

## Zuloagocardamum (Brassicaceae: Thelypodieae) a New Genus from the Andes Highlands of Northern Argentina

Diego L. Salariato<sup>1,3</sup> and Ihsan A. Al-Shehbaz<sup>2</sup>

<sup>1</sup>Instituto de Botánica Darwiniion, Labardén 200, Casilla de Correo 22, B1642HYD San Isidro, Buenos Aires, Argentina.

<sup>2</sup>Missouri Botanical Garden, P. O. Box 299, St. Louis, Missouri 63166–0299, U. S. A.

<sup>3</sup>Author for correspondence (dsalariato@darwin.edu.ar)

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**Abstract**—*Zuloagocardamum jujuyensis*, a new genus and species of Brassicaceae from Jujuy Province in Argentina, is described and illustrated, and its phylogenetic relationships to nearest relatives are demonstrated. It resembles some genera of the tribe Thelypodieae, such as *Chilocardamum* and *Weberbauera*, but differs mainly by having a well-developed woody caudex with reduced leafless stems, rosulate, awl-shaped or linear, sessile, parallel-veined basal leaves conspicuously ciliate with simple trichomes, racemes much shorter than the basal leaves, torulose fruits, and mucilaginous seeds. Phylogenetic analyses, based on DNA sequences of nuclear ITS and plastid *ndhF* and *trnL-F* regions, place *Z. jujuyensis* in the tribe Thelypodieae, where it is related to species of *Weberbauera*, *Englerocarlis*, and *Parodiodesoxa*. However, it is morphologically different from species of all four genera by the character combinations above.

**Keywords**—*Chilocardamum*, *Englerocarlis*, ITS, *ndhF*, *trnL-F*, *Weberbauera*.

Brassicaceae (Cruciferae) is a well-defined family distributed worldwide and includes approximately 321 genera and 3,660 species currently assigned to 49 tribes (Al-Shehbaz 2012a; Koch et al. 2012). Numerous phylogenetic studies (e.g. Bailey et al. 2006; Beilstein et al. 2006, 2008; Warwick et al. 2006, 2007, 2008, 2009, 2010, 2011; Al-Shehbaz and Warwick 2007; Koch et al. 2007; German and Al-Shehbaz 2008; Franzke et al. 2009; German et al. 2009; Khosravi et al. 2009; Koch and Al-Shehbaz 2009; Warwick and Hall 2009; Couvreur et al. 2010; Koch et al. 2012; Salariato et al. 2013) have recently resolved the phylogenetic and tribal position of approximately 94% of the genera (Warwick et al. 2010; Al-Shehbaz 2012a). Most species grow in temperate areas, with the highest diversity in the Irano-Turanian region, Mediterranean area, and western North America (Al-Shehbaz 1984; Appel and Al-Shehbaz 2003). The family is also well represented in southern South America, especially Argentina and Chile, where approximately 71 genera and 301 species grow (Al-Shehbaz 2008). Argentina has 59 genera and 222 species, of which 103 species are native and 57 are endemic (Al-Shehbaz 2012b).

During fieldtrips to the highlands of Jujuy in northern Argentina, in particular to Mina Aguilar in the department of Humahuaca, collections of an unknown Brassicaceae were made. This locality corresponds to the puna biogeographical province of Cabrera and Willink (1973) and was visited by Angel L. Cabrera, together with other botanists, in several collection trips mainly performed between 1948 and 1970, as part of the project “Flora de Jujuy”. Recently, several field trips were done to this region as part of the project “Flora of Argentina”. Mina Aguilar and its surroundings are included in an area of endemism that harbors nearly 1/3 of all vascular plants endemic to Argentina (Aagesen et al. 2012); three endemic species are restricted to this region: *Lobivia marsoneri* (Werderm.) Backeb. (Cactaceae), *Nototrichia macrotuba* Krapov. (Malvaceae), and *Senecio altoandinus* Cabrera (Asteraceae).

Morphological analyses of the vegetative and reproductive characters confirmed that the specimens represent an undescribed taxon, while preliminary phylogenetic studies using nuclear and plastid markers (see below) revealed that this new species belongs to the tribe Thelypodieae. The goals

of the present study were to determine the generic placement of this novelty and identify its nearest relatives in the tribe.

### MATERIALS AND METHODS

**Morphological Study**—Herbarium specimens at the Darwiniion Institution (SI) and fresh material collected during fieldtrips in northwestern Argentina were the basis of this study. Plant parts were re-hydrated prior to measurements, and flowers and fruits were measured using an ocular micrometer. Leaf trichomes, pollen, and seeds were examined on a Philips XL series 30 scanning electron microscope (SEM), operating at 10–15 kV at the Museum of Natural Sciences “Bernardino Rivadavia”. Leaf fragments and seeds were cleaned with xylene and subjected to sonication for 5 min, dried at room temperature for 12 hrs, mounted on aluminum stubs, and coated with gold:palladium 40:60%. Pollen grains were mounted directly on specimen stubs with double-sided adhesive and sputter-coated with gold:palladium 40:60%. Cross-sections of leaves preserved in 70% ethanol were prepared by hand, dyed with methylene blue, and mounted on semi-permanent microscope slides in polyvinyl alcohol (Omar et al. 1979). The presence of seed-coat mucilage was tested by placing them in a 0.01% (w/v) aqueous solution of ruthenium red.

**Molecular Sampling**—Sequences of the nuclear ribosomal internal transcribed spacer (ITS) region (ITS1–5.8S–ITS2), the chloroplast NADH dehydrogenase subunit F (*ndhF*) gene, and the the chloroplast tRNA-Leu (*trnL*) gene together the *trnL-trnF* intergenic spacer, were obtained for the new species from Zuloaga et al. 13574 and 14267 (vouchers at SI, see Appendix 1). The ITS, *ndhF*, and *trnL-F* Genbank numbers are KF569756, KF569759, and KF569762 for Zuloaga et al. 13574; and KF569757, KF569760, and KF569763 for Zuloaga et al. 14267, respectively. The ITS, *ndhF*, and *trnL-F* sequences were also obtained for one accession of *Chilocardamum patagonicum* (Speg.) O. E. Schulz [Zuloaga et al. 14102 (SI); KF569758, KF569761, and KF569764, respectively]. These nine new sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov>).

Molecular analyses were initially conducted to determine the tribal affiliation of this novelty within the Brassicaceae and later to establish the generic-level relationship within the tribe Thelypodieae. For the analyses at the tribal level, 96 (ITS), 113 (*ndhF*), and 89 (*trnL-F*) sequences of Brassicaceae, representing 45, 39 and 34 tribes, respectively, were downloaded from Genbank. *Cleome lutea* Hook. (ITS), *C. rutidosperma* DC. (*ndhF*) and *C. spinosa* Jacq. (*trnL-F*) were used as outgroups because Cleomaceae is widely recognized as the sister family to the Brassicaceae (Hall et al. 2002). The analyses within Thelypodieae included 66, 80, and 58, ITS, *ndhF*, and *trnL-F* sequences, respectively; representing ca. 92% of the genera currently assigned to this tribe (Al-Shehbaz 2012a). A few species of tribes Brassiceae, Isatideae, and Sisymbrieae were selected as the outgroup. All Genbank accession numbers are listed in the Appendix 1.

**Extraction, Amplification, and DNA Sequencing**—Total DNA was isolated from silica gel-dried leaves from natural populations using the modified (CTAB) procedure of Doyle and Doyle (1987). The ITS region was PCR-amplified using the ITS4 and ITS5 primers of Baldwin (1992),

whereas the *ndhF* gene was amplified using the pairs of primers 5F/972R and 972F/1666R (Olmstead and Sweeney 1994). The *trnL-F* region was amplified using primers C of Taberlet et al. (1991) and Fdw (5' CAGT CCTCTGCTCTACCAGC 3'). The PCR reactions were performed in 25  $\mu$ L final volumes with 50–100 ng template DNA, 0.2  $\mu$ M of each primer, 25  $\mu$ M dNTP, 5 mM MgCl<sub>2</sub>, 1  $\times$  buffer, and 1.5 U Taq polymerase provided by Invitrogen Life Technologies. PCR amplifications were set at: ITS 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 50°C for 1 min, and 72°C for 90 sec, with a final extension at 72°C for 7 min; *ndhF* 96°C for 4 min; 39 cycles of 94°C for 90 sec, 55°C for 1 min, and 72°C for 90 sec; and a final extension of 73°C for 7 min; *trnL-F* 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, 48°C for 1 min, and 72°C for 90 sec, with a final extension at 72°C for 10 min. Cleaning of PCR products and sequencing reactions were performed by Macrogen (Seoul, Korea). Sequences were assembled and edited using the software Chromas Pro v1.41 (Technelysium, South Brisbane, Australia).

**Sequence Alignments and Phylogenetic Analyses**—Alignments were generated with Muscle v3.6 (Edgar 2004) using a first round of multiple alignments and posterior rounds of refining under the default settings. Where necessary, the resulting alignments were checked and improved manually by visual refinement using the program Bioedit v7.0.9.0 (Hall 1999). All aligned matrices were submitted to TreeBASE (study URL: <http://purl.org/phylo/treebase/phylows/study/TB2:S14386>). Brassicaceae and Thelypodieae datasets from ITS, *ndhF*, and *trnL-F* were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Congruence among reduced ITS, *ndhF*, and *trnL-F* datasets of the tribe Thelypodieae (including sequences only shared by the three datasets, 47 sequences) were also analyzed by the incongruence length difference test of Farris et al. (1995) in TNT v1.1 (Goloboff et al. 2008) using the "ILD.run" script with 1,000 replications. The three data sets were combined because they were not significantly incongruent [ $p(\text{ITS vs } \text{trnL-F}) = 0.21$ ,  $p(\text{ITS vs } \text{ndhF}) = 0.27$ ,  $p(\text{ndhF vs } \text{trnL-F}) = 0.39$ ].

Gaps were treated as missing data. For MP analysis, tree searches were generated by the software TNT v1.1 using heuristic searches with

1,000 random addition sequences, tree bisection and reconnection (TBR) branch swapping, and holding 10 trees per replicate. Generated trees were then submitted to a new round of TBR branch swapping to completion. Support values for nodes were estimated by jackknife (JK) analysis (Farris et al. 1996) with 2,000 replicates of 10 random addition sequences, holding four trees per replicate and using the default removal probability (0.36). The ML analysis was conducted using RAxML v7.2.6 (Stamatakis 2006). The models of nucleotide substitution were selected by using the Akaike information criterion (AIC) implemented in jModeltest v0.1.1 (Posada 2008): GTR + I + G (ITS "Brassicaceae" and "Thelypodieae"), TVM + I + G (*ndhF* "Brassicaceae" and "Thelypodieae"), TVM + G (*trnL-F* "Brassicaceae"), and TPM1uf + G (*trnL-F* "Thelypodieae"). The algorithm implemented in RAxML was used to conduct non-parametric bootstrap (BS) analysis and searches for the best-scoring ML tree in a single run (Stamatakis et al. 2008). We executed 1,000 rapid bootstrap inferences and, thereafter, a thorough ML search under the GTR + GAMMA + I (ITS, *ndhF*) and the GTR + GAMMA (*trnL-F*) models. Bayesian analyses were conducted using MrBayes v3.2 (Ronquist et al. 2012). Models (GTR + I + G or GTR + G) were set in MrBayes with rate matrix parameters, state frequencies, gamma shape parameter, and proportion of invariable sites unlinked across partitions. Two simultaneous analyses, starting from different random trees and with four Markov Monte Carlo chains were run for eight million generations and sampled every 1,000 generations to ensure independence of the successive samples. The first 2,000 trees (25% of total trees) were discarded as burn-in. The convergence and effective sample size (ESS) of each replicate were checked using Tracer v. 1.5 (Rambaut and Drummond 2007). The remaining samples of each run were combined, and a maximum clade credibility tree was calculated.

## RESULTS

**Morphology**—Plants of this novelty are perennial with a well-developed woody caudex and much-reduced aerial

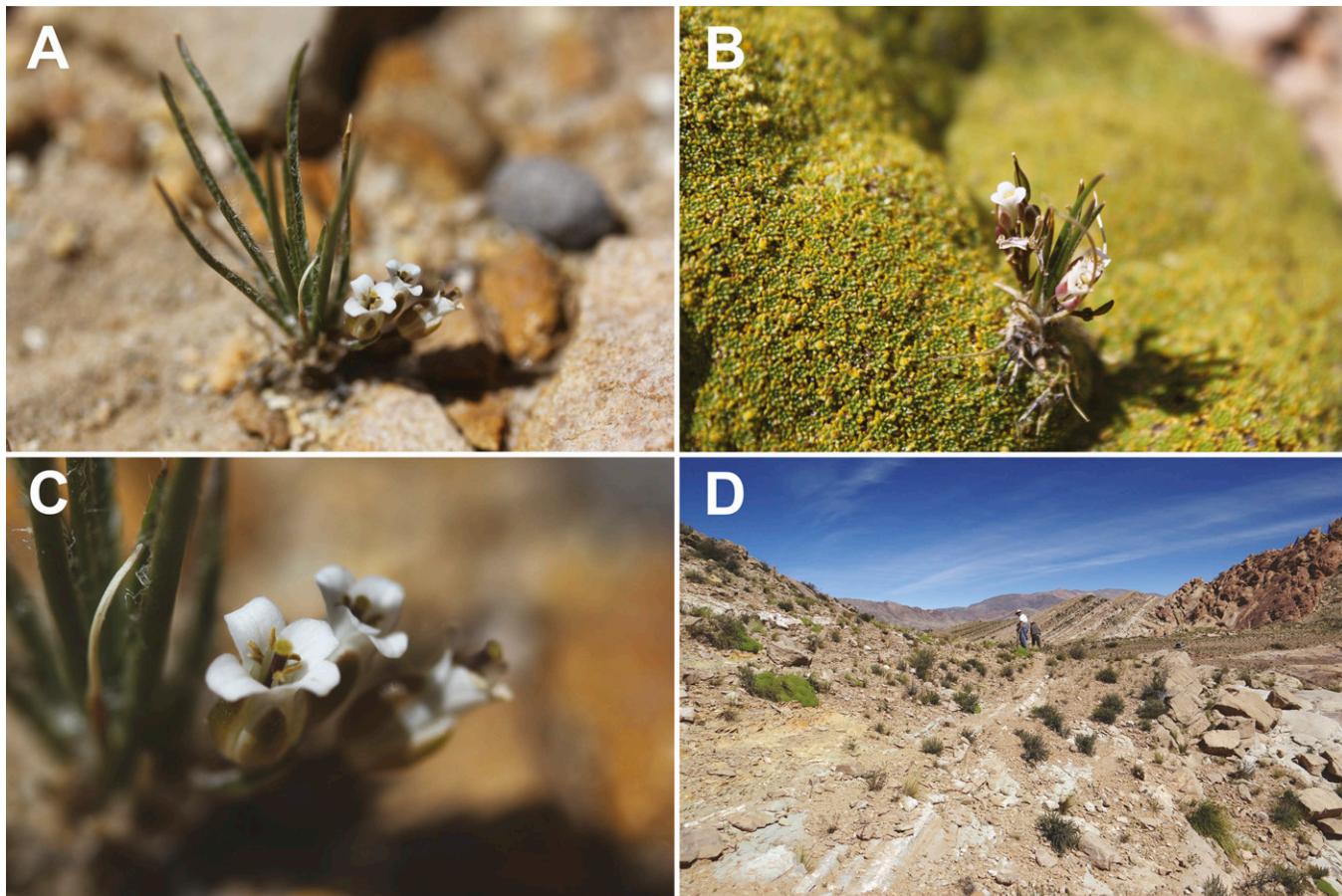


FIG. 1. *Zuloagocardamum jujuyensis*. A. Habit. B. With *Azorella compacta*. C. Flowers. D. Habitat.

stems (Fig. 1A–C). All leaves are basal, rosulate, leathery, sessile, awl-shaped to linear, parallel-veined, involute at the margin, and conspicuously ciliate with simple trichomes (Fig. 2A, B). Numerous second-order vascular bundles are located towards the abaxial surface, and palisade mesophyll

is present on both leaf sides (Fig. 3). The racemes are few-flowered and conspicuously shorter than the basal leaves (Fig. 1A). The flowers have non-saccate sepals, white petals, six stamens subequal in length, and a capitate, slightly 2-lobed stigma (Fig. 1C). Pollen grains are tricolporate (Fig. 4).

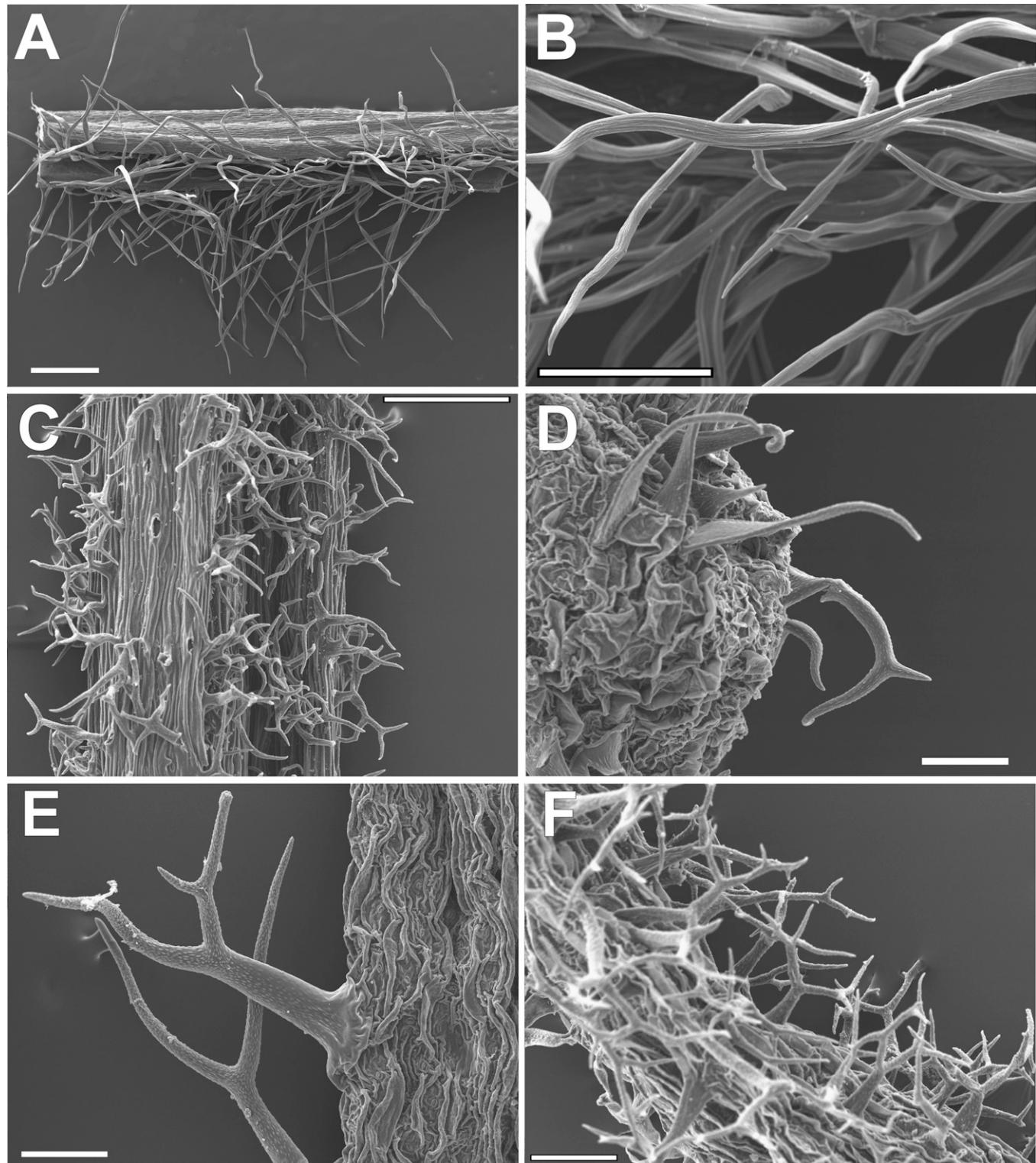


FIG. 2. Trichomes in *Zuloagocardamum jujuyensis* and species of *Chilocardamum*. A–B. Simple trichomes of *Z. jujuyensis* (Zuloaga et al. 13574). C–F. Branched trichomes in *Chilocardamum*. C. *Chilocardamum patagonicum* (Correa 2650). D. *Chilocardamum castellanossi* (Soriano 3882). E. *Chilocardamum onorudifolium* (Soriano 2067). F. *Chilocardamum longistylum* (Soriano 3861). Scale bars A: 500 µm, B–C: 200 µm, D–F: 100 µm.

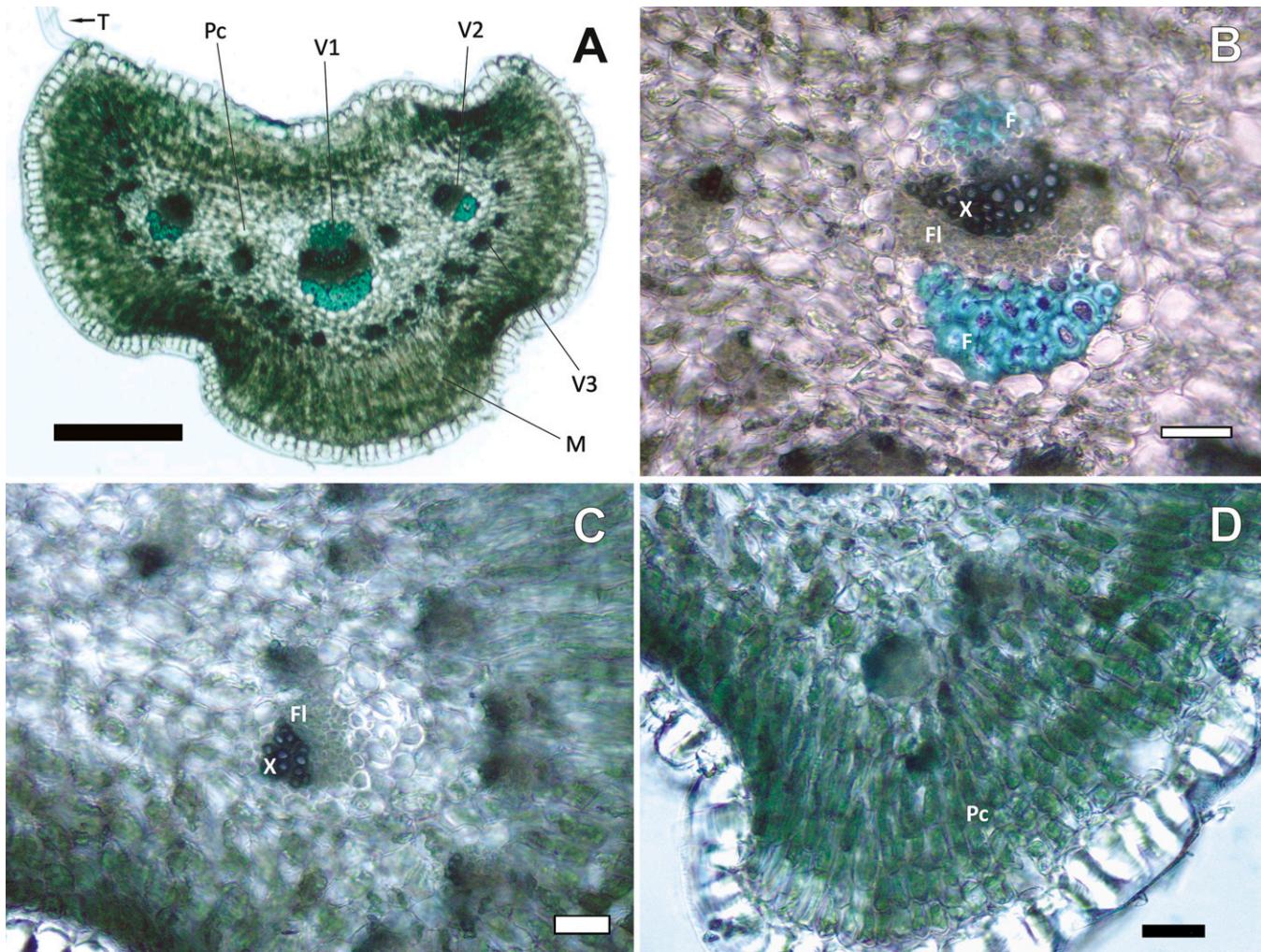


FIG. 3. Leaf blade cross-sections of *Zuloagocardamum jujuyensis*. A. General view. B. Midvein. C. Third-order vascular bundle. D. Mesophyll. Abbreviations: F—fibers, Fl—phloem, M—mesophyll, Pc—parenchyma, T—trichome, V1—midvein, V2—2° and V3—3° order vascular bundle, X—xylem. Scale bars A: 300  $\mu\text{m}$ , B–D: 50  $\mu\text{m}$ . (*Zuloaga et al.* 13574).

The fruits are terete, torulose, slightly curved siliques, and with 6–12 seeds that release mucilage when wetted (Fig. 5).

**Molecular Phylogenetics**—The ITS, *trnL-F*, and *ndhF* sequences were 600, 655, and 1,614 bp long, respectively. The ITS alignment for the Brassicaceae data set included 98 taxa and was 712 bp long, of which 340 (48%) were parsimony-informative. The MP analysis of this region resulted in 54 most parsimonious trees (MPT). The *trnL-F* data set included 91 taxa and was 1,406 bp long, of which 293 (20%) were parsimony-informative, and the MP analyses resulted in more than 10,000 trees. Finally, the *ndhF* data set included 116 sequences and was 2,090 bp long, of which 557 (26%) were parsimony-informative, and the MP analyses produced 1,044 MPT. Phylogenetic analyses of all three markers placed *Zuloaga et al.* 13574 and 14267 within the tribe Thelypodieae (Fig. 6). Monophyly of the tribe was supported both in ITS [jackknife support (JK): 98%, bootstrap support (BS): 90%, posterior probability (PP): 100%] and *ndhF* (JK: 90%, BS: 94%, PP: 100%) (Figs. 6A, C). In contrast, the *trnL-F* data set recovered the tribe in a polytomy together with tribes Brassiceae, Isatideae, and Sisymbrieae (Fig. 6B). However, sequences of *Zuloaga et al.* 13574 and 14267 were included in a supported clade (JK: 58%, BS: 63%, PP: 96%) with spe-

cies of the Thelypodieae genera *Englerocharis* Muschl. and *Weberbauera* Gilg & Muschl.

The alignments of Thelypodieae sequences were: ITS 575 bp long with 124 bp (21%) parsimony-informative, *trnL-F* 779 bp long with 41 bp (5%) parsimony-informative, and *ndhF* 1,522 bp long with 136 bp (9%) parsimony-informative. Maximum parsimony analysis for the three regions resulted in more than 10,000 MPT, and together with the ML and BI topologies, showed poorly resolved relationships within the tribe (Fig. 7A–C). However, all data sets showed a relationship of *Zuloaga et al.* 13574 and 14267 to species of *Weberbauera* and *Parodiodoxa* O. E. Schulz in ITS (JK, BS, PP: <50%) or to *Englerocharis* in *trnL-F* (JK: 56%, BS: 55%, PP: 85%) and *ndhF* (JK: 61%, BS: 70%, PP: 99%). Because the ITS, *ndhF*, and *trnL-F* data sets were not significantly incongruent, the three matrices were concatenated. Trees obtained from MP, ML, and BI analyses were similar and showed again the specimens *Zuloaga et al.* 13574 and 14267 included in a clade together with *W. herzogii* (O. E. Schulz) Al-Shehbaz, *W. rosulans* (O. E. Schulz) Al-Shehbaz and *E. pauciflora* Al-Shehbaz (Fig. 7D), however, in these analyses this clade was much more supported (JK: 83%, BS: 94%, PP: 100%).

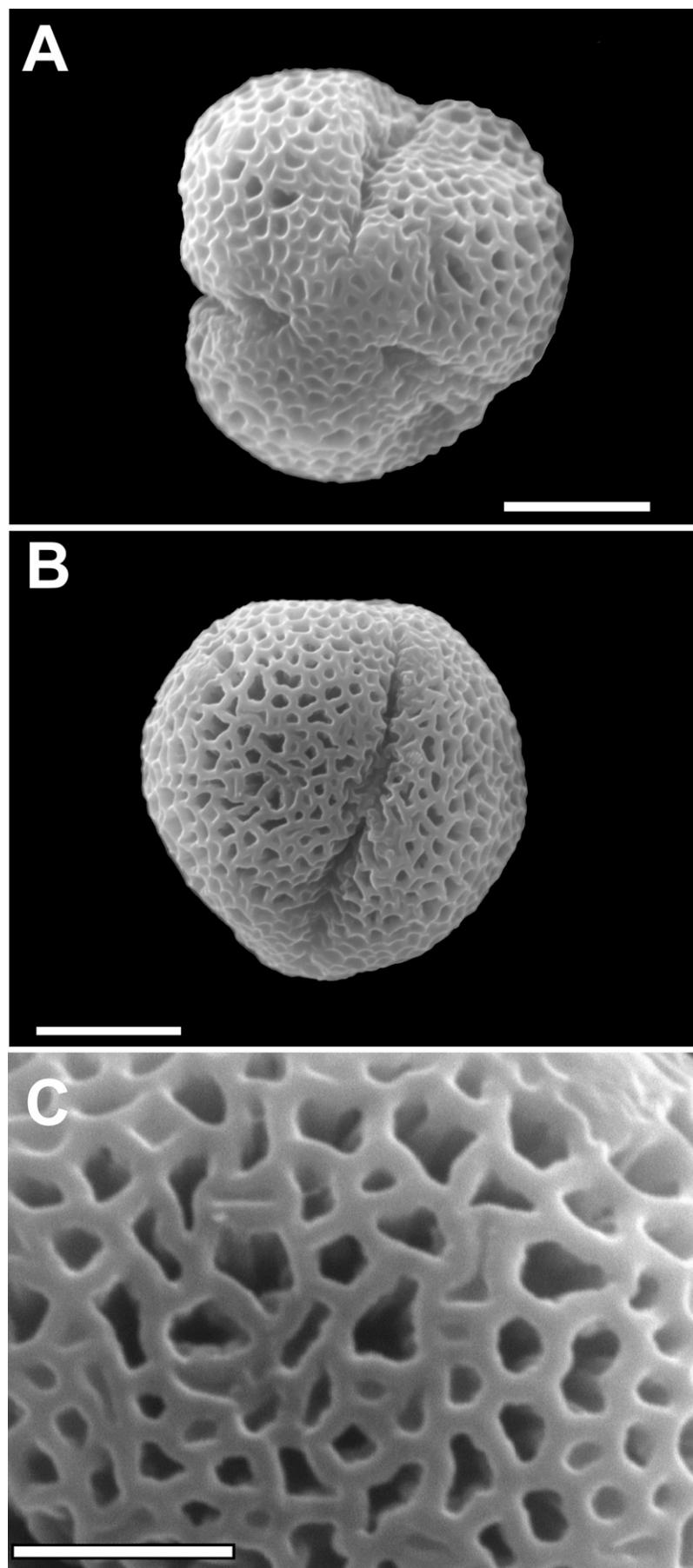


FIG. 4. Pollen of *Zuloagocardamum jujuyensis*. A. Polar view. B. Equatorial view. C. Exine sculpture. Scale bars A–B: 5  $\mu\text{m}$ , C: 2  $\mu\text{m}$ . (Zuloaga et al. 13574).

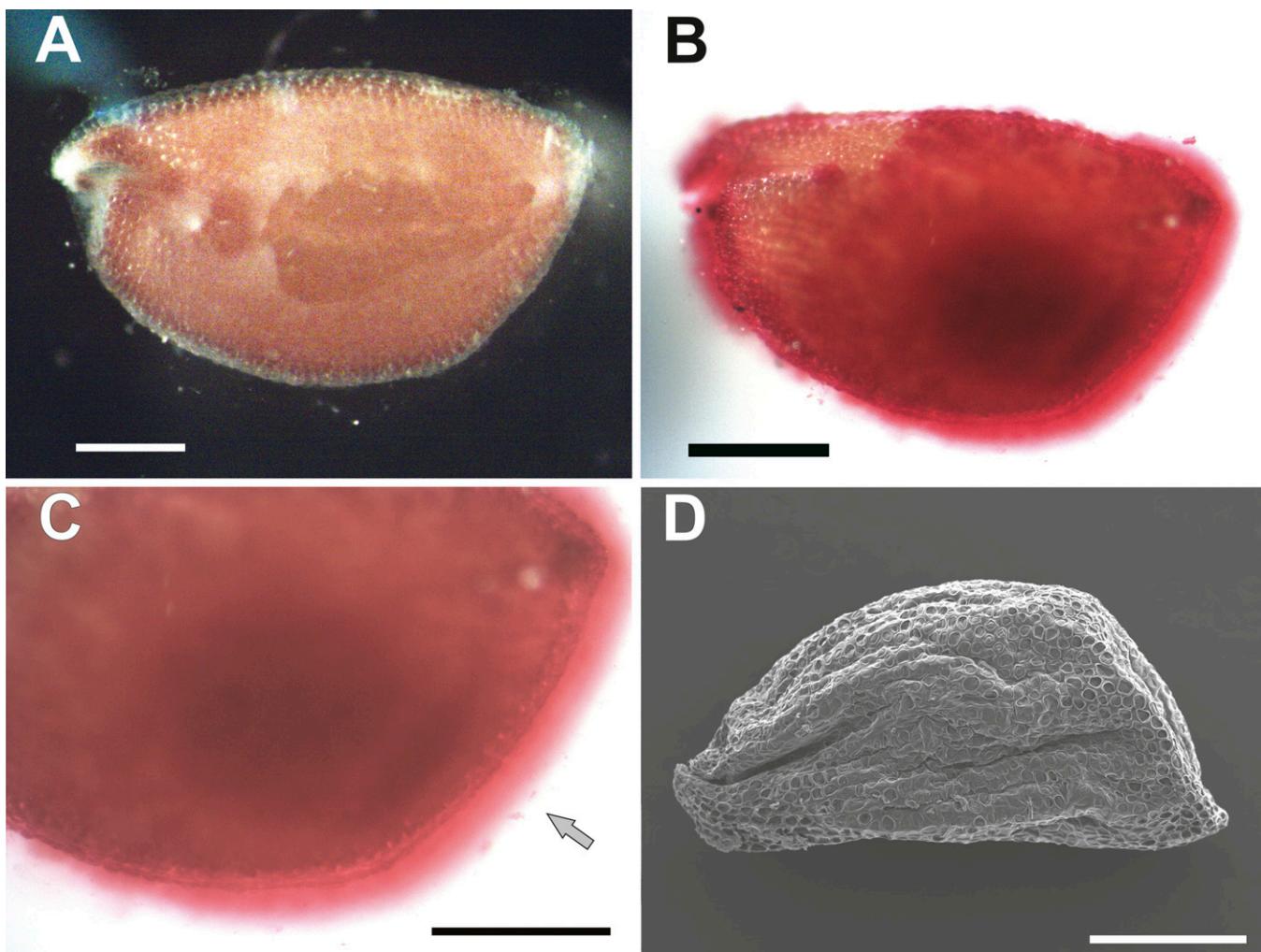


FIG. 5. Seeds of *Zuloagocardamum jujuyensis*. A. Hydrated seed. B. Hydrated seed stained with ruthenium red. C. Detail of mucilage stained with ruthenium red (arrow). D. Dry seed. Scale bars: A–D: 500 µm. (Zuloaga et al. 13574).

#### DISCUSSION

The collections Zuloaga et al. 13574 and 14267 resemble most taxa of tribe Thelypodieae, especially in their perennial habit with a well-developed woody caudex, rosulate basal leaves, simple trichomes, ebracteate racemes, white petals, dehiscent siliques that are terete to slightly latiseptate and torulose, slightly 2-lobed capitate stigmas, and incumbent cotyledons. Molecular phylogenies confirmed their tribal assignment, though relationships within the tribe remain unsolved. Previous phylogenetic studies (e.g. Warwick et al. 2009; Alexander et al. 2010; Warwick et al. 2011; Bartish et al. 2012) also demonstrated the lack of resolution using traditional DNA regions. Although molecular data are exceptionally useful in assigning genera to tribes, they are not helpful in delimiting genera of Thelypodieae. It has been suggested that the tribe is rather young in age and that the currently used molecular markers did not have enough time to diverge (Al Shehbaz 2012c).

*Chilocardamum* O. E. Schulz (four species) resembles the new taxon (Zuloaga et al. 13574 and 14267), hereafter *Zuloagocardamum*, in having sessile, awl-shaped or linear, parallel-veined basal leaves, but it differs by the leafy and well-developed stems (vs. leafless aborted stems), dendritic or forked (vs. simple) trichomes (Fig. 2C–F), 30–54 (vs. 6–12)

ovules per ovary, and non-torulose (vs. torulose) linear fruits. Plants of *Zuloagocardamum* grow at high elevations in the Jujuy Province of northwestern Argentina, whereas species of *Chilocardamum* are endemic to the Patagonian provinces of Neuquén, Río Negro, Chubut, and Santa Cruz. Furthermore, the nuclear and chloroplast molecular phylogenies do not support a relationship between the two genera. Instead, *Zuloagocardamum* shows more affinities to the exclusively South American *Weberbauera*, *Englerocarpha*, and *Parodiodesoxa*.

*Weberbauera* (23 species) is distributed along the Andes of Argentina, Bolivia, Chile, and Peru, and several of its species (e.g. *W. imbricatifolia* (Barnéoud) Al-Shehbaz, *W. minutipila* Al-Shehbaz, and *W. suffruticosa* (Barnéoud) Al-Shehbaz) have woody caudices, simple trichomes, linear basal leaves, and torulose siliques. However, these species clearly differ from *Zuloagocardamum* by having petiolate and not awl-shaped (vs. sessile and awl-shaped) basal leaves with reticulate (vs. parallel) venation, well-developed (vs. reduced) aerial stems with (vs. without) cauline leaves, and racemes conspicuously longer (vs. much shorter) than basal leaves.

The Peruvian *Englerocarpha* (four species) resembles *Zuloagocardamum* in having no cauline leaves, branched caudices densely covered with persistent leaf remains of previous years, and rosulate basal leaves with simple trichomes.

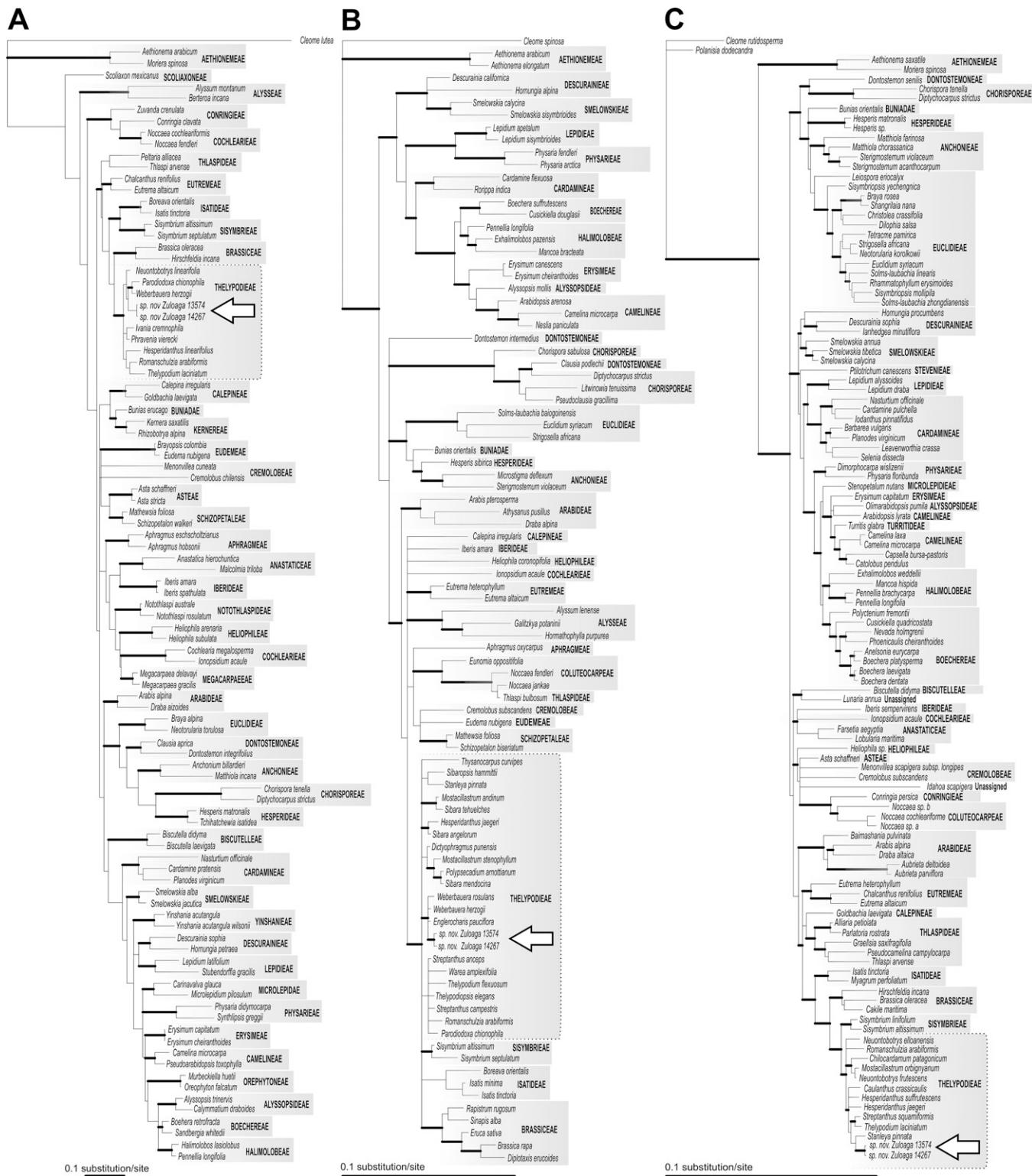


FIG. 6. Maximum clade credibility tree from 12,000 trees obtained in the Bayesian analysis at the tribal level of Brassicaceae. A. ITS. B. *trnL-F*. C. *ndhF*. Tribes are indicated to the right. Thick branches indicate internal branches with posterior probability  $\geq 95\%$ . The arrows show the phylogenetic position of Zuloaga et al. 13574 and Zuloaga et al. 14267 in Thelypodieae.

However, *Englerocharis* differs by its petiolate and not awl-shaped (vs. sessile and awl-shaped) basal leaves with reticulate (vs. parallel) venations, often bracteate (vs. ebracteate) racemes longer (vs. conspicuously shorter) than basal leaves, entire (vs. 2-lobed) stigmas, oblong to oblong-elliptic

and smooth silicles (vs. linear and torulose siliques), and non-mucilaginous (vs. mucilaginous) seeds.

The monotypic *Parodiodoxa* is endemic to northwestern Argentina (Catamarca, Jujuy, La Rioja, Salta, and Tucumán), where it grows on open, rocky soils at elevations of

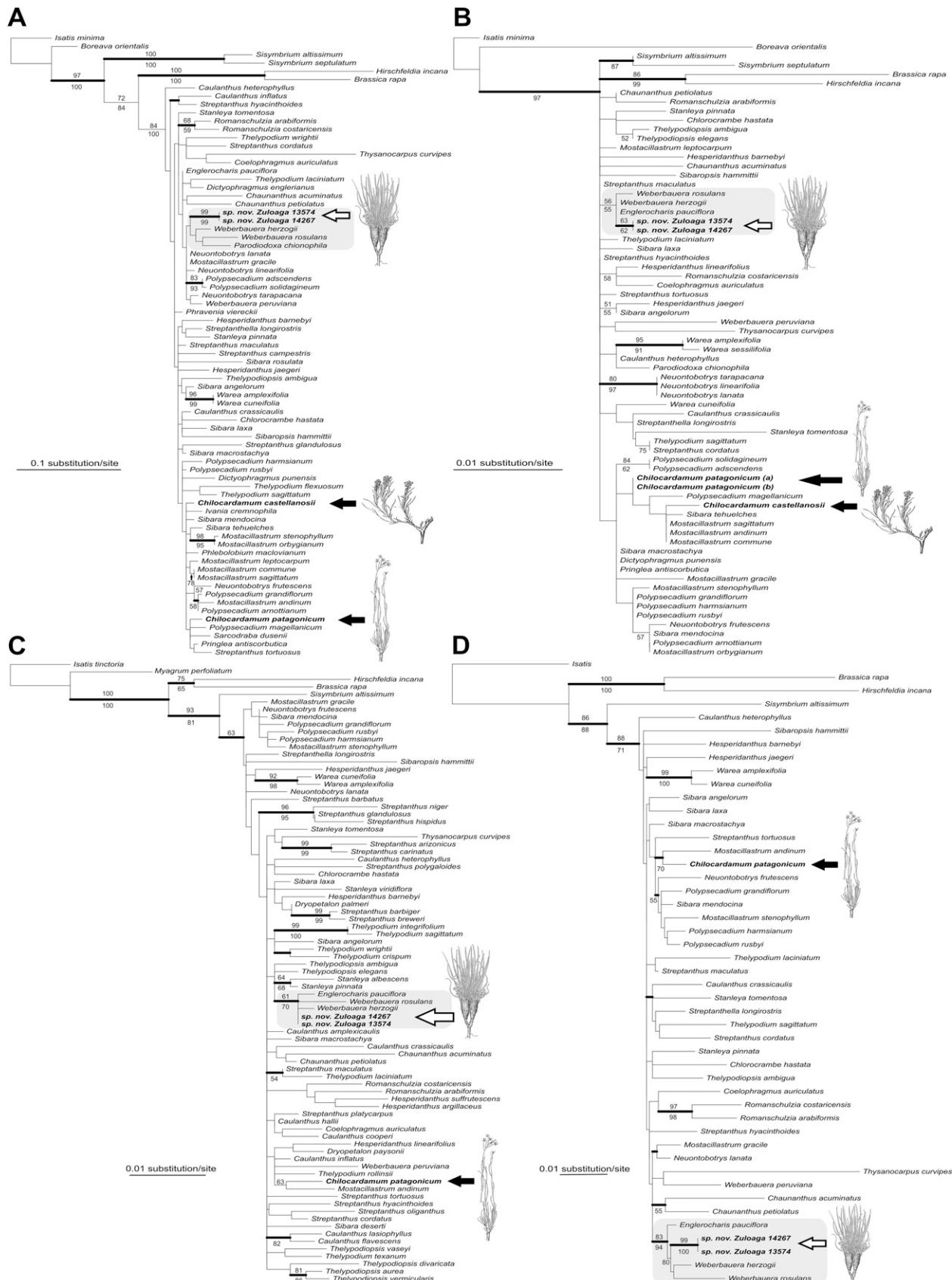


FIG. 7. Maximum likelihood trees obtained from the analysis at the generic level within Thelypodieae. A. ITS. B. *trnL-F*. C. *ndhF*. D. Combined dataset (ITS + *trnL-F* + *ndhF*). Values above and below branches correspond to parsimony jackknife/maximum likelihood bootstrap support, respectively. Thick branches indicate internal branches with posterior probability  $\geq 95\%$ . White arrows show the phylogenetic position of Zuloaga et al. 13574 and Zuloaga et al. 14267; black arrows show the positions of Chilocardamum.

TABLE 1. Morphological comparison of *Zuloagocardamum* with relatives in Thelypodieae.

Character	<i>Zuloagocardamum</i>	<i>Chilocardamum</i>	<i>Englerocarbis</i>	<i>Parodiodesoxa</i>	<i>Weberbauera</i>
Trichomes	Simple	Branched and dendritic, rarely some simple	Simple	Absent	Simple or branched
Basal leaves	Sessile, awl-shaped or linear	Sessile, awl-shaped or linear	Petiolate, lanceolate to oblanceolate or broadly spatulate	Petiolate, obovate to oblanceolate	Petiolate, linear to oblanceolate or oblong
Stems	Reduced, without caudex	Elongated, with caudex	Elongated, without caudex	Elongated, without caudex	Elongated, with or without caudex
Racemes	Shorter than basal leaves, ebracteate	Longer than basal leaves, ebracteate	Longer than basal leaves, ebracteate or bracteate basally	Longer than basal leaves, ebracteate or bracteate basally	Longer than basal leaves, ebracteate or bracteate basally
Fruits	Siliques linear, torulose	Siliques linear and not torulose	Silicles oblong to oblong-elliptic	Silicles oblong to elliptic and strongly angustiseptate	Siliques curved or straight and torulose or not
Seed mucilage	Present	Absent	Absent	Absent	Absent
Distribution	NW Argentina (Jujuy)	S Argentina (Patagonia)	Peru, rare in Bolivia	NW Argentina (Catamarca, Jujuy, La Rioja, Salta and Tucumán)	Argentina, Bolivia, Chile, and Peru

3,500–5,100 m (Salariato et al. 2013). It resembles *Zuloagocardamum* in being a scapose perennial with well-developed caudex and rosulate basal leaves. It differs by its long-petiolate, reticulate-veined, obovate to oblanceolate, and glabrous (vs. sessile, parallel-veined, linear to awl-shaped, and long ciliate) basal leaves, bracteate (vs. ebracteate) racemes much elongated (vs. not elongated) in fruit, entire (vs. 2-lobed) stigmas, oblong to elliptic and strongly angustiseptate smooth silicles (vs. linear, terete, torulose siliques), and non-mucilaginous (vs. mucilaginous) seeds.

As shown in Table 1, *Zuloagocardamum* has a unique combination of morphological characters not found in other genera of Thelypodieae. These include a well-developed woody caudex; reduced and leafless aerial stems; rosulate, leathery, awl-shaped, and parallel-veined basal leaves long ciliate with simple trichomes; racemes conspicuously shorter than basal leaves; 2-lobed stigmas; and slightly torulose siliques. Furthermore, although the seed-coat mucilage is common in numerous species of the Brassicaceae, it is quite rare in the Thelypodieae. The above characters seem to be an adaptation to arid environments (Fahn and Cutler 1992; Yang et al. 2012). The detailed morphological comparisons and molecular phylogenies presented herein clearly support the recognition of the novelty described below as a new genus.

#### TAXONOMIC TREATMENT

***Zuloagocardamum* Salariato & Al-Shehbaz, gen. nov.—**  
TYPE SPECIES: *Z. jujuyensis* Salariato & Al-Shehbaz

Perennial herbs with well-developed woody caudex covered with leaf remains of previous years. Trichomes simple. Multicellular glands absent. Stems much reduced, simple, glabrous. Basal leaves rosulate, sessile, entire, leathery, awl-shaped to linear, parallel veined, widest at base, strongly involute, conspicuously ciliate; caulin leaves absent. Racemes few-flowered, ebracteate, not elongated in fruit, considerably shorter than basal leaves; rachis obsolete or reduced. Sepals oblong, free, caducous, spreading, equal, glabrous, base of lateral pair not saccate; petals white, erect, longer than sepals, blade oblanceolate to narrowly spatulate, claw slightly dilated and minutely papillate at base; stamens 6, subequal; filaments

wingless, unappendaged, free, minutely papillate at base; anthers broadly ovate to oblong; nectar glands confluent, subtending bases of all filaments; ovules 6–12 per ovary. Fruits dehiscent, capsular siliques, linear to oblong, slightly curved, terete to slightly latiseptate, divaricate to ascending, glabrous, torulose; valves with a prominent mid-vein; replum rounded; septum complete, veinless; gynophore short to obsolete; style distinct; stigma capitate, slightly 2-lobed. Seeds uniseriate, oblong, wingless, mucilaginous when wetted; cotyledons incumbent.

**Etymology**—*Zuloagocardamum* is named after Dr. Fernando O. Zuloaga, director of the Darwinion Institution (Argentina) and a renowned agrostologist who discovered and collected plants of this new genus.

**Notes**—*Zuloagocardamum* is easily distinguished from other genera of Thelypodieae by having a well-developed woody caudex; much reduced and leafless stems; rosulate, awl-shaped or linear, parallel-veined, and long-ciliate basal leaves; ebracteate, few-flowered racemes conspicuously shorter than the basal leaves; torulose siliques with 2-lobed stigmas; and mucilaginous seeds (Table 1).

***Zuloagocardamum jujuyensis* Salariato & Al-Shehbaz, sp. nov.**—TYPE: ARGENTINA. Jujuy: Humahuaca, Mina Aguilar, Espinazo del Diablo, [23°13'02"S, 65°35'43"W, 3,670 m], 16 January 2012, Fernando O. Zuloaga, M. V. Nicola, S. Nomdedeu, & A. A. Suárez 13574 (holotype: SI!; isotype: MO!).

Perennial herbs, glabrous except for leaf margin. Caudex woody, to 8 mm diam., covered with leaf remains of previous years. Stems 0.5–1.2 cm, subdecumbent to decumbent, rarely ascending, simple, glabrous. Basal leaves (1.5–)2–5 cm × 0.5–1 mm; margin strongly involute, conspicuously ciliatipilose with simple trichomes (0.5–)1.2–1.5 mm long, straight basally and twisted distally. Racemes 6–12-flowered; rachis obsolete or <7 mm, glabrous; fruiting pedicels slender, erect to ascending or divaricate, glabrous, lowermost 0.5–1 cm long. Sepals 3.5–4 mm long, glabrous; petals 4.5–5.3 × 1.4–1.8 mm, claw 1–1.5 mm long; filaments minutely papillate basally, 3.5–4 mm long; anthers 0.8–1 mm long. Fruits 7–11 × 1.2–1.5 mm; gynophore 0.5–1(–1.2) mm long; style 1.5–2(–3) mm long. Seeds oblong, 1.5–2 × 0.8–1.2 mm. (Figs. 1, 8).

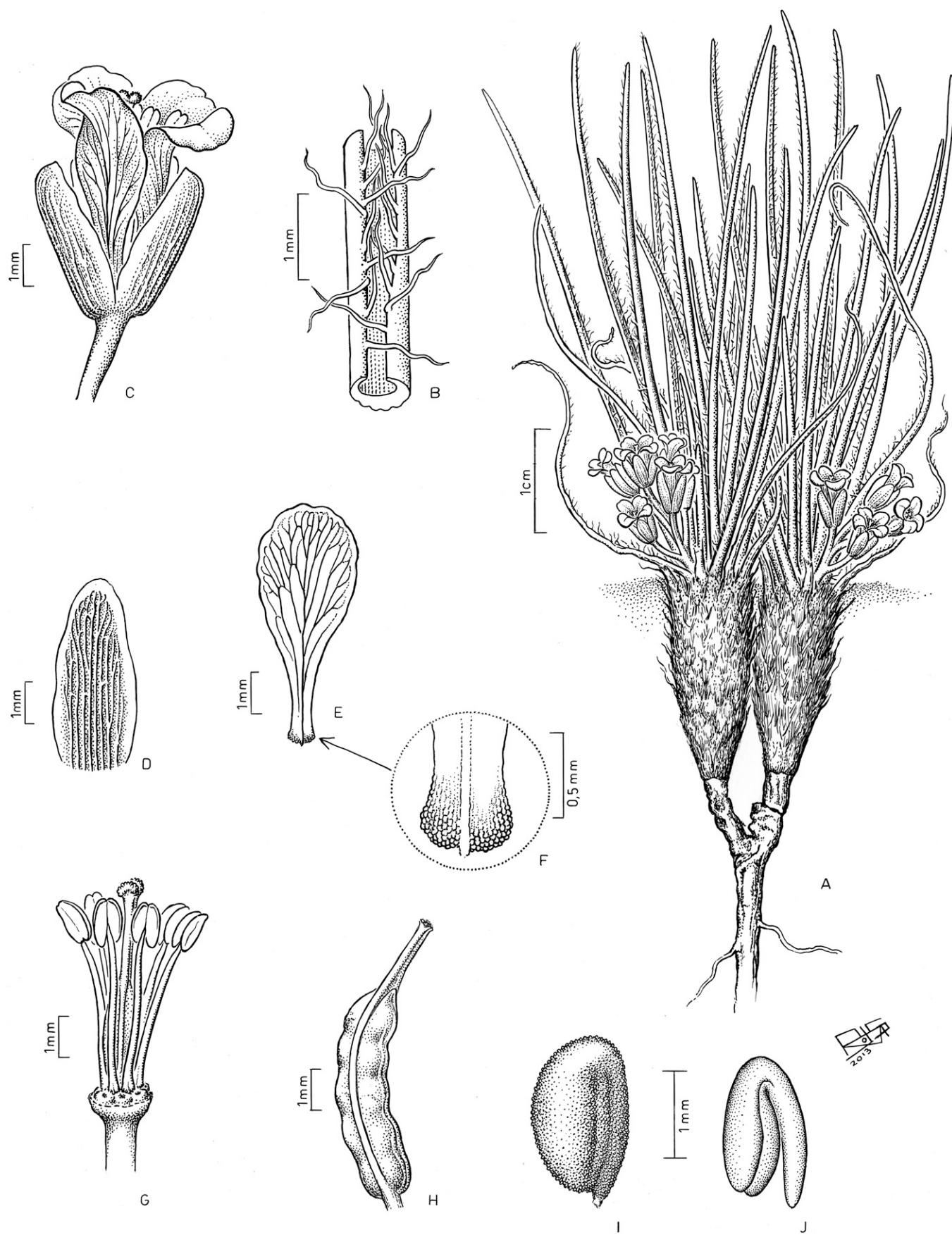


FIG. 8. *Zuloagocardamum jujuyensis*. A. Habit. B. Portion of the leaf. C. Flower. D. Sepal. E. Petal. F. Detail of petal base showing papillae. G. Nectar glands, stamens and ovary. H. Fruit. I. Seed. H. Embryo (Zuloaga et al. 13574).

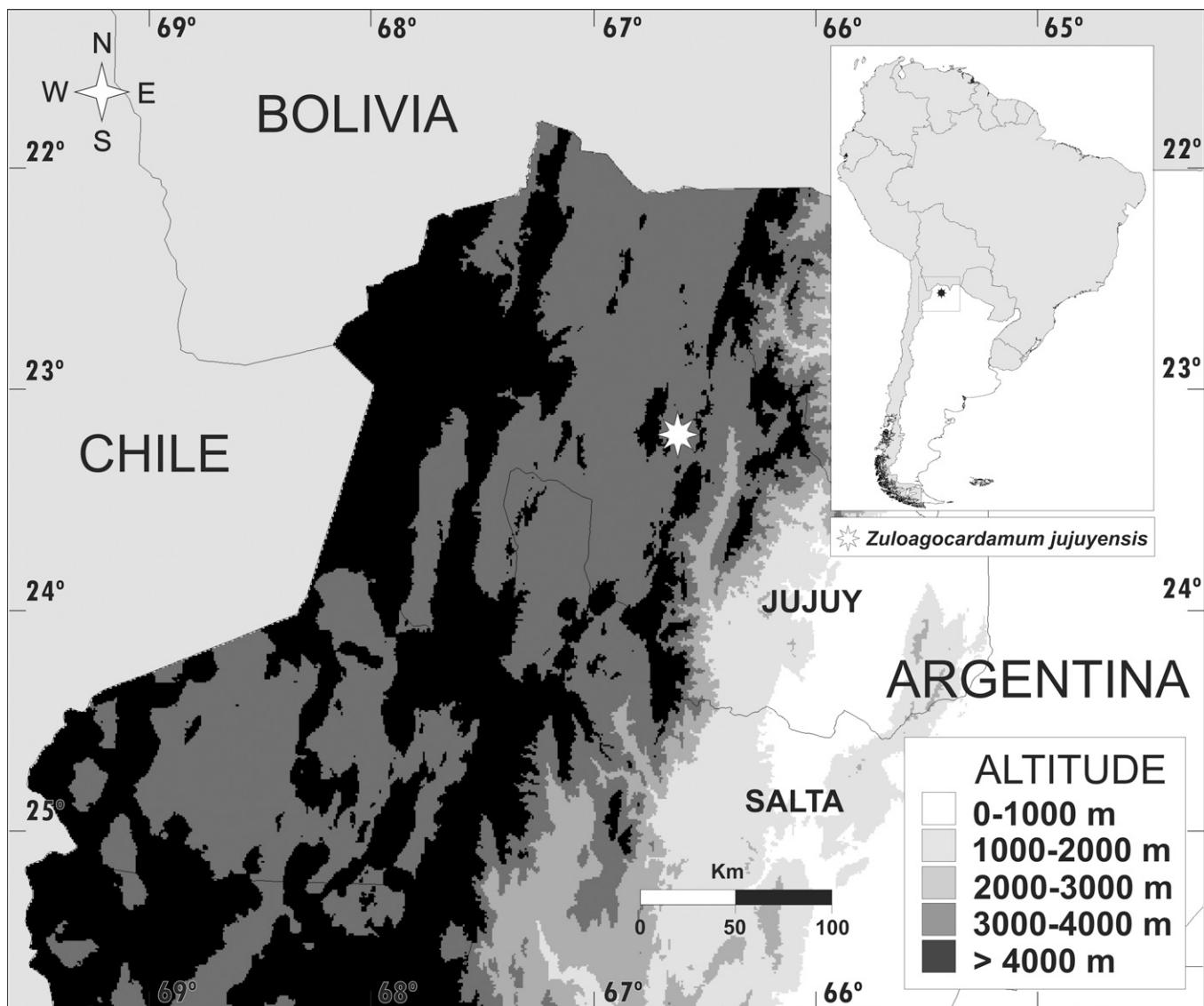


FIG. 9. Geographical distribution of *Zuloagocardamum jujuyensis*.

**Distribution and Habitat**—The species appears to be restricted to Espinazo del Diablo in Jujuy, Argentina (Figs. 1D, 9). This locality corresponds to the puna biogeographical province of Cabrera and Willink (1973) and has annual maximum, mean, and minimum temperatures of 22°C, 8.6°C, and -8°C, respectively; and an annual mean precipitation of 177 mm. Its populations grow at ca. 3,700 m on open, rocky soils together with species such as *Acantholippia salsolooides* Griseb. (Verbenaceae); *Alternanthera microphylla* R. E. Fr. and *Gomphrena meyeriana* Walp. (Amaranthaceae); *Astragalus hypsogenus* I. M. Johnst. and *Trifolium amabile* Kunth (Fabaceae); *Azorella compacta* Phil. (Apiaceae); *Cortaderia hieronymii* (Kuntze) N. P. Barker & H. P. Linder and *Deyeuxia breviaristata* Wedd. (Poaceae); *Ephedra rupestris* Benth. (Ephedraceae); *Evolvulus arizonicus* A. Gray (Convolvulaceae); *Lycium ciliatum* Schlecht. and *Solanum incisum* Griseb. (Solanaceae); *Nothoscordum andicolum* Kunth (Alliaceae); *Physaria pygmaea* O'Kane & Al-Shehbaz (Brassicaceae); *Phacelia pinnatifida* Griseb. ex Wedd. (Hydrophyllaceae); *Polygala corralitae* Tombesi & R. Kiesling and *P. jujuyensis* Grondona (Polygalaceae); and

*Trichocline auriculata* (Wedd.) Hieron., *Senecio jujuyensis* Cabrera, and *S. punae* Cabrera (Asteraceae).

**Phenology**—The species flowers from January to February, and produces fruits until March.

**Conservation Status**—This novelty has been collected only from the type locality, an area less than 1 km<sup>2</sup>. According to the IUCN red list categories and criteria (IUCN 2001), and until new localities are found, *Zuloagocardamum jujuyensis* can be referred to as an critically endangered species (CR).

**Additional Specimens Examined**—ARGENTINA. Jujuy: Humahuaca, Mina Aguilar, Espinazo del Diablo, 23°13'02"S 65°35'42"W, 3,660 m, 13 Mar 2013, F. O. Zuloaga *et al.* 14267 (SI).

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#### LITERATURE CITED

- Aagesen, L., M. J. Bena, S. Nomdedeau, A. Panizza, R. P. López, and F. O. Zuloaga. 2012. Areas of endemism in the southern central Andes. *Darwiniana* 50: 218–251.
- Alexander, P. J., M. D. Windham, R. Govindarajulu, I. A. Al-Shehbaz, and C. D. Bailey. 2010. Molecular phylogenetics and taxonomy of the genus *Thysanocarpus* (Brassicaceae). *Systematic Botany* 35: 559–577.
- Al-Shehbaz, I. A. 1984. The tribes of Cruciferae (Brassicaceae) in the southeastern United States. *Journal of the Arnold Arboretum* 65: 343–373.
- Al-Shehbaz, I. A. 2008. Brassicaceae in Catalogue of the vascular plants of the southern cone (Argentina, southern Brazil, Chile, Paraguay and Uruguay): Dicotyledoneae: Acanthaceae-Fabaceae (*Abarema-Schizolobium*), vol. 2, eds. F. O. Zuloaga, O. Morrone, and M. J. Belgrano. *Monographs in Systematic Botany from the Missouri Botanical Garden* 107: 1663–1709.
- Al-Shehbaz, I. A. 2012a. A generic and tribal synopsis of the Brassicaceae (Cruciferae). *Taxon* 61: 931–954.
- Al-Shehbaz, I. A. 2012b. Brassicaceae. Pp 1–273, in *Flora Argentina*, vol. 8, eds. M. A. Anton and F. O. Zuloaga. Buenos Aires: Sigma.
- Al-Shehbaz, I. A. 2012c. Notes on miscellaneous species of the tribe Thelypodieae (Brassicaceae). *Harvard Papers in Botany* 17: 3–10.
- Al-Shehbaz, I. A. and S. I. Warwick. 2007. Two new tribes (Dontostemoneae and Malcolmiae) in the Brassicaceae (Cruciferae). *Harvard Papers in Botany* 12: 429–433.
- Appel, O. and I. A. Al-Shehbaz. 2003. Cruciferae. Pp 75–174 in *The families and genera of vascular plants*, eds. K. Kubitzki and C. Bayer. Berlin: Springer-Verlag.
- Bailey, C. D., M. A. Koch, K. Mummenhoff, M. Mayer, S. L. O'Kane, S. I. Warwick, M. D. Windham, and I. A. Al-Shehbaz. 2006. Toward a global phylogeny of the Brassicaceae. *Molecular Biology and Evolution* 23: 2142–2160.
- Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.
- Bartish, I. V., A. Aïnouche, D. Jia, D. Bergstrom, S. L. Chown, R. C. Winkworth, and F. Hennion. 2012. Phylogeny and colonization history of *Pringlea antiscorbutica* (Brassicaceae), an emblematic endemic from the south Indian Ocean Province. *Molecular Phylogenetics and Evolution* 65: 748–756.
- Beilstein, M. A., I. A. Al-Shehbaz, and E. A. Kellogg. 2006. Brassicaceae phylogeny and trichome evolution. *American Journal of Botany* 93: 607–619.
- Beilstein, M. A., I. A. Al-Shehbaz, S. Mathews, and E. A. Kellogg. 2008. Brassicaceae phylogeny inferred from phytochrome A and *ndhF* sequence data: tribes and trichomes revisited. *American Journal of Botany* 95: 1307–1327.
- Cabrera, A. L. and A. Willink. 1973. *Biogeografía de América Latina*. Organización de los Estados Americanos, Serie Biológica, Monografía 13. Washington, D. C.: Organización de los Estados Americanos.
- Couvreur, T. L. P., A. Franzke, I. A. Al-Shehbaz, F. T. Bakker, M. A. Koch, and K. Mummenhoff. 2010. Molecular phylogenetics, temporal diversification and principles of evolution in the mustard family (Brassicaceae). *Molecular Biology and Evolution* 27: 55–71.
- Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Farris, J. S., V. A. Albert, M. Källersjö, D. Lipscomb, and A. G. Kluge. 1996. Parsimony jack-knifing outperforms neighbor-joining. *Cladistics* 12: 99–124.
- Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Testing significance of incongruence. *Cladistics* 10: 315–319.
- Fahn, A. and D. F. Cutler. 1992. *Xerophytes*. Berlin: Gebrüder Borntraeger.
- Franzke, A., D. German, I. A. Al-Shehbaz, and K. Mummenhoff. 2009. *Arabidopsis* family ties: molecular phylogeny and age estimates in the Brassicaceae. *Taxon* 58: 425–437.
- German, D. A. and I. A. Al-Shehbaz. 2008. Five additional tribes (Aphragmeae, Biscutelleae, Calepineae, Corringiae, and Erysimeae) in the Brassicaceae (Cruciferae). *Harvard Papers in Botany* 13: 165–170.
- German, D. A., N. Friesen, B. Neuffer, I. A. Al-Shehbaz, and H. Hurka. 2009. Contribution to ITS phylogeny of the Brassicaceae, with a special reference to some Asian taxa. *Plant Systematics and Evolution* 283: 33–56.
- Goloboff, P. A., J. S. Farris, and K. Nixon. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.
- Hall, T. A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hall, J. C., K. J. Sytsma, and H. H. Iltis. 2002. Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *American Journal of Botany* 89: 1826–1842.
- IUCN. 2001. *IUCN red list categories and criteria: version 3.1*. IUCN Species Survival Commission. Gland, Switzerland and Cambridge, U. K.: IUCN.
- Koch, M. and I. A. Al-Shehbaz. 2009. Molecular systematics and evolution of "wild" crucifers (Brassicaceae or Cruciferae). Pp 1–18 in *Biology and breeding of Crucifers*, ed. S. Gupta. Boca Raton: CRC Press.
- Koch, M., C. Dobč, C. Keifer, R. Schmidl, L. Klimes, and M. A. Lysak. 2007. Supernetwork identifies multiple events of plastid *trnF*(GAA) pseudogene evolution in the Brassicaceae. *Molecular Biology and Evolution* 24: 63–73.
- Koch, M., M. Kiefer, D. A. German, I. A. Al-Shehbaz, A. Franzke, K. Mummenhoff, and R. Schmidl. 2012. BrassiBase: Tools and biological resources to study characters and traits in the Brassicaceae – version 1.1. *Taxon* 61: 1001–1009.
- Khosravi, A. R., S. Mohsenzadeh, and K. Mummenhoff. 2009. Phylogenetic relationships of Old World Brassicaceae from Iran based on nuclear ribosomal DNA sequences. *Biochemical Systematics and Ecology* 37: 106–115.
- Olmstead, R. G. and J. A. Sweere. 1994. Combining data in phylogenetics systematics: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* 43: 467–481.
- Omar, M. B., L. Bolland, and W. A. Heather. 1979. A permanent mounting medium for fungi. *Bulletin of the British Mycological Society* 13: 31–32.
- Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.
- Rambaut, A. and A. J. Drummond. 2007. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>.
- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a Large Model Space. *Systematic Biology* 61: 539–542.
- Salariato, D. L., F. O. Zuloaga, and I. A. Al-Shehbaz. 2013. Revision and tribal placement of the Argentinean genus *Parodiodoxa* (Brassicaceae). *Plant Systematics and Evolution* 299: 305–316.
- Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 57: 758–771.
- Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Warwick, S. I. and J. C. Hall. 2009. Phylogeny of *Brassica* and wild relatives. Pp 19–36 in *Biology and breeding of Crucifers*, ed. S. Gupta. Boca Raton: CRC Press.
- Warwick, S. I., I. A. Al-Shehbaz, and C. A. Sauder. 2006. Phylogenetic position of *Arabis arenicola* and generic limits of *Eutrema* and *Aphragmus* (Brassicaceae) based on sequences of nuclear ribosomal DNA. *Canadian Journal of Botany* 84: 269–281.
- Warwick, S. I., K. Mummenhoff, C. A. Sauder, M. A. Koch, and I. A. Al-Shehbaz. 2010. Closing the gaps: Phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS. *Plant Systematics and Evolution* 285: 209–232.
- Warwick, S. I., C. A. Sauder, and I. A. Al-Shehbaz. 2008. Phylogenetic relationships in the tribe Alyssae (Brassicaceae) based on nuclear ribosomal ITS DNA sequences. *Canadian Journal of Botany* 86: 315–336.
- Warwick, S. I., C. A. Sauder, and I. A. Al-Shehbaz. 2011. Systematic position of *Ivania*, *Scoliaxon*, and *Phravenia* (Brassicaceae). *Taxon* 60: 1156–1164.
- Warwick, S. I., C. A. Sauder, I. A. Al-Shehbaz, and F. Jacquemoud. 2007. Phylogenetic relationships in the Brassicaceae tribes Anchonieae, Chorisporae, Euclidieae, and Hesperideae based on nuclear

- ribosomal ITS DNA sequences. *Annals of the Missouri Botanical Garden* 94: 56–78.
- Warwick, S. I., C. A. Sauder, M. S. Mayer, and I. A. Al-Shehbaz. 2009. Phylogenetic relationships in the tribes Schizopetaleae and Thelypodieae (Brassicaceae) based on nuclear ribosomal ITS region and chloroplast *ndhF* DNA sequences. *Botany* 87: 961–985.
- Yang, X., M. J. Baskinc, C. C. Baskinc, and H. Zhenying. 2012. More than just a coating: Ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. *Perspectives in Plant Ecology, Evolution and Systematics* 14: 434–442.

#### APPENDIX 1.

Taxa and GenBank accession numbers for the ITS, trnL-F and *ndhF* sequences used in the phylogenetic analyses (ITS, trnL-F, *ndhF*). All sequences were downloaded from GenBank, except those marked with an asterisk, which were generated for this study.

- CLEOMACEAE.** *Cleome lutea* Hook. (AF137588, -, -); *Cleome spinosa* Jacq. (-, DQ649093, -); *Cleome rutidosperma* DC (-, -, DQ288755); *Polanisia dodecandra* (L.) DC. (-, -, DQ288815).
- BRASSICACEAE.** Tribe **Aethionemeae.** *Aethionema arabicum* Andrz. ex DC. (AY254539, DQ180218, -); *Aethionema elongatum* Boiss. (-, DQ180216, -); *Aethionema saxatile* (L.) R. Br. (-, -, DQ288726); *Moriera spinosa* Boiss. (GQ424545, -, DQ288798). Tribe **Alyssae.** *Alyssum lenense* Adams (-, FN677633, -); *Alyssum montanum* L. (AY237938, -, -); *Berteroia incana* (L.) DC. (EF514632, -, -); *Galitzkya potaninii* (Maxim.) V. V. Botschantz. (-, FN677635, -); *Hormathophylla purpurea* (Lag. & Rodr.) P. Kämpfer (-, FN677738, -). Tribe **Alyssopsideae.** *Alyssopsis mollis* O. E. Schulz (-, FJ188227, -); *Alyssopsis trinervis* Botsch. & Seifulin (GQ497846, -, -); *Calymmatium draboides* O. E. Schulz (GQ497854, -, -); *Olimarabidopsis pumila* (Stephan) Al-Shehbaz, O'Kane & R. A. Price (-, -, DQ288807). Tribe **Anastaticeae.** *Anastatica hierochuntica* L. (GQ424524, -, -); *Farsetia aegyptia* Turra (-, -, DQ288769); *Lobularia maritima* (L.) Desv. (-, -, DQ288791); *Malcolmia triloba* Spreng. (DQ357561, -, -). Tribe **Anchonieae.** *Anchonium billardieri* DC. (DQ357512, -, -); *Matthiola chorassanica* Bunge ex Boiss. (-, -, DQ288795); *Matthiola farinosa* Bunge ex Boiss. (-, -, DQ288796); *Matthiola incana* (L.) W. T. Aiton (AJ628339, -, -); *Microstigma deflexum* Juz. (-, FN677641, -); *Sterigmostemum acanthocarpum* Kuntze (-, -, DQ288834); *Sterigmostemum violaceum* (Botsch.) H. L. Yang (-, FN677640, DQ288808). Tribe **Aphragmeae.** *Aphragmus eschscholtzianus* Andrz. ex DC. (DQ165334, -, -); *Aphragmus hobsonii* (H. Pearson) Al-Shehbaz & Warwick (DQ165357, -, -); *Aphragmus oxycarpus* (Hook. f. & Thomson) Jafri (-, DQ518350, -). Tribe **Arabideae.** *Arabis alpina* L. (AF137559, EF449508, DQ288731); *Athyrsanus pusillus* (Hook.) Greene (-, GU246241, -); *Aubrieta deltoidea* (L.) DC. (-, -, DQ288734); *Aubrieta parviflora* Boiss. (-, -, DQ288735); *Baimashania pulvinata* Al-Shehbaz (-, -, DQ288736); *Draba aizoides* L. (AF146512, -, -); *Draba alpina* L. (-, DQ467004, -); *Draba altaica* (C. A. Mey.) Bunge (-, -, DQ288765). Tribe **Asteae.** *Asta schaffneri* (S. Watson) O. E. Schulz (HQ541168, -, DQ288733); *Asta stricta* Rollins (HQ541171, -, -). Tribe **Biscutelleae.** *Biscutella didynima* L. (DQ452058, -, DQ288738); *Biscutella laevigata* L. (DQ452056, -, -). Tribe **Boechereae.** *Anelsonia eurycarpa* (A. Gray) J. F. Macbr. & Payson; *Boechera dentata* (Raf.) Al-Shehbaz & Zarucchi (-, -, DQ288741); *Boechera laevigata* (Muhl. ex Willd.) Al-Shehbaz (-, -, DQ288739); *Boechera retrofacta* (Graham) Á. Löve & D. Löve (AF183105, -, -); *Boechera platysperma* (A. Gray) Al-Shehbaz (-, -, DQ288740); *Boechera suffrutescens* (S. Watson) Dorn (-, DQ013046); *Cusickiella douglasii* (A. Gray) Rollins (-, AF307557, -); *Cusickiella quadricostata* (Rollins) Rollins (-, -, DQ288758); *Nevada holmgrenii* (Rollins) N. H. Holmgren (-, -, DQ288829); *Phoenicaulis cheiranthoides* Nutt. (-, -, DQ288812); *Polyctenium fremontii* (S. Watson) Greene (-, -, DQ288816); *Sandbergia whitedii* (Piper) Greene (AJ628295, -, -). Tribe **Brassicaceae.** *Brassica oleracea* L. (AY722423, -, DQ288742); *Brassica rapa* L. (JN564039, GQ268033, DQ200044); *Cakile maritima* Scop. (-, -, DQ288745); *Diplotaxis erucoides* (L.) DC. (-, AY751763, -); *Eruca sativa* Lam. (-, AY751765, -); *Hirschfeldia incana* (L.) Lagr.-Fossat (AY722470, EU620407, DQ288778); *Rapistrum rugosum* (L.) All. (-, AY751769, -); *Sinapis alba* L. (-, JQ041854, -). Tribe **Buniidae.** *Bunias erucago* L. (GQ497885, -, -); *Bunias orientalis* L. (-, FN677645, DQ288744). Tribe **Calepineae.** *Calepina irregularis* (Asso) Thell. (DQ249822, AY751760, -); *Goldbachia laevigata* (M. Bieb.) DC. (DQ357545, -, DQ288771). Tribe **Camelineae.** *Arabidopsis arenosa* (L.) Lawalrée (-, GQ386472, -); *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz (-, -, DQ288730); *Camelina laxa* C. A. Mey. (-, -, DQ288747); *Camelina microcarpa* (AF137574, DQ821412; DQ288746); *Capsella bursa-pastoris* (L.) Medik. (-, -, DQ288748); *Catolobus pendulus* (L.) Al-Shehbaz (-, -, DQ288732); *Neslia paniculata* (L.) Desv. (-, DQ310518, -); *Pseudoarabidopsis toxophylla* (M. Bieb.) Al-Shehbaz, O'Kane & R. A. Price (AF137558, -, -). Tribe **Cardamineae.** *Barbarea vulgaris* W. T. Aiton (-, -, DQ288737); *Cardamine flexuosa* With. (-, AB247985, -); *Cardamine pratensis* L. (AY245995 and AY246025, -, -); *Cardamine pulchella* (Hook. f. & Thomson) Al-Shehbaz & G. Yang (-, -, DQ288749); *Iodanthus pinnatifidus* (Michx.) Steud. (-, -, DQ288784); *Leavenworthia crassa* Rollins (-, -, DQ288787); *Nasturtium officinale* W. T. Aiton (AY254531, -, DQ288801); *Planodes virginicum* (L.) Greene (GQ424554, -, DQ288814); *Rorippa indica* (L.) Hiern (-, EF426788, -); *Selenia dissecta* Torr. & A. Gray (-, -, DQ288822). Tribe **Chorisporaceae.** *Chorispora sabulosa* Cambess. (-, FN677724, -); *Chorispora tenella* (Pall.) DC. (DQ357526, -, DQ288753); *Diptychocarpus strictus* (Fisch. ex M. Bieb.) Trautv. (DQ357534, FN677717, DQ288762); *Litwinowia tenuissima* (Pall.) Woronow ex Pavlov (-, FN677714, -); *Pseudoclausia gracillima* A. N. Vassiljeva (-, FN677652, -). Tribe **Cochlearieae.** *Cochlearia megalosperma* Vogt (AF336208, -, -); *Ionopsis acaule* (Desf.) DC. ex Rchb. (AF336210 and AF336211, HQ268714, DQ288785). Tribe **Coluteocarpeae.** *Eunomia oppositifolia* DC. (-, AY122456, -); *Noccaea* sp. a (-, -, DQ288805); *Noccaea* sp. b (-, -, DQ288806) *Noccaea cochleariformis* (DC.) Á. Löve & D. Löve (DQ249838, -, DQ288804); *Noccaea fendleri* (A. Gray) Holub (AY154824, AY154786, -); *Noccaea jankae* (A. Kern.) F. K. Mey. (-, AY154796, -). Tribe **Conringieae.** *Conringia clavata* Boiss. (AY722505, -, -); *Conringia persica* Boiss. (-, -, DQ288756); *Zuvanda crenulata* Askerova (DQ357606, -, -). Tribe **Cremolobeae.** *Cremolobus chilensis* (Lag. ex DC.) DC. (GQ424530, -, -); *Cremolobus subscandens* Kuntze (-, EU620348, DQ288757); *Menonvillea cuneata* (Gillies & Hook.) Rollins (GQ497868, -, -); *Menonvillea scapigera* subsp. *longipes* (Rollins) Prina (-, -, DQ288797). Tribe **Descurainiae.** *Descuraria californica* (A. Gray) O. E. Schulz (-, GU246239, -); *Descuraria sophia* (L.) Webb ex Prantl (DQ418727, -, DQ288759); *Hornungia alpina* (Sievers) O. Appel (-, DQ310515, -); *Hornungia petraea* Rchb. (AJ628293 and AJ628294, -, -); *Hornungia procumbens* (L.) Hayek (-, -, DQ288779); *Ianhedgea minutiflora* (Hook. f. & Thomson) Al-Shehbaz & O'Kane (-, -, DQ288780). Tribe **Dontostemoneae.** *Clausia aprica* (Stephan) Korn. -Trotzky (DQ357529, -, -); *Clausia podlechii* Dvořák (-, FN677719); *Dontostemon integrifolius* (L.) C. A. Mey. (DQ357536, -, -); *Dontostemon intermedius* Vorosch. (-, FN677644, -); *Dontostemon*

*senilis* Maxim. (-, -, DQ288764). **Tribe Erysimeae.** *Erysimum canescens* Roth (-, EU170623, -); *Erysimum capitatum* (Douglas ex Hook.) Greene (DQ357540, -, DQ288766); *Erysimum cheiranthoides* L. (DQ005989, EU170622, -). **Tribe Euclidieae.** *Braya alpina* Sternb. & Hoppe (AY353095, -, -); *Braya rosea* (Turcz.) Bunge (-, -, DQ288743); *Christolea crassifolia* Cambess. (-, -, DQ288754); *Dilophia salsa* Thomson (-, -, DQ288761); *Euclidium syriacum* (L.) W. T. Aiton (-, EF426780, DQ288767); *Leiospora eriocalyx* (Regel & Schmalh.) Dvořák (-, -, DQ288788); *Neotorularia korolkowii* (Regel & Schmalh.) Hedge & J. Léonard (-, -, DQ288803); *Neotorularia torulosa* (Desf.) Hedge & J. Léonard (AY353164, -, -); *Rhammatophyllum erysimoides* (Kar.) Al-Shehbaz & O. Appel (-, -, DQ288818); *Sisymbriopsis mollipila* (Maxim.) Botsch. (-, -, DQ288824); *Sisymbriopsis yechengnica* (C. H. An) Al-Shehbaz, Z. X. An & G. Yang (-, -, DQ288825); *Shangrilaia nana* Al-Shehbaz, J. P. Yue & H. Sun (-, -, DQ288823); *Solms-laubachia baioigoinensis* (K. C. Kuan & C. H. An) J. P. Yue, Al-Shehbaz & H. Sun (-, DQ523315, -); *Solms-laubachia linearis* (N. Busch) J. P. Yue, Al-Shehbaz & H. Sun (-, -, DQ288760); *Solms-laubachia zhongdianensis* J. P. Yue, Al-Shehbaz & H. Sun (-, -, DQ288830) *Strigosella africana* (L.) Botsch. (-, EU170625, DQ288793); *Tetracme pamirica* Vassilcz. (-, -, DQ288837). **Tribe Eudemeae.** *Brayopsis colombiana* Al-Shehbaz (EU620283, -, -); *Eudema nubigena* Bonpl. (EU620297, EU620354, -). **Tribe Eutremiae.** *Chalcanthus renifolius* (Boiss.) Boiss. (GQ424528, -, DQ288752); *Eutrema altaicum* (C. A. Mey.) Al-Shehbaz & Warwick (DQ165364, DQ649087, DQ288836); *Eutrema heterophyllum* (W. W. Sm.) H. Hara (-, DQ649086, DQ288768). **Tribe Halimolobeae.** *Exhalimolobos pazensis* (Rusby) Al-Shehbaz & C. D. Bailey (-, AF307547, -); *Exhalimolobos weddellii* (E. Fourn.) Al-Shehbaz & C. D. Bailey (-, -, DQ288773); *Halimolobos lasiolobus* O. E. Schulz (-, AF307647, -); *Mancoa bracteata* (S. Watson) Rollins (-, AF307556, -); *Mancoa hispida* Wedd. (-, -, DQ288794); *Pennellia brachycarpa* Beilstein & Al-Shehbaz (-, -, DQ288811); *Pennellia longifolia* (Benth.) Rollins (AF307627, AF307549, DQ288810). **Tribe Heliophileae.** *Heliophila arenaria* Sond. (AJ863600, -, -); *Heliophila coronopifolia* L. (-, DQ518369, -); *Heliophila* sp. (-, -, DQ288775); *Heliophila subulata* Burch. & DC. (AJ863580 and AJ864835, -, -). **Tribe Hesperideae.** *Hesperis matronalis* L. (DQ357547, -, DQ288776); *Hesperis sibirica* L. (-, FN677642, -); *Hesperis* sp. (-, -, DQ288777) *Tchihatchewia isatidea* Boiss. (GQ497882, -, -). **Tribe Iberideae.** *Iberis amara* L. (AJ440311, AY122455, -); *Iberis sempervirens* L. (-, -, DQ288781); *Iberis spathulata* Bergeret (AJ440312, -, -). **Tribe Isatideae.** *Boreava orientalis* Jaub. & Spach (DQ249859, DQ518353, -); *Isatis minima* Bunge (GQ131320, DQ821409, -); *Isatis tinctoria* L. (DQ249851, DQ518370, DQ288786); *Myagrum perfoliatum* L. (-, -, DQ288800). **Tribe Kernereae.** *Kernera saxatilis* (L.) Rchb. (AF401118 and AF401119, -, -); *Rhizobotrya alpina* Tausch (AJ440315, -, -). **Tribe Lepidieae.** *Lepidium alyssoides* A. Gray (-, -, Q288789); *Lepidium apetalum* Willd. (-, DQ821406, -); *Lepidium draba* L. (-, -, DQ288790); *Lepidium latifolium* L. (AJ582447 and AJ582521, -, -); *Lepidium sisymbrioides* Hook. f. (-, DQ997056, -); *Stubendorffia gracilis* Botsch. & Vved. (DQ780944 and DQ780945, -, -). **Tribe Megacarpareae.** *Megacarpaea delavayi* Franch. (AJ628325 and AJ628326, -, -); *Megacarpaea gracilis* Lipsky (AJ628327 and AJ628328, -, -). **Tribe Microlepidae.** *Carinavalva glauca* Ising (GQ424527, -, -); *Microlepидium pilosulum* F. Mull. (GQ497869, -, -); *Stenopetalum nutans* F. Mull. (-, -, DQ288833). **Tribe Notothlaspideae.** *Notothlaspi australe* Hook. f. (AF100689, -, -); *Notothlaspi rosulatum* Hook. f. (AF100690, -, -). **Tribe Oreophytoneae.** *Murbeckiella huetii* Rothm. (GQ424546, -, -);

*Oreophytum falcatum* O. E. Schulz (GQ424549, -, -). **Tribe Physarieae.** *Dimorphocarpa wislizenii* (Engelm.) Rollins (-, -, DQ288763); *Physaria arctica* (Wormsk. ex Hornem.) O'Kane & Al-Shehbaz (-, GQ245072, -); *Physaria didymocarpa* (Hook.) A. Gray (AF137583, -, -); *Physaria fendleri* (A. Gray) O'Kane & Al-Shehbaz (-, AF055266, -); *Physaria floribunda* Rydb. (-, -, DQ288813); *Synthipsis greggii* A. Gray (AF137590, -, -). **Tribe Schizopetaleae.** *Mathewsia foliosa* Hook. & Arn. (DQ357563, EU620360, -); *Schizopetalon biseriatum* Phil. (-, EU620375, -); *Schizopetalon walkeri* Sims (EU620315, -, -). **Tribe Scoliaxoneae.** *Scoliaxon mexicanum* (S. Watson) Payson (HQ541174, -, -). **Tribe Sisymbrieae.** *Sisymbrium altissimum* L. (AF531560, AY958545, DQ288826); *Sisymbrium linifolium* (Nutt.) Nutt. ex Torr. & A. Gray (-, -, DQ288821); *Sisymbrium septulatum* DC. (AF531600, AY958565, -). **Tribe Smelowskieae.** *Smelowskia alba* (Pall.) Regel (AY230562, -, -); *Smelowskia annua* Rupr. (-, -, DQ288831); *Smelowskia calycina* (Stephan) C. A. Mey. (-, JF298538, DQ288828); *Smelowskia jacutica* (Botsch. & Karav.) Al-Shehbaz & Warwick (AY230646, -, -); *Smelowskia sisymbrioides* (Regel & Herder) Lipsky ex Paulsen (-, JF298539, -); *Smelowskia tibetica* (Thomson) Lipsky (-, -, DQ288774). **Tribe Stevenieae.** *Ptilotrichum canescens* (DC.) C. A. Mey. (-, -, DQ288728). **Tribe Thelypodieae.** *Caulanthus amplexicaulis* S. Watson (-, -, EU718527); *Caulanthus cooperi* (S. Watson) Payson (-, -, EU718528); *Caulanthus crassicaulis* (Torr.) S. Watson (EU620267 and EU620252, EU620341, DQ288750); *Caulanthus flavescens* (Hook.) Payson (-, -, EU718548); *Caulanthus hallii* Payson (-, -, EU718531); *Caulanthus heterophyllum* (Nutt.) Payson (EU620267, EU620342; EU718532); *Caulanthus inflatus* S. Watson (AF531617, -, EU718533); *Caulanthus lasiophyllus* (Hook. & Arn.) Payson (-, -, EU718534); *Chaunanthus acuminatus* (Rollins) R. A. Price & Al-Shehbaz (EU620344, EU620344, EU718536); *Chaunanthus petiolatus* (Hemsl.) O. E. Schulz (EU620289, EU620345, EU718537); *Chilocardamum castellanosii* (O. E. Schulz) Al-Shehbaz (AY958592, AY958548, -); *Chilocardamum patagonicum* (Speg.) O. E. Schulz (a) ARGENTINA: Santa Cruz, Güer Aike, 3 km al N de Río Gallegos, Zuloaga et al., 14102 (SI) (KF569758\*, KF569761\*, KF569764\*), (b) (-, AY958561, -); *Chlorocratome hastata* (S. Watson) Rydb. (EU620254 and EU620269, EU620346; EU718538); *Coelophragmus auriculatus* (A. Gray) O. E. Schulz (AF531598, EU620347, EU718539); *Dictyophragmus englerianus* (Muschl.) O. E. Schulz (EU620293, -, -); *Dictyophragmus punensis* (Romanczuk) Al-Shehbaz (EU620294, EU620349, -); *Dryopetalon palmeri* (S. Watson) O. E. Schulz (-, -, EU718541); *Dryopetalon paysonii* (Rollins) Al-Shehbaz (EU620270 and EU620255, EU620350, EU718542); *Englerocharis pauciflora* Al-Shehbaz (EU620295, EU620351, EU718543); *Hesperidanthus argillaceus* (S. L. Welsh & N. D. Atwood) Al-Shehbaz (-, -, EU718549); *Hesperidanthus barnebyi* (S. L. Welsh & N. D. Atwood) Al-Shehbaz (EU620271 and EU620256, EU620356, EU718550); *Hesperidanthus jaegeri* (Rollins) Al-Shehbaz (GQ424569, EU620357, DQ288751); *Hesperidanthus linearifolius* (A. Gray) Rydb. (AF531612, EU620358, EU718553); *Hesperidanthus suffrutescens* (Rollins) Al-Shehbaz (-, -, DQ288770); *Ivania cremnophila* (I. M. Johnston) O. E. Schulz (HQ541176, -, -); *Mostacillastrum andinum* (Phil.) Al-Shehbaz (AF531649, EU620363, EU718557); *Mostacillastrum commune* (Speg.) Al-Shehbaz (AY958593, AY958549, -); *Mostacillastrum gracile* (Wedd.) Al-Shehbaz (EU620304, AY958553, EU874869); *Mostacillastrum leptocarpum* (Hook. & Arn.) Al-Shehbaz (AF531632, AY958556, -); *Mostacillastrum orbignyanum* (E. Fourn.)

Al-Shehbaz (AF531583, -, DQ288799); *Mostacillastrum sagittatum* (Hook. & Arn.) Al-Shehbaz (AF531599, AY958564, -); *Mostacillastrum stenophyllum* (Gilles ex Hook. & Arn.) O. E. Schulz (EU620305, EU620364, EU718558); *Neuontobotrys elloanensis* Al-Shehbaz (-, -, DQ288802); *Neuontobotrys frutescens* (Gillies ex Hook. & Arn.) Al-Shehbaz (AY958595, AY958551, DQ288827); *Neuontobotrys lanata* (Walp.) Al-Shehbaz (AF531652, EU620366, EU718559); *Neuontobotrys linearifolia* (Kuntze) Al-Shehbaz (EU620306, EU620367, -); *Neuontobotrys tarapacana* (Phil.) Al-Shehbaz (KC174372, EU620368, -); *Parodiodoxa chionophila* (Speg.) O. E. Schulz (JX971121, JX971122); *Phlebolobium maclovianum* (d'Urv.) O. E. Schulz (GQ497873, -, -); *Phravenia viereckii* (O. E. Schulz) Al-Shehbaz & S. I. Warwick (HQ541181, -, -); *Polypsecadium arnottianum* (Gillies ex Hook. & Arn.) Al-Shehbaz (AF531629, EU620369, -); *Polypsecadium adscendens* (O. E. Schulz) Al-Shehbaz (EU620308, AY958544, -); *Polypsecadium grandiflorum* Romanczuk & Boelcke (EU620309, EU620370, EU718560); *Polypsecadium harmsianum* (Muschl.) O. E. Schulz (EU620310, EU620371, EU718561); *Polypsecadium magellanicum* (Juss. ex Pers.) Al-Shehbaz (AF531589, AY958558, -); *Polypsecadium rusbyi* (Britton) Al-Shehbaz (AF531597, EU620372, EU718562); *Polypsecadium solidagineum* (Triana & Planch.) Al-Shehbaz (AF531602, EU620373, -); *Pringlea antiscorbutica* R. Br. ex Hook. f. (AF531619, AY533521, -); *Romanschulzia arabiformis* (DC.) Rollins (AF531635, AY958538, DQ288819); *Romanschulzia costaricensis* (Standl.) Rollins (AF531636, AY958539, EU718564); *Sarcodraba dusenii* (O. E. Schulz) Al-Shehbaz (GQ424568, -, -); *Sibara angelorum* (S. Watson) Greene (EU620317, EU620379, EU718566); *Sibara deserti* (M. E. Jones) Rollins (-, -, EU718568); *Sibara laxa* (S. Watson) Greene (AF531637, -, EU718569); *Sibara macrostachya* (O. E. Schulz) Al-Shehbaz (EU620337, EU620403, EU718619); *Sibara mendocina* (Boelcke) Al-Shehbaz (EU620338, EU620404, EU718620); *Sibara tehuelches* (Speg.) Al-Shehbaz (EU620311, EU620374, -); *Sibara rosulata* Rollins (AF531648; -, -); *Sibaropsis hammittii* S. Boyd & T. S. Ross (EU620318, EU620380, EU718571); *Stanleya albescens* M. E. Jones (-, -, EU718573); *Stanleya pinnata* (Pursh) Britton (EU620319, EU620381, EU718574); *Stanleya tomentosa* Parry (EU620273 and EU620258, EU620382, EU718576); *Stanleya viridiflora* Nutt. (-, -, EU718577); *Streptanthus anceps* (Payson) Hoover (-, JF827264, -); *Streptanthella longirostris* (S. Watson) Rydb. (EU620320, EU620383, EU718578); *Streptanthus arizonicus* S. Watson (-, -, EU718580); *Streptanthus barbatus* S. Watson (-, -, EU718581); *Streptanthus barbiger* Greene (-, -, EU718583); *Streptanthus breweri* A. Gray (-, -, EU718584); *Streptanthus campestris* S. Watson (EU620321, AY958571, -); *Streptanthus carinatus* C. Wright ex A. Gray (-, -, EU718585); *Streptanthus cordatus* Nutt. (EU620322, EU620384, EU718586); *Streptanthus glandulosus* Hook. (JF827208, -, EU718587); *Streptanthus hispidus* A. Gray (-, -, EU718588); *Streptanthus hyacinthoides* Hook. (EU620276 and EU620261, EU620385, EU718589); *Streptanthus maculatus* Nutt. (EU620326, EU620386, EU718591); *Streptanthus niger* Greene (-, -, EU718592); *Streptanthus oliganthus* Rollins (-, -, EU718589); *Streptanthus platycarpus* A. Gray (-, -, EU718594); *Streptanthus polygaloides* A. Gray (-, -, EU718595); *Streptanthus squamiformis* Goodman (-, -, DQ288835); *Streptanthus tortuosus* Kellogg (EU620277 and EU620262, EU620387, EU718597); *Thelypodiopsis ambigua* (S. Watson) Al-Shehbaz (EU620278 and EU620263, EU620389, EU718599); *Thelypodiopsis aurea* (Eastw.) Rydb. (-, -, EU718600); *Thelypodiopsis elegans* (M. E. Jones) Rydb. (-, EU620391, EU718602); *Thelypodiopsis divaricata* (Rollins) S. L. Welsh & Reveal (-, -, EU718601); *Thelypodiopsis vaseyi* (S. Watson ex B.L. Rob.) Rollins (-, -, EU718603); *Thelypodiopsis vermicularis* (S. L. Welsh & Reveal) Rollins (-, -, EU718604); *Thelypodium crispum* Greene ex Payson (-, -, EU718605); *Thelypodium flexuosum* B. L. Rob. (-, AY958582, -); *Thelypodium integrifolium* (Nutt.) Endl. (-, -, EU718606); *Thelypodium laciniatum* (Hook.) Endl. (EU620328, EU620392, DQ288838); *Thelypodium rollinsii* Al-Shehbaz (-, -, EU718608); *Thelypodium sagittatum* (Nutt.) Endl. (EU620279 and EU620264, EU620393, EU718609); *Thelypodium texanum* (Cory) Rollins (-, -, EU718610); *Thelypodium wrightii* A. Gray (EU620329, -, EU718611); *Thysanocarpus curvipes* Hook. (EU620330, GU246194, EU718613); *Warea amplexifolia* (Nutt.) Nutt. (EU620280 and EU620265, EU620397, EU718614); *Warea cuneifolia* (Muhl. ex Nutt.) Nutt. (EU620281 and EU620266, EU620398, EU718615); *Warea sessilifolia* Nash (-, EU620399, -); *Weberbauera herzogii* (EU620334, EU620400, EU718616); *Weberbauera peruviana* (DC.) Al-Shehbaz (EU620336, EU620402, EU718617); *Weberbauera rosulans* (O. E. Schulz) Al-Shehbaz (EU620284, EU620340, EU718526); *Zuloagocardamum jujuyensis* Salarato & Al-Shehbaz ARGENTINA: Jujuy, Humahuaca, Mina Aguilar (a) Zuloaga et al. 13574 (SI) (KF569756\*, KF569759\*, KF569762\*), (b) Zuloaga et al. 14267 (SI) (KF569757\*, KF569760\*, KF569763\*).

**Tribe Thlaspideae.** *Alliaria petiolata* (M. Bieb.) Cavara & Grande, (-, -, DQ288727); *Graellsia saxifragifolia* Boiss. (-, -, DQ288772); *Parlatoria rostrata* Boiss. (-, -, DQ288809); *Peltaria alliacea* Jacq. (DQ249855, -, -); *Pseudocamelina campylocarpa* (Boiss.) N. Busch (-, -, DQ288817); *Thlaspi arvense* L. (AF336152S1 and AF336152S2, -, DQ288839); *Thlaspi bulbosum* Boiss. (-, AY154798, -). **Tribe Turritideae.** *Turritis glabra* L. (-, -, DQ288840). **Tribe Yinshaniaeae.** *Yinshania acutangula* (O. E. Schulz) Y. H. Zhang (AH007969, -, -); *Yinshania acutangula* subsp. *wilsonii* (O. E. Schulz) Al-Shehbaz, G. Yang, L. L. Lu & T. Y. Cheo (AH007968, -, -). **Unassigned.** *Idahoa scapigera* (Hook.) A. Nelson & J. F. Macbr. (-, -, DQ288783); *Lunaria annua* L. (-, -, DQ288792).