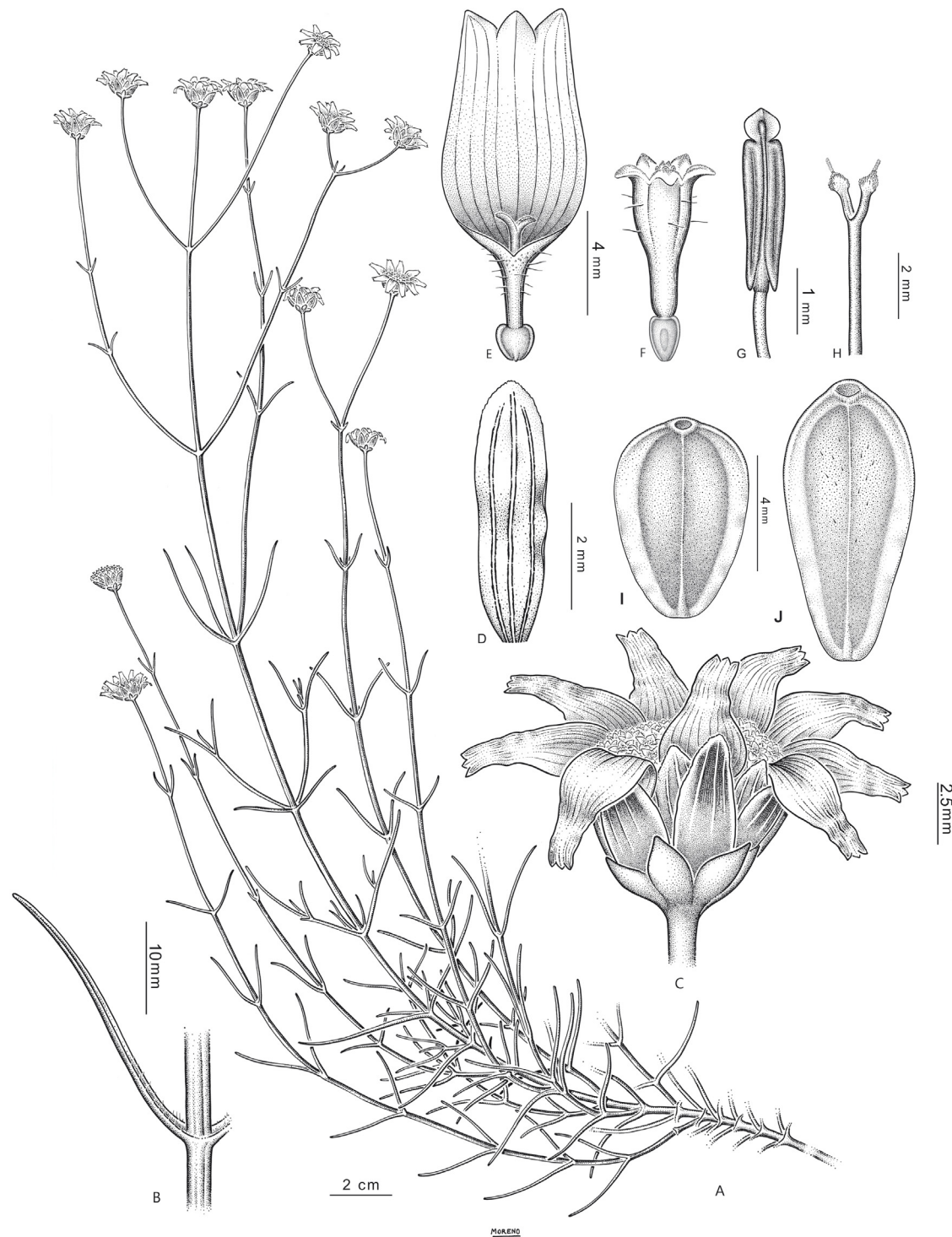
**FIGURE 5.** *Heterosperma*. A–F. *H. nanum*. A–B. Habit. C. Floriferous stems. D. Capitulum showing ray and disc florets. E. Capitulum showing inner and outer phyllaries. F. Capitulum showing cypselae. G–H. *H. ovatifolium*. G. Capitulum showing maturate cypselae. H. Habit [B. from Lizarazu & Satto 125 (SI). C. from Lizarazu & Satto 142 (SI). G–H. from Lizarazu 87 (SI). F. from Zuloaga *et al* 14207 (SI), and D–E, from Zanotti *et al.* 863 (SI)]. Photo credit: Lizarazu 2017 (A–C, G–H), Zuloaga 2013 (F), and Zanotti 2016 (D–E).

***Heterosperma mexicanum*** (A. Gray) Lizarazu & S.E. Freire, **comb. nov.** *Leptosyne mexicana* A. Gray ex Watson (1887: 429). *Coreopsis cyclocarpa* Blake (1914 [1913]: 339).

TYPE:—MEXICO. Jalisco, Rio Blanco, 17 Sept 1886, *E. Palmer* 568 (holotype US-00128148!; isotypes GH-00009645!, K-0004979490!, MEXU-012220337!, MO-1840292!, NDG-60484!, NY-00180551!, NY-00180552!, P-02140481!, PH-00014921!, US-00931206!, YU-002167!). Fig. 6.

Perennial *herbs*, erect, 60–70 cm tall, stems numerous from a thick woody base, slightly glabrous to pubescent below. *Leaves* opposite, sessile, entire, linear to filiform, 20–60 × 1.5–2 mm, glabrous. *Capitula* in open corymbiform cymes, peduncles 2–7 cm long, glabrous to rarely sparsely hispid. Involucre hemispheric. *Phyllaries* 2–3 seriate: outer 5–6, narrowly lanceolate, 2–3 × 1.5–2 mm, glabrous, fleshy; inner 8–12, ovate to lanceolate, 5–9 × 4 mm, striate, glabrous, scarious, yellow, reddish and pale on margins. *Receptacle* convex, paleate; palea oblanceolate, 4–5 × 1.5 mm, obtuse, 3-nerved, membranaceous, glabrous. *Ray florets* yellow, 8–10, female, fertile, rays 3-lobed, 11–13 × (3–)5.5–7 mm,

12–14-nerved; tube narrow, with glandular and non-glandular trichomes. *Disc florets* 15–25(–35), bisexual, fertile; corolla yellow, tubulose, 5-lobed, 3.5–5 mm long, with glandular and eglandular trichomes; anthers 1.5–1.7 mm long, subauriculate at the base, appendages deltate; style arm apices deltate, papillose with long appendages. *Cypselae* graded monomorphic (sub-heteromorphic), obcompressed, winged, epappose, smooth and glabrous, dark brown to black; ray cypselae orbicular to obovate, 3.5–5 × 2.2–3.5 mm; disc cypselae narrowly orbicular to obovate, 6.5–7 × 5–3 mm.



**FIGURE 6.** *Heterosperma mexicanum* (A. Gray) Lizarazu & S.E. Freire var. *mexicanum*. A. Habit. B. Entire leaf. C. Capitulum. D. Palea. E. Ray floret. F. Disc floret. G. Stamen. H. Style branches. I. Ray cypsel. J. Disc cypsel (from Carman 68-120 (TEX)).



**Geographic distribution and habitat:**—Endemic of Mexico (Jalisco, Nayarit, Zacatecas); in grassy oak woods, on granitic or rocky slopes; 1500–2200 m (Fig. 7).

**Additional specimens examined:**—MEXICO. Jalisco: Near Guadalajara, 4 Oct 1903, *Pringle 11546* (SI-paratype); on rocky slopes of Cerro Etzatlan directly S of Etzatlan, 9 Sep 1966, *Melchert et al. 6359* (TEX); in grassy oak woods on granitic slopes overlooking Guadalajara from the west, 15 Sep 1974, *Cronquist 11195* (TEX); Guadalajara: Route 15, ca 5 mi west Guadalajara between K-693 and K 694, 20 Sep 1968, *Carman 68-120* (TEX); Nayarit. 105 Km WNW of Huejuquilla, El Alto along road to Jesus Maria near side road to Santa Lucia de la Sierra, 9 Sep 1984, *Breedlove 61476* (TEX); Zacatecas: 32 km W on Mexican Hwy 41 just S of Jalpa along road to Tlatenango, 8 Sep 1986, *Breedlove 63942* (TEX).

***Heterosperma mexicanum*** (A. Gray) Lizarazu & S.E. Freire var. ***pinnatisectum*** (S.F. Blake) Lizarazu & S.E. Freire, **comb. nov.** *Coreopsis pinnatisecta* Blake (1914 [1913]: 339). *Coreopsis cyclocarpa* var. *pinnatisecta* (S.F. Blake) Crawford (1970b: 166). *Leptosyne pringlei* Robinson & Greenman (1895: 155–156).

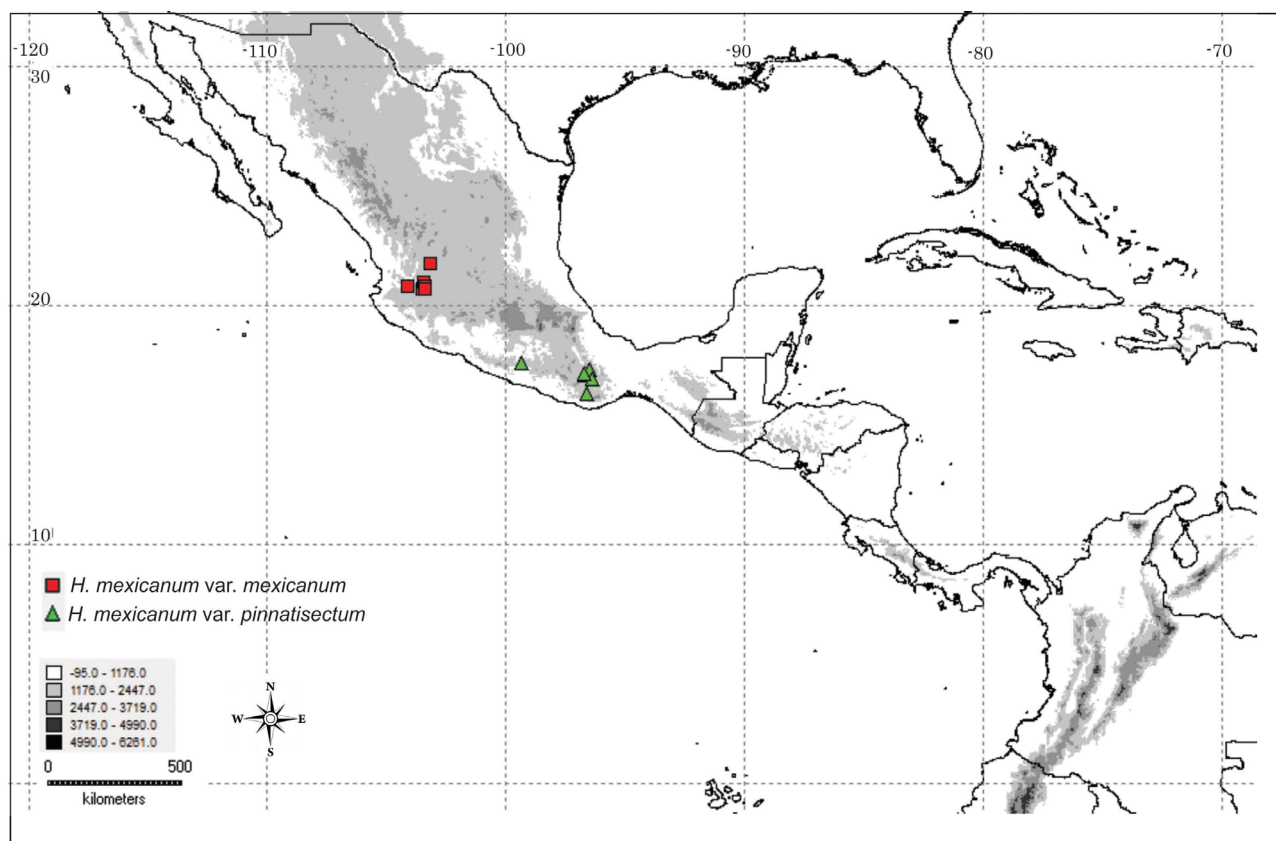
**TYPE:**—MEXICO. Oaxaca: Sierra de San Felipe, 2743 msl, 7 Aug 1894, *C.G. Pringle 4871* (holotype GH-9648!; isotypes AC-319470!, BKL-162!, BR-5430482!, CM-2442!, E-413827!, GOET-1395!, JE-4599!, M-30051!, MEXU-1220335!, MEXU-1220336!, MO-1840278!, NDG-60486!, NY-180553!, P-2140482!, P-2140483!, P-2140484!, PH-14922!, RSA-1371!, S-G-3608!, TEX-369!, UC-128166!, UVMVT-27580!).

Leaves pinnatisect, 25–35 × 15–30 mm, petiole 6–8 mm long, segments linear to filiform, 10–15 × 1–1.5 mm.

**Distribution:**—Endemic to Mexico (Guerrero and Oaxaca). Fig. 7.

**Taxonomic notes:**—*Heterosperma mexicanum* var. *pinnatisectum* is morphologically similar to *H. achaetum* and *H. pinnatum* in its pinnatisect leaves. It differs by being a perennial plant, with inner phyllaries longer than the outer ones, ray florets 11–13 mm long, disc florets 3.5–5 mm long (vs. plant annual, with inner phyllaries shorter than the outer ones, ray florets (1.5–)3–4.5 mm long, disc florets 1–1.2 mm long in *H. achaetum* and *H. pinnatum*). *Heterosperma mexicanum* also resembles *H. achaetum* in its graded monomorphic (sub-heteromorphic) cypselae.

We agree with Crawford (1970a) who observed that *H. mexicanum* var. *mexicanum* (= *Coreopsis cyclocarpa* var. *cyclocarpa*) and *H. mexicanum* var. *pinnatisectum* (= *C. cyclocarpa* var. *pinnatisecta*) are very similar morphologically and appear to differ only by a feature of their leaves, with simple linear to filiform leaves in *H. mexicanum* var. *mexicanum*, and pinnatisect cauline leaves in *H. mexicanum* var. *pinnatisectum*.



**FIGURE 7.** Distribution of *Heterosperma mexicanum*: *H. mexicanum* var. *mexicanum* (squares), *H. mexicanum* var. *pinnatisectum* (triangles).

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**Appendix 1.** List of species used in the phylogenetic analysis including the fourth new vouchers, in bold. Voucher information and GenBank accession numbers (ITS, *trnL*) are shown.

**Tribe Coreopsideae. Subtribe Coreopsidinae:** *Bidens cronquistii* (Sherff) Melchert (AF330102, EU047838). *B. hintonii* (Sherff) Melchert (AF330101, EU047839). *B. schimperii* Sch. Bip. ex Walp. (GU736437, EU047840). *B. segetum* Mart. ex Colla (U67112, EU047836). *Coreocarpus arizonicus* (A. Gray) S.F. Blake (AF330092, EU047842). *C. congregatus* (S.F. Blake) E.B. Sm. (AF330089, EU047843). *C. parthenioides* Benth. (ITS, EU047844). *C. sonorans* Sherff (AF330097, EU047845). *Coreopsis basalis* (A. Dietr.) S.F. Blake (KM347929, KR817893). *C. connata* Cabrera (KM269114, EU047865). *C. cyclocarpa* S.F. Blake **Carman 68–120 (TEX) (new, new)**, (AY561300, EU047846). *C. grandiflora* Hogg ex Sweet (KM347931, EU047850). *C. lanceolata* Michx. (KM347926, KR817896). *C. latifolia* (KM347915, EU047871). *C. major* Walter (KM347911, EU047860). *C. notha* S.F. Blake & Sherff (KM269119, EU053649). *C. pervelutina* Sagást. (AY429083, EU047866). *C. petrophila* A. Gray & S. Watson (KM269136, EU047867). *C. petrophiloides* B.L. Rob. & Greenm. (KM269132, AY216030). *C. rosea* Nutt. (KM347938, EU047859). *C. senaria* S.F. Blake & Sherff (KM269139, EU047869). *C. tinctoria* Nutt. (KM347935, EU047847). *Cosmos bipinnatus* Cav. (KM347948, EU047873). *C. sulphureus* Cav. (KM347949, EU047874). *Dalia coccinea* Cav. (ITS, EU047875). *Dicranocarpus parviflorus* A. Gray (AY429087, EU047876). *Electranthera mutica* (DC.) Mesfin, D.J. Crawford & Pruski (AY561298, EU047854). *Henricksonia mexicana* B.L. Turner **Henrickson 17475 (US) (new, new)**, **Henrickson 15350 (US) (new, new)**. *Heterosperma achaetum* S.F. Blake **Merrill King 5915 (NY) (new, new)**. *H. diversifolium* Kunth **Lizarazu 119 (SI) (new, new)**, **Lizarazu 133 (SI) (new, new)**. *H. nanum* (Nutt.) Sherff **Lizarazu 123 (SI) (new, new)**, **Zanotti 863 (SI) (new, new)**. *H. ovale* S.F. Blake **Liogier 12606 (NY) (new, new)**. *H. ovatifolium* Cav. **Lizarazu 87 (SI) (new, new)**, **Lizarazu 90 (SI) (new, new)**. *H. pinnatum* Cav. **Guirardo Cañas 5865 (COL) (new, new)**, (AY429094, EU047879), *H. tenuisectum* (Griseb.) Cabrera **Lizarazu 79 (SI) (new, new)**. *Leptosyne californica* Nutt. (KM275385, EU047864). *L. calliopsidea* (DC.) A. Gray (ITS, EU047870). *L. gigantea* Kellogg (KM275383, EU047872). *Narvalina domingensis* (Cass.) Less. (ITS, EU047880). *Selleophytum buchii* Urb. (AY429082, EU047881), *Thelesperma marginatum* Rydb (AY429100, EU047882). *Thelesperma megapotamicum* (Spreng.) Kuntz. (AY429101, EU047883) **Tribe Heliantheae. Subtribe Ecliptinae:** *Wedelia asperrima* Benth. (JX564771, JX564691)