Molecular phylogeny of *Menonvillea* and recognition of the new genus *Aimara* (Brassicaceae: Cremolobeae)

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Abstract Monophyly and phylogeny of *Menonvillea* were studied using molecular phylogenetic analyses of nuclear ribosomal (ITS) and chloroplast (*trnL-F*) DNA sequences. The phylogenies obtained were contrasted with the morphology of the genus. *Menonvillea*, excluding *M. rollinsii*, is monophyletic, and this species is sister to *Cremolobus*. Molecular and morphological data support the segregation of *M. rollinsii* into the new genus *Aimara*. Within the *Menonvillea* clade, three strongly supported and morphologically well-defined lineages were obtained. The geographical distribution of *Aimara* and the *Menonvillea* lineages are discussed.

Keywords *Aimara*; Brassicaceae; Cremolobeae; *Cremolobus*; ITS; *Menonvillea*; *trnL-F*

Supplementary Material The alignment files are available in TreeBase (http://www.treebase.org/treebase; study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S14157).

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■ INTRODUCTION

Menonvillea DC. includes about 25 species distributed mainly along the Andes of Chile and western Argentina, with some taxa growing in southern Patagonia (Santa Cruz Province in Argentina southward into Región Magallanes and Antarctica in Chile) and others restricted to the Antofagasta desert of northern Chile (Al-Shehbaz & Marticorena, 1990; Al-Shehbaz, 2008, 2010). Several taxa now included in *Menonvillea* were originally described in *Hexaptera* Hook., *Dispeltophorus* Lehm., *Decaptera* Turcz. and *Cymatoptera* Turcz. Hooker (1830) included in *Hexaptera* taxa with 3-winged fruit valves (one dorsal and two lateral wings) and restricted *Menonvillea* to species with two lateral-winged valves. Turczaninow (1846) included in *Decaptera* taxa with 5-winged fruit valves. In both *Cymatoptera* and *Dispeltophorus* the fruit valves are 2-winged and therefore do not differ from *Menonvillea*. Rollins (1955) revised *Menonvillea*, united the four genera above, and recognized 29 species. He indicated that the number of fruit wings was variable and unreliable at the generic level and characterized the genus to include species with schizocarpic silicles that break into two 1-seeded, indehiscent, 2-, 3-, or 5-winged (or rarely wingless) mericarps. Recently, Al-Shehbaz (2008) accepted 23 species in the genus and later (Al-Shehbaz, 2010) added *M. zuloagaensis* Al-Shehbaz as a new species from Argentina.

Menonvillea is highly diverse morphologically, especially in habit (shrubs, herbs), leaves (size, margin, shape, indumentum), flowers (size, color, nectar gland type, indumentum of petal and filament bases), and fruits (size, wing number, callosities; Fig. 1). The ecological range of the genus is remarkably wide, with species growing at Andean high altitudes (up to 5300 m), in the dry coastal desert of the Chilean Atacama, or in high rainfall regions in southwestern Argentina and Chile.

Schulz (1936) placed *Menonvillea* and *Cremolobus* DC. in the South American Cremolobeae, a tribe he distinguished from the rest of Brassicaceae by having schizocarpic, angustiseptate, often longitudinally winged fruits that split at maturity into two indehiscent and 1-seeded mericarps. *Menonvillea* differs from *Cremolobus* (7 spp. along the Andes from Colombia to northern Argentina and Chile; Khanna & Rollins, 1965) by its 2-, 3-, or 5-winged or rarely wingless (vs. 1-winged), dorsiventrally (vs. laterally) flattened mericarps and absence (vs. presence) of the fruit septum.

Family-wide phylogenies of Brassicaceae (Couvreur & al., 2010; Warwick & al., 2010) showed that *Menonvillea* is related to *Cremolobus* and tribes Eudemeae and Schizopetaleae. However, these studies included only one species of *Menonvillea*. Since the monophyly and phylogenetic relationships within *Menonvillea* remained unresolved, it is important to study the molecular phylogeny of the genus using both nuclear and chloroplast data. Sequences from ITS and the *trnL-F* region were shown to be useful in evaluating relationships among Brassicaceae, particularly at the generic level (e.g., Koch & Mummenhoff, 2001; Warwick & al., 2002, 2006, 2007, 2008, 2009, 2010, 2011; O'Kane & Al-Shehbaz, 2003; Bailey & al., 2006; Alexander & al., 2010; Moazzeni & al., 2010; German & al., 2011).

The principal goals of this study are to test the monophyly of *Menonvillea* and to establish phylogenetic relationships among its species. To achieve this, molecular phylogenies using DNA sequences of the nuclear ITS and the chloroplast

Fig. 1. Habit, inflorescence and habitat of *Menonvillea* species. **A,** *M. cuneata*, raceme (*Zuloaga 12775*); **B,** *M. cuneata*, mature fruiting plant (*Teillier 5558*); **C,** *M. purpurea*, raceme (*Gosewijn s.n.*); **D,** *M. patagonica*, mature flowering plant (*Villamil 11174*); **E,** *M. nordenskjoeldii*, mature flowering plant (*Iribarren s.n.*); **F**, *M. scapigera* subsp. *scapigera*, mature flowering plant (*Zuloaga 12364*); **G,** *M. scapigera* subsp. *scapigera*, raceme (*Álvarez 11*); **H,** *M. cicatricosa*, mature flowering plant (*Álvarez 2*); **I,** *M. scapigera* subsp. *longipes*, mature flowering plant (*Johnson 10-130*); **J,** *M. scapigera* subsp. *scapigera*, raceme (*Álvarez 3*); **K,** *M. spathulata*, mature flowering plant (*Zuloaga 12258*); **L,** *M. virens*, mature flowering plants (*Donadío 124*).

trnL-F region were obtained. Also, the main morphological characters to differentiate groups of species were analyzed and discussed in a phylogenetic context.

■ MATERIALS AND METHODS

Taxon sampling. — Forty-six accessions representing 22 species and four subspecies of *Menonvillea* (ca. 90% of taxa) from all major geographical areas and covering all morphological variation were sampled. Only samples of *M. constitutionis* (Phil.) Rollins, *M. macrocarpa* (I.M. Johnst.) Rollins and *M. zuloagaensis* were not available. The findings of German & al. (2009), Couvreur & al. (2010), Beilstein & al. (2010) and Warwick & al. (2010) were used as the basis for selecting an outgroup of 19 species of 12 tribes (Aethionemeae, Arabideae, Boechereae, Chorisporeae, Cremolobeae, Crucihimalayeae, Dontostemoneae, Eudemeae, Halimolobeae, Schizopetaleae, Sisymbrieae, Thelypodieae) sensu Al-Shehbaz (2012), representing all main lineages of the family. Sequences of *Cremolobus* were obtained in this work, and those of the rest of the outgroup were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

PCR amplification and DNA sequencing. — Total DNA was isolated from leaves (collected in the field and dried in silica gel) using a modified (CTAB) protocol by Doyle & Doyle (1987), or from herbarium material using a DNeasy Plant Mini kit (Qiagen, Hilden, Germany). The nuclear ribosomal ITS region (ITS1-5.8S-ITS2) was amplified by PCR in one or two fragments using the ITS2, ITS3, ITS4 and ITS5 primers of Baldwin (1992); the chloroplast region (*trnL* intron/*trnL-F* spacer) was amplified in one or two fragments using primers C, D, and E of Taberlet & al. (1991) and Fdw (5′ CAGTCCTCT GCTCTACCAGC 3′). PCR reactions were performed in 25 µL final volumes with 50–100 ng of template DNA, 0.2 µM of each primer, 25 µM dNTP, 5 mM $MgCl_2$, 1× buffer and 1.5 units of *Taq* polymerase provided by Invitrogen Life Technologies. PCR amplifications were set at the following conditions for most of the species: (ITS) a first period of denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 60 s, and extension at 72°C for 90 s, with a final extension at 72°C for 7 min; (*trnL-F*) a first period of denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 60 s, and extension at 72°C for 90 s, with a final extension at 72°C for 10 min. In addition, a variety of PCR additives and enhancing agents (e.g., bovine serum albumin, dimethyl sulfoxide, formamide) were used to increase the yield, specificity and consistency of PCRs. Cleaning of PCR products was done by Macrogen, Inc. (Seoul, Korea) using the Montage PCR purification kit from Millipore and following the manufacturer's protocol. Sequencing reactions were also performed by Macrogen using the ABI PRISM BigDye Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul, Korea) following the protocols supplied by the manufacturer. Sequences were assembled and edited using the program Chromas Pro v.1.41 (Technelysium Pty, Ltd), which was also used for checking the presence of single peaks in the chromatograms, especially in the ITS sequences. Eigthy-two new sequences were obtained and submitted to GenBank (http://www.ncbi.nlm.nih.gov). Voucher information and GenBank accession numbers are provided in the Appendix 1. Alignments were generated with Muscle v.3.6 (Edgar, 2004) using a first round of multiple alignment and posterior rounds of refinement under the default settings. Alignments were then checked and improved manually where necessary by visual refinement using the program Bioedit v.7.0.9.0 (Hall, 1999). All aligned matrices were submitted to TreeBase (http://www.treebase.org/treebase; study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S14157).

Phylogenetic analyses. — Datasets from ITS and *trnL-F* were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). The ITS and *trnL-F* datasets were also analyzed by using the incongruenc length difference (ILD) test of Farris & al. (1995) in PAUP* v.4.0b10 (Swofford, 2003) ("HomPart" command) with 1000 replications. Both datasets were combined because they were non-significantly incongruent ($P = 0.182$). Gaps were coded as present or absent using the "simple indel coding" method implemented by Simmons & Ochoterena (2000) using the program FastGap v.1.2 (Borchsenius, 2009), and included only in the MP analyses. For these analyses, tree searches were done with the program TNT v.1.1 (Goloboff & al., 2008) using heuristic searches with 1000 random addition sequences, tree-bisection-reconnection branch swapping (TBR) and holding ten trees per replicate; generated trees were then submitted to a new round of TBR branch swapping to completion. Support values for nodes were estimated using Jackknife (JK) analysis (Farris & al., 1996) with 2000 replicates of ten random addition sequences, holding four trees per replicate and using the default removal probability (0.36). Maximum likelihood analyses were conducted using RAxML v.7.2.6 (Stamatakis, 2006). The model of nucleotide substitution was selected by the Akaike information criterion (AIC) implemented in jModeltest v.0.1.1 (Posada, 2008): SYM+G (ITS) and TPM1uf+G (*trnL-F*). The algorithm implemented in RAxML was used for carrying out nonparametric bootstrap (BS) analyses and searches for the best-scoring ML tree in one single run (Stamatakis & al., 2008). We executed 1000 rapid bootstrap inferences and thereafter thorough ML search under the GTRGAMMA model. Bayesian analyses were conducted using MrBayes v.3.2 (Ronquist & al., 2012). Models were set in MrBayes as GTR+G 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 1000 generations to ensure independence of the successive samples. The first 2000 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 & Drummond, 2007). The remaining samples of each run were combined, and a maximum clade credibility tree was calculated. Additionally, the hypothesis of monophyly of *Menonvillea* including and

excluding *M. rollinsii* was tested using the SH test (Shimodaira & Hasegawa, 1999) implemented in PAUP* v.4.0b10 (Swofford, 2003). Searches of constrained topologies were conducted in RAxML with 1000 replicates and the GTRGAMMA model, and the significance of differences between the best ML tree and the best ML constrained tree was determined in PAUP* v.4.0b10 using 1000 BS replicates and rejecting the hypothesis when $P < 0.05$.

Morphological and biogeographical data. — Morphological and biogeographical studies of all species included in *Menonvillea* were based on 864 specimens from BAA, BAB, BCRU, CONC, CORD, GH, K, LIL, LP, MERL, SI and SRFA and on fresh material collected during field trips in Chile and Argentina. These results are part of the taxonomic revision of the genus (in preparation) but are discussed here to characterize the clades obtained in the molecular phylogenies (Figs. 4–8).

■ RESULTS

Monophyly and phylogenetic relations in *Menonvillea*. — Characteristics of ITS and *trnL-F* sequences are summarized in Table 1. The resulting ITS multiple alignments for 62 taxa was 684 bp long, of which 235 bp (≈34%) were parsimony-informative (Table 1). Length of the ITS sequences varied within the ingroup from 596 bp in *M. famatinensis* (Boelcke) Rollins to 602 in *M. minima* Rollins. Thirthy-four informative indels ranging from 1 to 37 bp were introduced in the alignment, of which 12 were 1 bp long. The MP analyses using substitution data and gaps resulted in 228 most parsimonious trees, and the ML and BI analyses (using only substitution data) recovered similar topologies showing the same strongly supported clades. *Menonvillea* was not recovered as monophyletic (Fig. 2A–B) when it included *M. rollinsii* Al-Shehbaz & Martic. This species formed a highly supported clade with species of *Cremolobus* (JK = 90%, BS = 93%, PP = 100%). The remaining species of *Menonvillea* formed three well-supported clades: (1) the chilensis clade (CHI) including all Chilean endemics minus *M. rollinsii* (JK, BS and PP = 100%), (2) the scapigera clade (SCA) including *M. famatinensis* and *M. scapigera* (Phil.) Rollins (JK, BS and PP = 100%), and (3) the cuneata clade (CUN) including species distributed along the Argentinean-Chilean Andes (JK, BS and PP = 100%). Relationships between these clades were not recovered in the MP analysis, and in the ML and BI analyses the scapigera and cuneata clades were unsupported sisters. When only gaps were analyzed with MP, the chilensis, scapigera and cuneata clades were also recovered (Fig. 2C)

The *trnL-F* multiple alignments included 60 taxa (*C. chilensis* (Lag. ex DC.) DC. could not be sequenced) and were 1321 bp long, of which 128 bp (≈10%) were parsimony-informative. Length of the *trnL-F* sequences varied within the ingroup from 725 bp (several species) to 778 bp (*M. frigida* (Phil.) Rollins). Twenty-five informative indels were introduced, with lengths ranging from 1 to 86 bp. The topologies obtained by MP (60 MPT), ML, and BI analyses were similar to those of the ITS sequences, and they recovered the same highly supported four clades of *Menonvillea*: the *M. rollinsii*+*Cremolobus* clade (JK = 85%, BS = 84%, PP = 99%), the chilensis clade (JK = 99%, BS = 99%, PP = 100%), the scapigera clade (JK = 99%, BS = 100%, PP = 100%), and the cuneata clade (JK = 99%, BS = 99%, PP = 100%; Fig. 2D–E). In these topologies, the scapigera and cuneata clades appeared as sister groups (JK = 77%, BS = 80%, PP =98%), and *Menonvillea* minus *M. rollinsii* formed a monophyletic group (JK < 50%, BS = 54%, PP = 0.93). Gap data of the *trnL-F* alignment also recovered the chilensis and cuneata clade in the MP analysis (Fig. 2F).

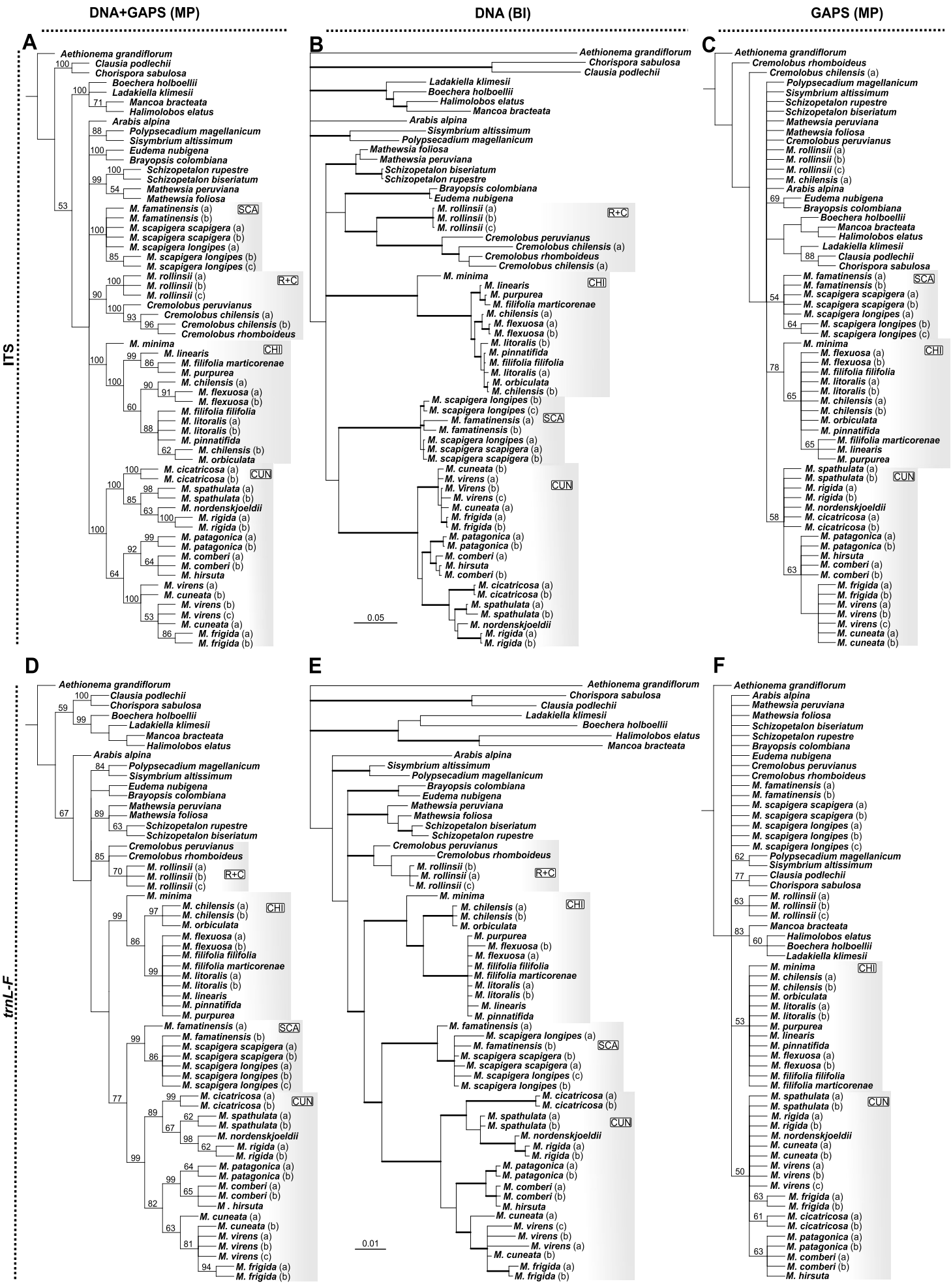
The hypothesis of monophyly of *Menonvillea* including *M. rollinsii* was rejected under the SH test for both ITS and *trnL-F* datasets ($\Delta\mathrm{Dln}L = 22.86$, $P = 0.022$; $\Delta\mathrm{Dln}L = 16.04$. $P = 0.04$, respectively). When monophyly of *Menonvillea* excluding *M. rollinsii* was tested with the ITS data, the SH test did not reject it ($\Delta\mathrm{Dln}L = 3.43$, $P = 0.15$). This last hypothesis was not tested with the *trnL-F* sequences because monophyly of *Menonvillea* excluding *M. rollinsii* was recovered in optimal trees.

Since the ILD test found that the ITS and *trnL-F* data were non-significantly incongruent ($P = 0.182$), the two datasets were combined. Results from MP, ML and BI using the combined dataset (≈2 kb) yielded highly congruent topologies, with all species of *Menonvillea* minus *M. rollinsii* included in a weakly supported clade (JK < 50%, BS = 61%, PP = 88%; Fig. 3). The

Table 1. Sequence characteristics and model of DNA evolution

	ITS	*trnL-trnF*
Number of terminals	62	60
Length of the alignment [bp]	684	1321
Length of sequences (ingroup) [bp]	596 (*M. famatinensis*) - 602 (*M. minima*)	725 (several species) - 778 (*M. frigida*)
No. of parsimony-informative characters	235	128
No. of informative gaps in the alignment	34	25
Maximum sequence divergence within ingroup (%)*	16	4
Model selected by AIC	SYM+G	TPM1uf+G

* calculated as p distance


DNA+GAPS (MP)
DNA (BI)
GAPS (MP)
ITS
trnL-F
A
B
C
D
E
F
Aethionema grandiflorum
Clausia podlechii
Chorispora sabulosa
Boechera holboellii
Ladakiella klimesii
Mancoa bracteata
Halimolobos elatus
Arabis alpina
Polypsecadium magellanicum
Sisymbrium altissimum
Eudema nubigena
Brayopsis colombiana
Schizopetalon rupestre
Schizopetalon biseriatum
Mathewsia peruviana
Mathewsia foliosa
M. famatinensis (a)
M. famatinensis (b)
M. scapigera scapigera (a)
M. scapigera scapigera (b)
M. scapigera longipes (a)
M. scapigera longipes (b)
M. scapigera longipes (c)
M. rollinsii (a)
M. rollinsii (b)
M. rollinsii (c)
Cremolobus peruvianus
Cremolobus chilensis (a)
Cremolobus chilensis (b)
Cremolobus rhomboideus
M. minima
M. linearis
M. filifolia marticorenae
M. purpurea
M. chilensis (a)
M. flexuosa (a)
M. flexuosa (b)
M. filifolia filifolia
M. litoralis (a)
M. litoralis (b)
M. pinnatifida
M. chilensis (b)
M. orbiculata
M. cicatricosa (a)
M. cicatricosa (b)
M. spathulata (a)
M. spathulata (b)
M. nordenskjoeldii
M. rigida (a)
M. rigida (b)
M. patagonica (a)
M. patagonica (b)
M. comberi (a)
M. comberi (b)
M. hirsuta
M. virens (a)
M. cuneata (b)
M. virens (b)
M. virens (c)
M. cuneata (a)
M. frigida (a)
M. frigida (b)
SCA
R+C
CHI
CUN
0.05
0.01

chilensis, scapigera and cuneata clades were highly supported (JK, BS and PP = 100%), and the last two were sister groups (JK = 54%, BS = 83%, PP = 100%). *Menonvillea rollinsii* was included in a strongly supported clade as sister to *Cremolobus* (JK = 99%, BS = 100%, PP = 100%). The SH test rejected the monophyly of *Menonvillea* including *M. rollinsii* ($\Delta \mathrm{Dln}L$ = 31.65462, P = 0.01).

■ DISCUSSION

The present molecular analysis of *Menonvillea* clearly establishes monophyly of the genus, excluding *M. rollinsii* (*Menonvillea* s.str.). *Cremolobus* appeared closely related to *Menonvillea*, but monophyly of tribe Cremolobeae sensu Schulz (1936) and Warwick & al. (2010) was not recovered.

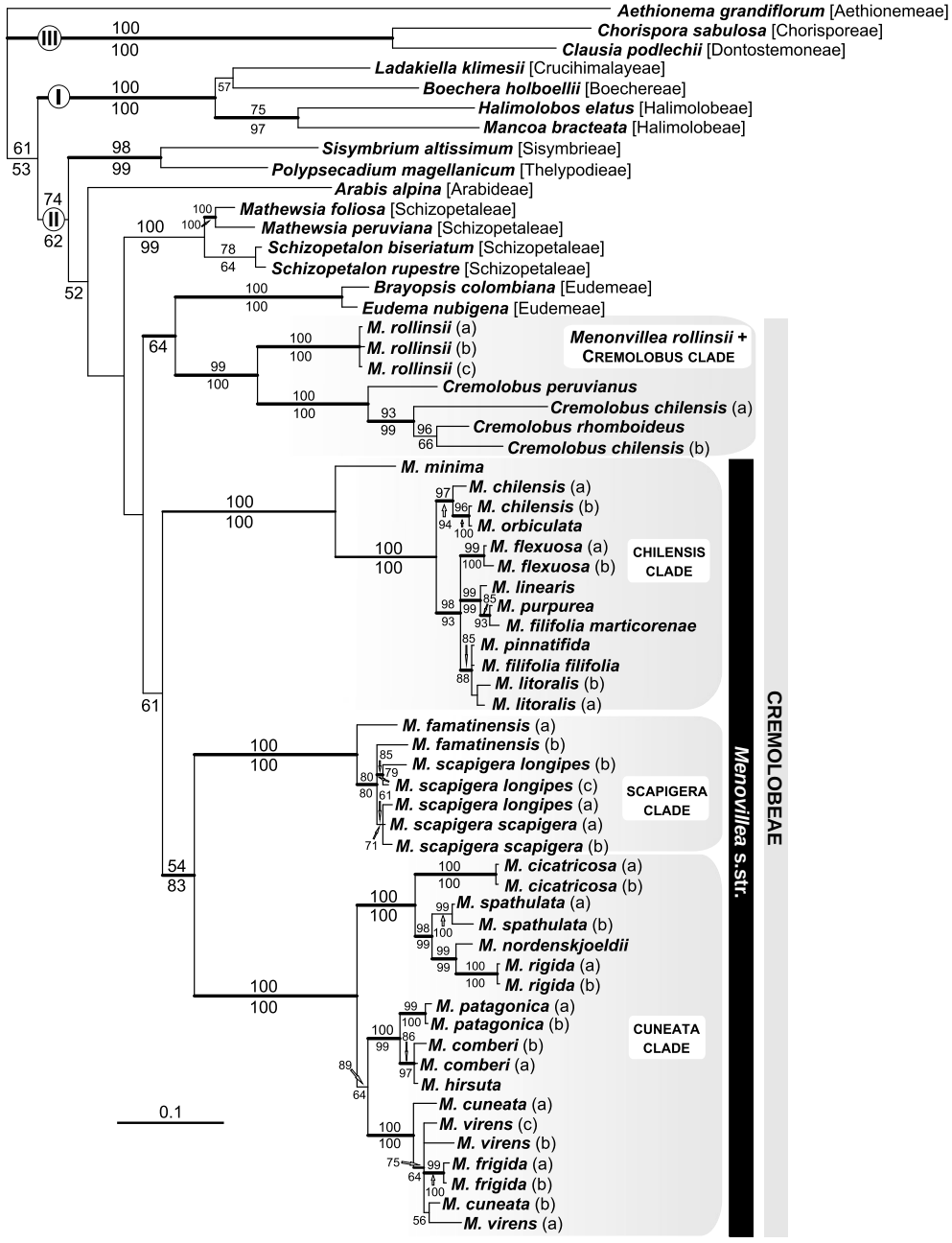


Fig. 3. Maximum clade credibility tree from 12,002 trees obtained in the Bayesian analysis of the combined ITS+*trnL-F* dataset. Values above and below branches correspond to parsimony jackknife/maximum likelihood bootstrap values, respectively. Thick branches indicate >0.95 Bayesian posterior probability. Names in brackets indicate tribal assigment of taxa and circles with I, II, and III indicate main lineages of Brassicaceae.

◀ **Fig. 2.** Phylogenetic trees obtained in the maximum parsimony and Bayesian analyses using ITS **(A–C)** and *trnL-F* sequences **(D–F). A, D,** maximum parsimony strict consensus tree using DNA substitutions and gaps; **B, E,** Bayesian maximum clade credibility tree using only DNA substitutions; **C, F,** maximum parsimony strict consensus tree using only gaps. Values above branches in A, C, D and F represent parsimony jackknife values. Thick branches in B and E indicate >0.90 Bayesian posterior probability. CHI = chilensis-clade, CUN = cuneata clade, R+C = *Menonvillea rollinsii*+Cremolobus clade, SCA = scapigera clade.

All species in Cremolobeae (*Cremolobus* and *Menonvillea*) have schizocarpic, strongly angustiseptate silicles that break into two 1-seeded, indehiscent mericarps (Fig. 4). However *Cremolobus* is easily separated from *Menonvillea* by its laterally (vs. dorsiventrally) flattened mericarps (Fig. 4A). Our analysis thus confirms the robustness of fruit morphology as a diagnostic character.

Menonvillea rollinsii, segregated by the molecular data from *Menonvillea* and sister to *Cremolobus* in all analyses conducted, also has laterally compressed mericarps as *Cremolobus* (Fig. 4B). It is a shrub with well-developed cork, minutely scabrous trichomes (Fig. 5A), petals crisped at their margins, and four tooth-like median nectar glands; all of these characters are absent in the remaining species of *Menonvillea* (Table 2).

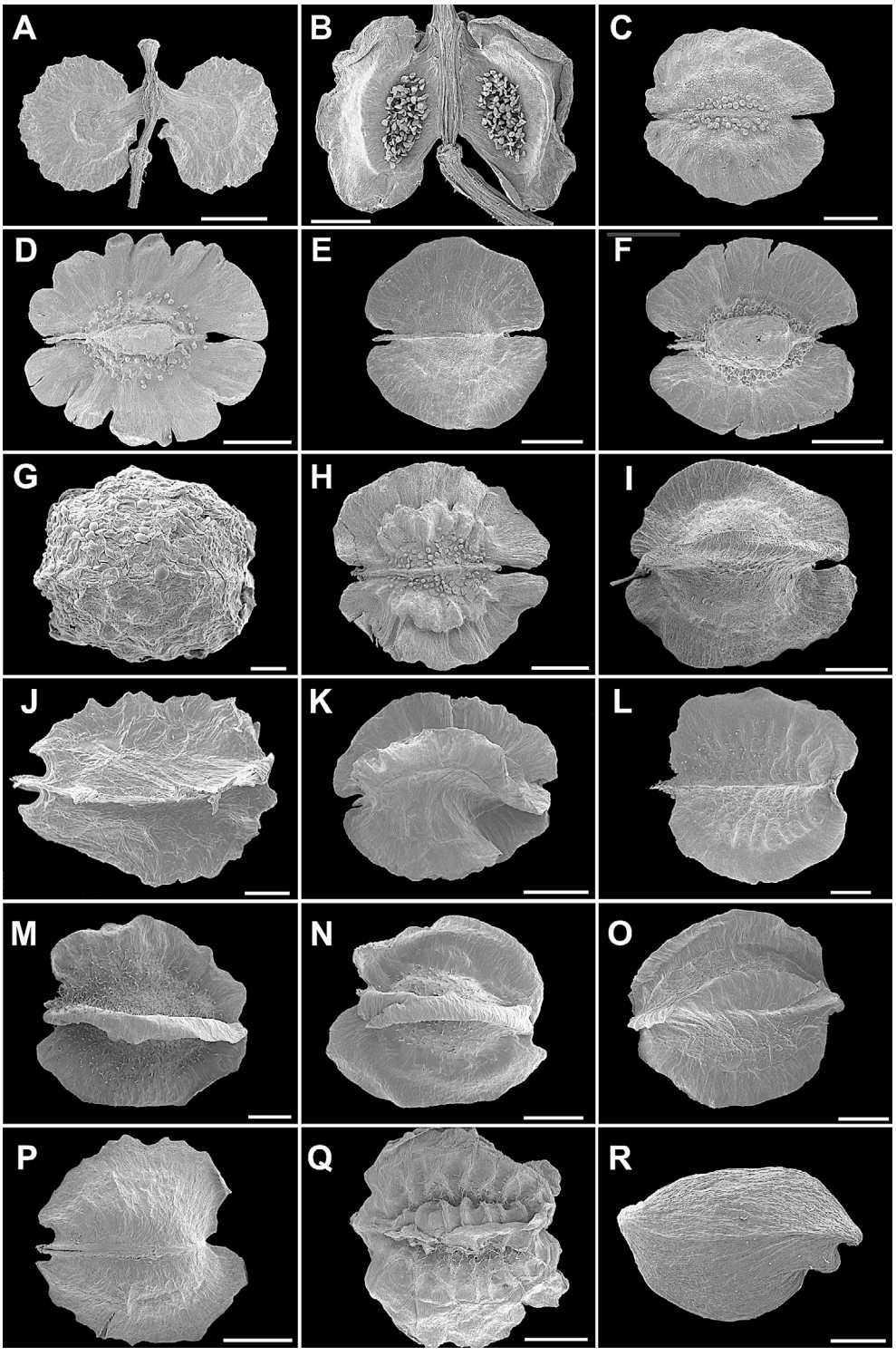


Fig. 4. Mericarps of *Cremolobus* **(A)** and species of *Menonvillea* **(B–R). A,** *Cremolobus peruvianus* (*Boeke 947*, SI); **B,** *Menonvillea rollinsii* (*Baumann 148*, CONC); **C,** *M. chilensis* (*Garaventa 7095*, SI); **D,** *M. filifolia* subsp. *filifolia* (*Garaventa 2746*, SI); **E,** *M. flexuosa* (*Arancio 93163*, ULS); **F,** *M. litoralis* (*Garaventa 8005*, SI); **G,** *M. minima* (*Marticorena & al. 1840*, CONC); **H,** *M. orbiculata* (*Werdermann 391*, LIL); **I,** *M. purpurea* (*Teillier & Márquez 5301*, CONC); **J,** *M. famatinensis* (*Hieronymus & Niederlein 379*, SI); **K,** *M. scapigera* subsp. *longipes* (*Al-Shehbaz 815*, SI); **L,** *M. hirsuta* (*Zöllner 12024*, BAA); **M,** *M. cuneata* (*Zuloaga & al. 12775*, SI); **N,** *M. frigida* (*Arroyo 135995*, CONC); **O,** *M. nordenskjoeldii* (*Guerrido 613*, SI); **P,** *M. patagonica* (*Ruíz Leal 24473*, MERL); **Q,** *M. spathulata* (*Prina 3624*, SI); **R,** *M. zuloagaensis (Nicora & al. 8262*, BAA). — Scale bars: A, K = 2000 μm; B–F, H, I, L–Q = 1000 μm; G = 200 μm; J, R = 500 μm.

Menonvillea rollinsii differs from *Cremolobus* by its 2- (vs. 1-) winged mericarps and incumbent (vs. accumbent) cotyledons (Table 2; Fig. 6). Molecular phylogenies and morphological differences between *M. rollinsi* and species of *Menonvillea* and *Cremolobus* justify the recognition of this species as a separate genus. It is restricted to the Atacama Plateau in northwestern Chile (Fig. 7), where it grows at altitudes between 2500 and 3350 m with high temperature fluctuations, with ca. 3°C in winter, and with high aridity with ca. 20 mm annual precipitation. The shrubby habit with a well-developed cork, and the reduced and fleshy leaves seem to be important adaptations that ensure its survival in such a harsh environment. Typical shrubs are uncommon in Brassicaceae (Appel & Al-Shehbaz, 2003), though they undoubtedly evolved independently many times in the family.

As delimited by molecular data, *Menonvillea* s.str. comprises three well-defined clades (Figs. 2–3, 6). The chilensis clade includes herbs with linear leaves covered with simple or branched arachnoid trichomes (Fig. 5B–C), white or yellow petals, and petaloid nectar glands (except in *M. flexuosa* Phil.). The mericarps can be glabrous or papillate and with or without conspicuous callosities, while simple trichomes are absent (Fig. 4C–I). All species have incumbent cotyledons, except *M. pinnatifida* Barnéoud with oblique to accumbent cotyledons (Fig. 6). Members of this clade are endemic to Chile along the central depression (between the Andes and the Chilean coast ranges), and their distribution extends to the Chilean coast (Fig. 7). Taxa of this clade are distributed mainly from the Antofagasta region southwards into the central regions of Santiago, Libertador O'Higgins and Maule, although *M. purpurea* (Hastings) Rollins and *M. linearis* DC. reach the southern regions of Araucanía and Los Ríos, respectively. Species such as *M. chilensis* (Turcz.) B.D. Jacks., *M. filifolia* Fisch. & C.A. Mey., *M. orbiculata* Phil., *M. litoralis* (Barnéoud) Rollins, and *M. minima* Rollins inhabit lowlands near sea level along the Chilean coast, while *M. flexuosa*, *M. linearis*, *M. pinnatifida*

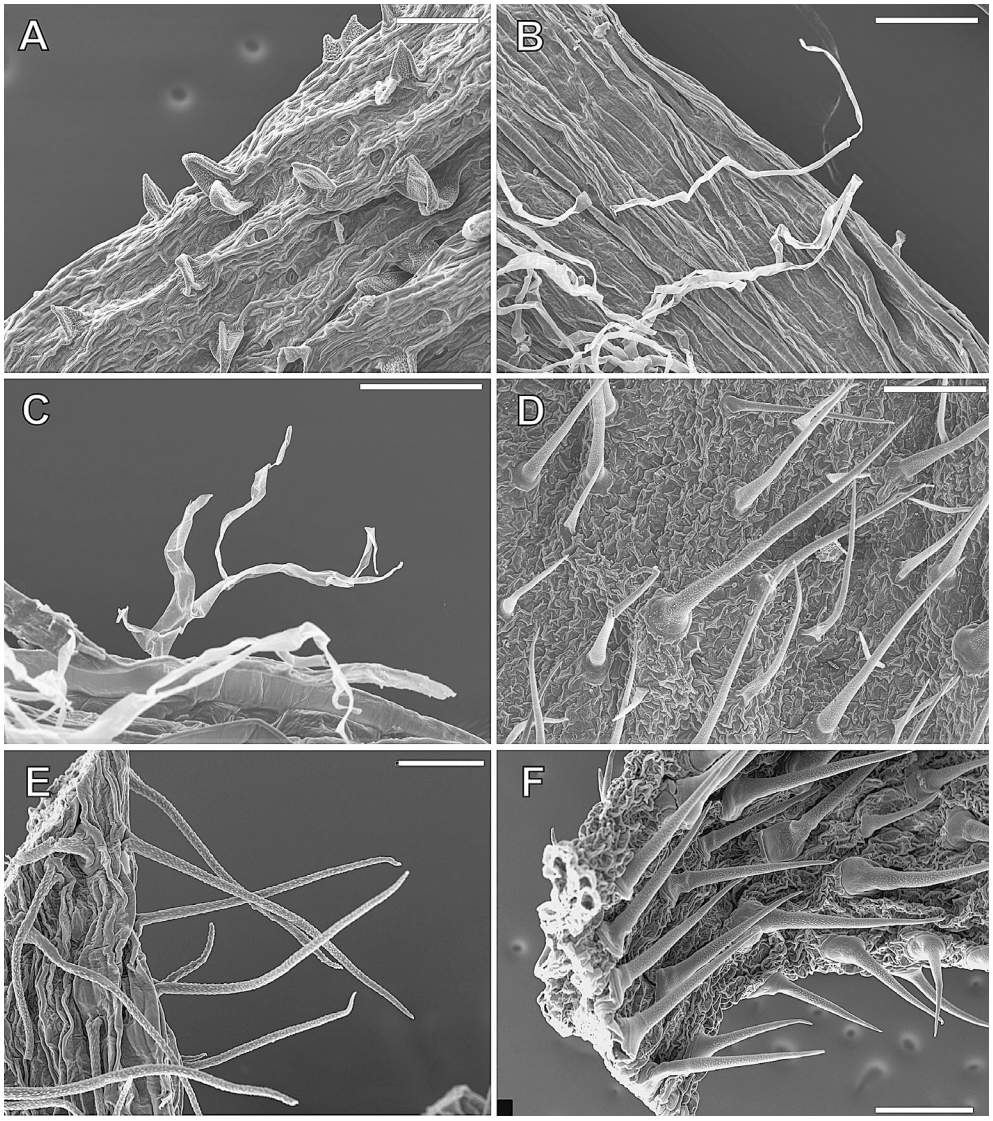


Fig. 5. Trichomes in *Menonvillea*. **A,** *Menonvillea rollinsii* (*Baumann 148*, CONC); **B,** *M. filifolia* subsp. *filifolia* (*Schlegel 3830*, CONC); **C,** *M. pinnatifida* (*Pisano & Bravo 895*, CONC); **D,** *M. scapigera* subsp. *longipes* (*Morrone 5958*, SI); **E,** *M. virens* (*Krapovickas 6312*, BAB); **F,** *M. spathulata* (*Prina 2920*, SI). — Scale bars: A–C, E = 100 µm; D, F = 200 µm.

Table 2. Comparison of *Aimara* with related taxa.

	Aimara	*Cremolobus*	*Menonvillea*
Habit	Shrubs	Herbs, lianas, shrubs	Herbs
Trichome type when present	Scabrous	Simple terete, rarely branched	Simple terete, simple arachnoid and branched arachnoid
Nectar glands	Four tooth-like	Two or four tooth-like or inconspicuous	One and confluent, four and petaloid or inconspicuous
Compression of mericarps	Lateral	Lateral	Dorsal
Wing number of mericarp	Two	One, rarely wingless	Wingless, two, three, less frequently five
Cotyledonary position	Incumbent	Accumbent	Incumbent and accumbent

and *M. purpurea* show a similar distribution pattern but also grow in Andean highlands. The annual mean temperature in these areas is 9°C–16°C, and the annual mean precipitation ranges from ca. 15 mm in Atacama to 100–800 mm in the southernmost regions. The chilensis clade includes four annual species (*M. chilensis*, *M. minima*, *M. filifolia* Fisch. & C.A. Mey., *M. litoralis* (Barnéoud) Rollins) adapted to warm/hot environments with dry summers and mild to cool wet winters, and the areas they occupy may experience four months of drought in the southern part to nine in the north (Grau, 1995).

Species of the scapigera and cuneata clades differ from those of the chilensis clade mainly by having non-linear leaves, simple and terete trichomes (Fig. 5D–F), white petals, one confluent nectar gland, and fruits without papillae or callosities (Fig. 4J–R). The scapigera clade includes perennial herbs, generally with a scapose habit (except *M. scapigera* (Phil.) Rollins subsp. *longipes* (Rollins) Prina with cauline leaves), long-petiolate leaves, connate median staminal filaments, glabrous mericarps (Fig. 4J–K), and incumbent cotyledons (Fig. 6). By contrast, members of the cuneata clade are annuals or perennials with cauline leaves, pubescent mericarps with simple trichomes (except *M. cicatricosa* (Phil.) Rollins and *M. patagonica* with glabrous fruits) (Fig. 4M–Q), and accumbent cotyledons (Fig. 6).

Species of the scapigera and cuneata clades are distributed along the Andes and their foothills of Argentina and Chile, and they reach high altitudes in the northern part and low altitudes southwards (Fig. 7). Members of the scapigera clade occupy the central Andes of Chile and Argentina in the Altoandina, Prepuna and Puna biogeographical provinces (sensu Cabrera & Willink, 1973; Morrone, 2001) and northern Patagonia. They are perennials that usually grow at high altitudes, reaching ca. 4500 m in areas where the temperature is low throughout the year (annual mean −1°C to 12ºC), and produce a deep underground caudex that allows them to survive in the coldest season. Species of the cuneata clade are widely distributed and occupy the most variable ecological ranges in the genus, ranging from the northern Altoandina and Puna regions of Jujuy in Argentina and Antofagasta in Chile, to southern Patagonia in Santa Cruz and the Magallanes region. For example, *M. virens* (Phil.) Rollins and *M. cuneata* (Gillies & Hook.) Rollins grow in the north-central Andes at altitudes of 2000–5300 m, whereas *M. nordenskjoeldii* inhabits the southernmost end of the Andes at 500–2100 m. Species of the cuneata clade are perennials except for *M. comberi* Sandwith, *M. hirsuta* Rollins and *M. patagonica*, which are distributed in central-western Patagonia between 500–2500 m.

The three species not sampled for the molecular analyses include *M. constitutionis* (Phil.) Rollins, which shares features such as narrow leaves, arachnoid trichomes, yellow petals, petaloid nectar glands, and incumbent cotyledons with members of the chilensis clade. *Menonvillea macrocarpa* (I.M. Johnst.) Rollins resembles the cuneata clade by its lanceolate cauline leaves with simple and terete trichomes, one confluent nectar gland, pubescent fruits, and accumbent cotyledons. Finally, *M. zuloagaensis* is morphologically similar to members of the scapigera clade and has a scapose habit with a compactly branched woody caudex, long-petiolate and palmately-pinnately lobed leaves, white petals, one confluent nectar gland, and incumbent cotyledons. The last species differs from members of the scapigera clade in having persistent petals, free median stamens, and wingless mericarps (Al-Shehbaz, 2010) (Fig. 4R). Therefore, its phylogenetic position should be confirmed by molecular data.

■ TAXONOMIC TREATMENT

Key to the genera of tribe Cremolobeae

1 Fruit valves dorsally compressed, 2-, 3-, or 5-winged, rarely wingless; herbs …… ***Menonvillea***
1 Fruits with valves laterally compressed, 1- or 2-winged, rarely wingless; herbs, lianas, shrubs …… 2
2 Fruit valves 1-winged or rarely wingless; cotyledons accumbent; annual herbs, scandent perennials or shrubs; leaves neither fleshy nor subulate, dentate to pinnatifid …… ***Cremolobus***
2 Fruit valves 2-winged; cotyledons incumbent; shrubs; leaves fleshy, subulate-linear, entire …… ***Aimara***

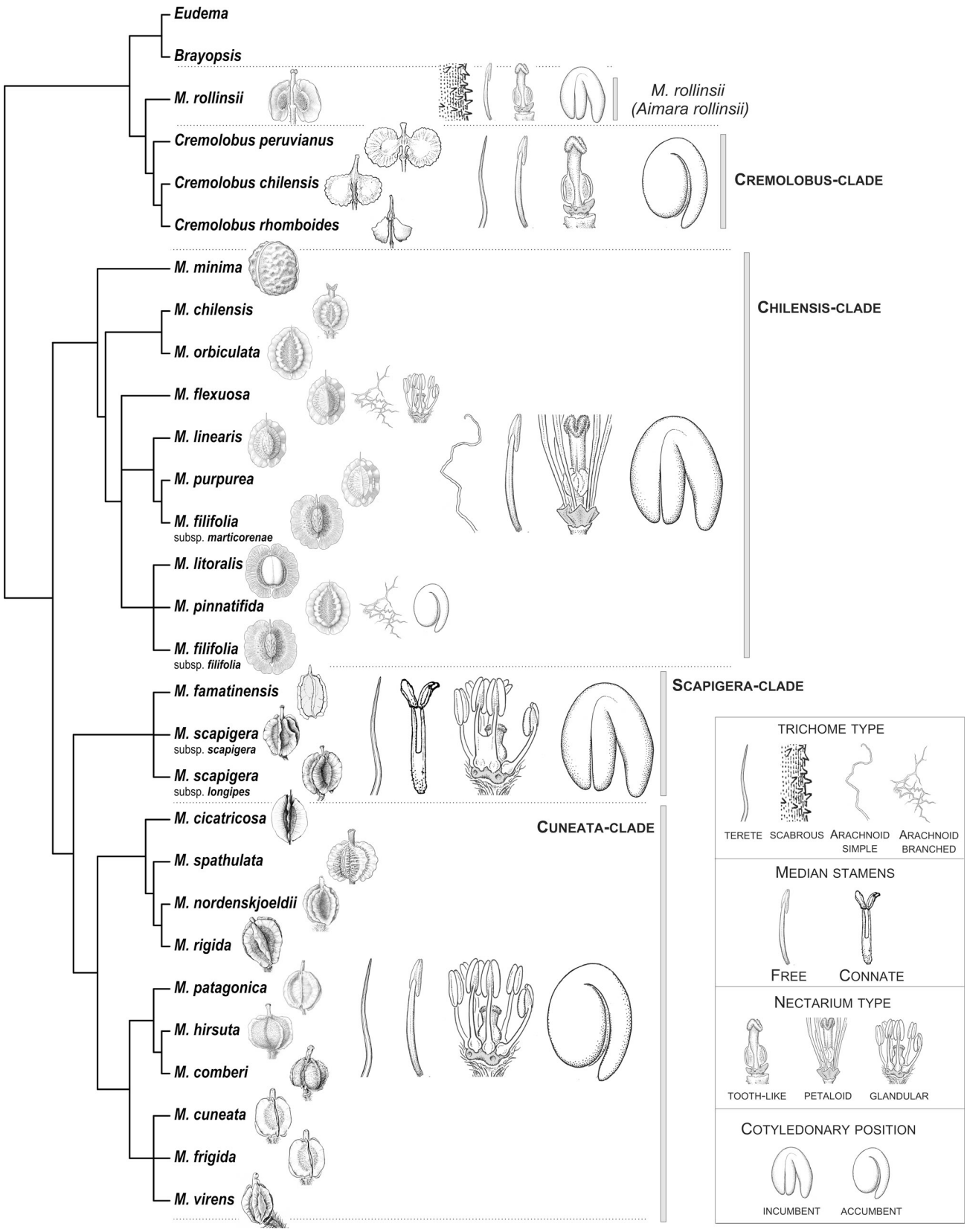


Fig. 6. Principal clades in *Menonvillea* and main morphological characters shared by its species. Backbone of the tree based on the maximum clade credibility tree obtained with the combined ITS+*trnL-F* dataset. Large drawings show character states shared by all member of a clade, and small drawings show characters of individual species. Drawings by Francisco Rojas.

Aimara Salariato & Al-Shehbaz, **gen. nov.** – Type: *Aimara rollinsii* (Al-Shehbaz & Martic.) Salariato & Al-Shehbaz.

Shrubs, perennial. **Stems** branched, erect, woody, older ones stramineus, with well-developed cork. **Trichomes** simple, sparsely and minutely scabrous throughout. **Multicellular glands** absent. **Leaves** cauline, sessile; leaf blades subulate-linear, fleshy, straight or incurved, scabrous, margin entire. **Racemes** terminal, few-flowered, ebracteate, corymbose, elongated considerably in fruit, longer than leaves; rachis scabrous, straight; fruiting pedicels divaricate, straight, scabrous, persistent. **Sepals** oblong, free, caducous, erect, equal, scabrous, not saccate, scarious margined. **Petals** white, slightly longer than sepals; blade oblong, uniform in width, crisped at margins; claw undifferentiated from blade, glabrous at base, unappendaged. **Stamens** 6, erect, slightly tetradynamous; filaments wingless, unappendaged, with a slender and glabrous base, free; anthers oblong to slightly sagittate, not apiculate. **Median nectar glands** solitary, toothlike; lateral glands ringlike. **Ovules** 2 per ovary, placentation subapical. **Fruits** schizocarpic silicles, breaking into two 1-seeded mericarps, ovoid, laterally compressed, didymous, strongly angustiseptate, not inflated, unsegmented; valves indehiscent, thickened, woody, not keeled, glabrous; valves obscurely to prominently 2-winged, inconspicuously veined, triangular in cross section, coarsely papillate adaxially between wing and replum, minutely papillate abaxially on both sides of central ridge; gynophore distinct; replum persistent, thickened, rounded, the two sides nearly connate, not expanded apically, without septal perforation; septum absent; style distinct, cylindric, thick, persistent, scabrous or glabrous; stigma capitate, 2-lobed, unappendaged. **Seeds** 1 per mericarp, wingless, ovate, plump; seed coat minutely reticulate, not mucilaginous when wetted; cotyledons incumbent.

Etymology. – *Aimara* is the indigenous ethnic group of people that inhabit the Andes and Altiplano regions of Western Bolivia, southern Peru and northern Chile for over 2000 years.

Distribution. – A monotypic genus of northwestern Chile (Fig. 7).

Aimara rollinsii (Al-Shehbaz & Martic.) Salariato & Al-Shehbaz, **comb. nov.** ≡ *Menonvillea rollinsii* Al-Shehbaz & Martic. in J. Arnold Arbor. 71: 135. 1990 – Holotype: CHILE. [Región de Antofagasta]. Camino de Chuquicamata a Conchi, 2700 m, 5 Apr. 1961, *M. Ricardi, C. Marticorena & O. Matthei 445* (CONC No. 93507!; isotype: GH! [fragm. ex CONC]).

Aimara rollinsii is illustrated in Figure 8.

Phenology. – It flowers between January and October.

Distribution and habitat. – *Aimara rollinsii* is endemic to regions of Antofagasta (II) and Atacama (III) of northwestern Chile and grows at 2500–3550 m in dry habitats with an annual rainfall of no more than 9 mm (Al-Shehbaz & Marticorena, 1990) (Fig. 7).

Observations. – *Aimara rollinsii* is the most distinctive species in tribe Cremolobeae by its shrubby habit, minute trichomes almost throughout, sessile and subulate-linear fleshy leaves, marginally crisped petals, and laterally compressed fruit valves triangular in cross section and with adaxial papillae. The species was previously known only from the type collection.

Additional specimens examined. – CHILE. II ANTOFAGASTA. EL LOA: Com. Calama, Cerro Camino de Conchi a Chuquicamata, Cerro Platero, *Arroyo & al. 97661* (CONC); Quebrada de Paqui, *Arroyo & al. 97665* (CONC); Cerro Platero, lado O de la Quebrada de Paqui, *Arroyo & al. 97670* (CONC); Lado O de Cerro Bayo, *Arroyo & al. 97673* (CONC); 23 km de Conchi, *Martin 221* (SI); Santa Barbara, Tapiche, *Martin 309* (SI); Quebrada camino a Conchi, desague de Cere, 30 km de Conchi, *Martin 493* (SI); Com. San Pedro de Atacama, Cordillera de Domeyko, Cerro Quimal, en lado O, *Baumann 148* (CONC). III ATACAMA. CHAÑARAL: Com. Diego de Almagro, Inca de oro, cerca de Diego de Almagro, *Garfia & Bustamante s.n.* (CONC 173204).

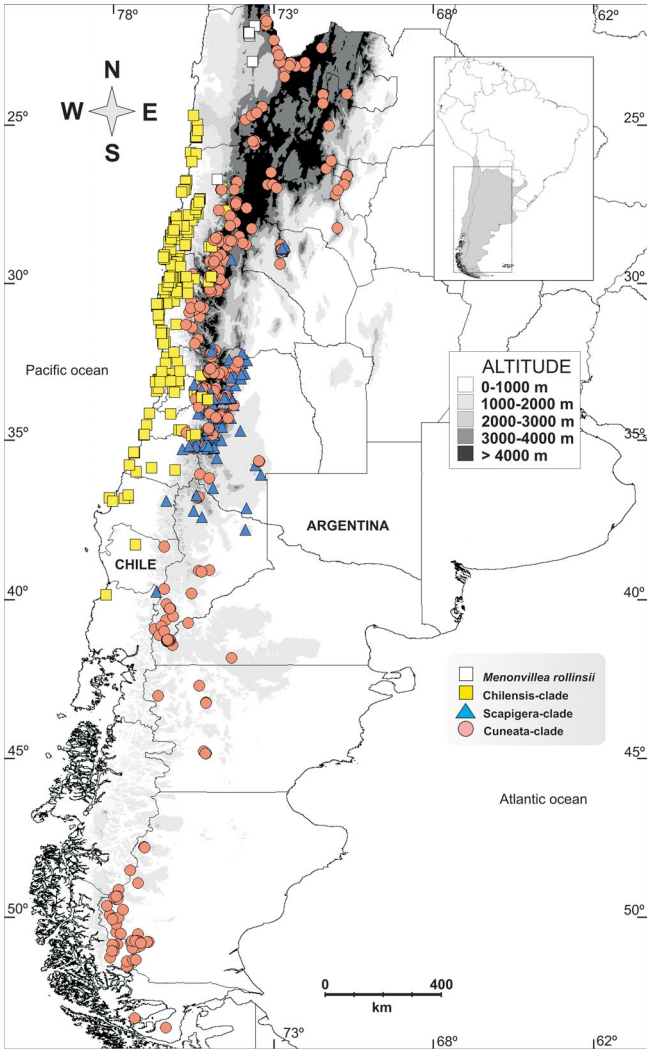


Fig. 7. Geographical distribution of the main clades of *Menonvillea*.

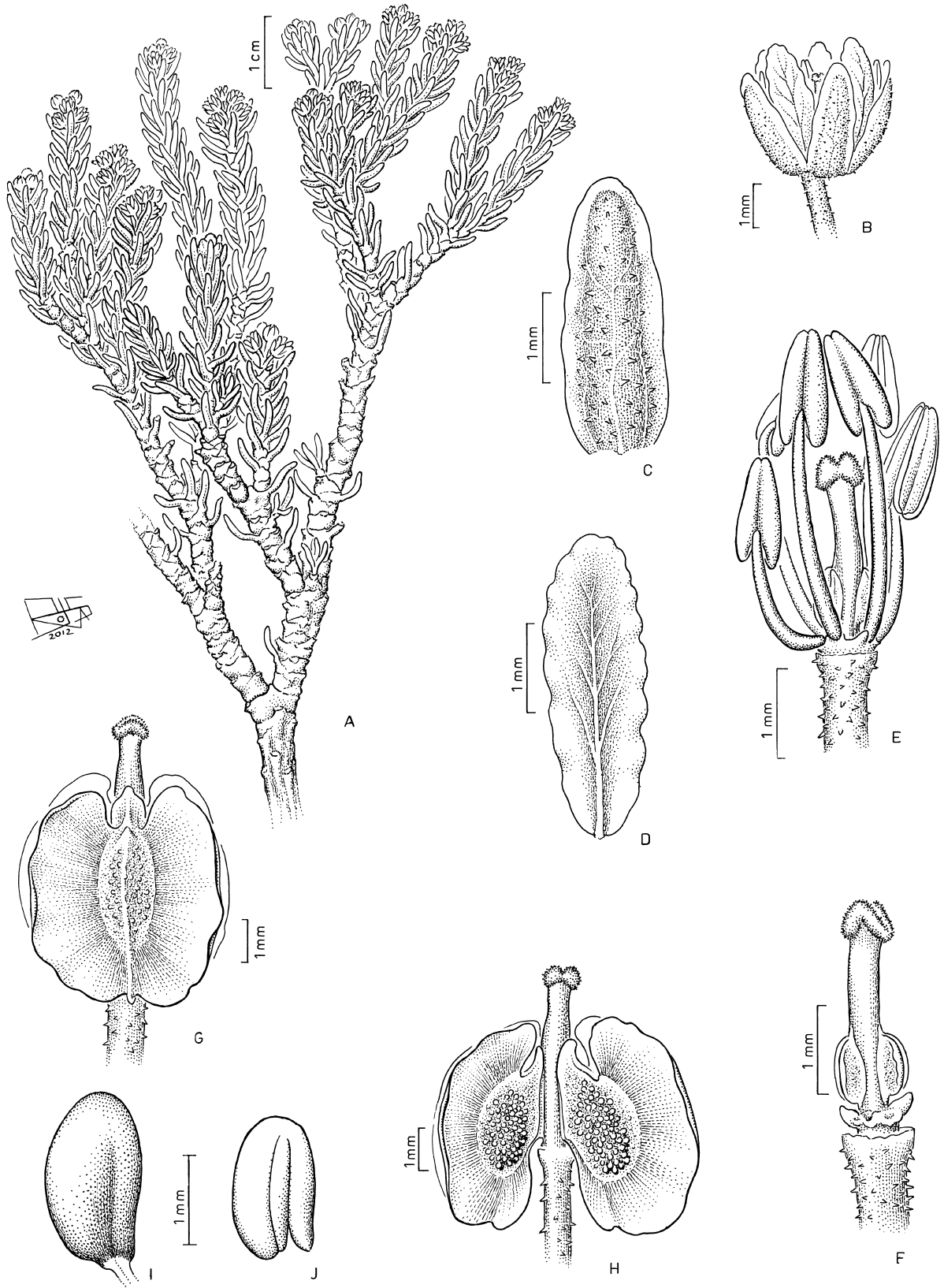


Fig. 8. *Aimara rollinsii* (Al-Shehbaz & Martic.) Salariato & Al-Shehbaz. **A,** plant; **B,** flower; **C,** sepal; **D,** petal; **E,** stamens and ovary; **F,** ovary and nectar glands at base; **G,** fruit, dorsal view; **H,** fruit, lateral view; **I,** seed; **J,** embryo. Drawn by Francisco Rojas from *Arroyo & al. 97665* (CONC).

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■ LITERATURE CITED

Alexander, P.J., Windham, M.D., Govindarajulu, R., Al-Shehbaz, I.A. & Bailey, C.D. 2010. Molecular phylogenetics and taxonomy of the genus *Thysanocarpus* (Brassicaceae). *Syst. Bot.* 35: 559–557. http://dx.doi.org/10.1600/036364410792495926

Al-Shehbaz, I.A. 2008. Brassicaceae. Pp. 1663–1709 in: Zuloaga, F.O., Morrone, O. & Belgrano, M.J. (eds.), *Catalogue of the vascular plants of the southern cone (Argentina, southern Brazil, Chile, Paraguay and Uruguay)*, vol. 2, *Dicotyledoneae: Acanthaceae–Fabaceae (Abarema–Schizolobium).* Monographs in Systematic Botany from the Missouri Botanical Garden 107. St. Louis: Missouri Botanical Garden Press.

Al-Shehbaz, I.A. 2010. *Menonvillea zuloagaensis* and *Mostacillastrum hunzikeri* (Brassicaceae), two new species from Argentina. *Darwiniana* 48: 59–63.

Al-Shehbaz, I.A. 2012. A generic and tribal synopsis of the Brassicaceae (Cruciferae). *Taxon* 61: 931–954.

Al-Shehbaz, I.A. & Marticorena, C. 1990. *Menonvillea rollinsii* (Brassicaceae), a new shrubby species from Chile. *J. Arnold Arbor.* 71: 135–138.

Appel, O. & Al-Shehbaz, I.A. 2003. Cruciferae. Pp. 75–174 in: Kubitzki, K. & Bayer, C. (eds.), *The families and genera of vascular plants*, vol. 5. Berlin: Springer.

Bailey, C.D., Koch, M.A., Mummenhoff, K., Mayer, M., O'Kane, S.L., Jr., Warwick, S.I., Windham, M.D. & Al-Shehbaz, I.A. 2006. Toward a global phylogeny of the Brassicaceae. *Molec. Biol. Evol.* 23: 2142–2160. http://dx.doi.org/10.1093/molbev/msl087

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molec. Phylogen. Evol.* 1: 3–16. http://dx.doi.org/10.1016/1055-7903(92)90030-K

Beilstein, M.A, Nagakingum, N.S., Clements, M.D., Manchester, S.R. & Mathews, S. 2010. Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 107: 18724–18728. http://dx.doi.org/10.1073/pnas.0909766107

Borchsenius, F. 2009. FastGap, version 1.2. Department of Biosciences, Aarhus University, Denmark. at http://www.aubot.dk/FastGap_home.htm

Cabrera, A.L. & Willink, A. 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., Franzke, A., Al-Shehbaz, I.A., Bakker, F.T., Koch, M.A. & Mummenhoff, K. 2010. Molecular phylogenetics, temporal diversification and principles of evolution in the mustard family (Brassicaceae). *Molec. Biol. Evol.* 27: 55–71. http://dx.doi.org/10.1093/molbev/msp202

Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull. Bot. Soc. Amer.* 19: 11–15.

Edgar, R.C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* 32: 1792–1797. http://dx.doi.org/10.1093/nar/gkh340

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. 1995. Testing significance of incongruence. *Cladistics* 10: 315–319, http://dx.doi.org/10.1111/j.1096-0031.1994.tb00181.x

Farris, J.S., Albert, V.A., Källersjö M., Lipscomb, D. & Kluge, A.G. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124. http://dx.doi.org/10.1111/j.1096-0031.1996.tb00196.x

German, D.A., Friesen, N., Neuffer, B., Al-Shehbaz, I.A. & Hurka, H. 2009. Contribution to ITS phylogeny of the Brassicaceae, with a special reference to some Asian taxa. *Pl. Syst. Evol.* 283: 33–56. http://dx.doi.org/10.1007/s00606-009-0213-5

German, D.A., Grant, J.R., Lysak, M.A & Al-Shehbaz, I.A. 2011. Molecular phylogeny and systematics of the tribe Chorisporeae (Brassicaceae). *Pl. Syst. Evol.* 294: 65–86. http://dx.doi.org/10.1007/s00606-011-0452-0

Goloboff, P.A., Farris, J.S. & Nixon, K. 2008. TNT, a free program for phylogenetics analysis. *Cladistics* 24: 774–786. http://dx.doi.org/10.1111/j.1096-0031.2008.00217.x

Grau, J. 1995. Aspectos geográficos de la flora de Chile. Pp. 63–83 in: Marticorena C. & Rodríguez R. (eds.), *Flora de Chile*, vol. 1. Concepción: Universidad de Concepción.

Hall, T.A. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95–98.

Hooker, W.J. 1830. On a new genus of plants of the Nat. Ord. Cruciferae from the Andes of Chili and Mendoza. *Bot. Misc.* 1: 349–352.

Khanna, K.R. & Rollins, R.C. 1965. A taxonomic revision of *Cremolobus* (Cruciferae). *Contr. Gray Herb.* 195: 135–157.

Koch, M. & Mummenhoff, K. 2001. *Thlaspi* s.str. (Brassicaceae) versus *Thlaspi* s.l.: Morphological and anatomical characters in the light of ITS nrDNA sequence data. *Pl. Syst. Evol.* 227: 209–225. http://dx.doi.org/10.1007/s006060170049

Moazzeni, H., Zarre, S., Al-Shehbaz, I.A. & Mummenhoff, K. 2010. Phylogeny of *Isatis* (Brassicaceae) and allied genera based on ITS sequences of nuclear ribosomal DNA and morphological characters. *Flora* 205: 337–343. http://dx.doi.org/10.1016/j.flora.2009.12.028

Morrone, J.J. 2001. *Biogeografía de América latina y el Caribe*. Zaragoza: M&T-Manuales & Tesis SEA.

O'Kane, S.L. & Al-Shehbaz, I.A. 2003. Phylogenetic position and generic limits of *Arabidopsis* (Brassicaceae) based on sequences of nuclear ribosomal DNA. *Ann. Missouri Bot. Gard.* 90: 603–612. http://dx.doi.org/10.2307/3298545

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molec. Biol. Evol.* 25: 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Prina, A.O. 2001. Nuevas combinaciones en *Menonvillea* (Brassicaceae). *Hickenia* 3: 93–94.

Rambaut, A. & Drummond, A.J. 2007. Tracer, version 1.4. http://beast.bio.ed.ac.uk/Tracer

Rollins, R.C. 1955. A revisionary study of the genus *Menonvillea* (Cruciferae). *Contr. Gray Herb.* 177: 3–57.

Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61: 539–542. http://dx.doi.org/10.1093/sysbio/sys029

Salariato, D.L., Zuloaga, F.O. & Al-Shehbaz, I.A. 2012. Morphometric studies and taxonomic delimitation in *Menonvillea scapigera* and related species (Cremolobeae: Brassicaceae). *Pl. Syst. Evol* 298: 1961–1976. http://dx.doi.org/10.1007/s00606-012-0694-5

Schulz, O.E. 1936. Cruciferae. Pp. 227–658 in: Engler, A. & Harms, H.

(eds.), *Die natürlichen Pflanzenfamilien*, 2nd ed, vol. 17B. Leipzig: Engelmann.

Shimodaira, H. & Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molec. Biol. Evol*: 16, 1114–1116. http://dx.doi.org/10.1093/oxfordjournals.molbev.a026201

Simmons, M.P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* 49: 369–381. http://dx.doi.org/10.1093/sysbio/49.2.369

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. http://dx.doi.org/10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Syst. Biol.* 57: 758–771. http://dx.doi.org/10.1080/10635150802429642

Swofford, D.L. 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sunderland, Massachusetts: Sinauer.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109. http://dx.doi.org/10.1007/BF00037152

Turczaninow, N.S. 1846. Decas secunda generum adhuc non descriptorum, adjectis descriptionibus nonnullarum specierum Byttneriaceum. *Bull. Soc. Imp. Naturalistes Moscou* 19(2): 497–510.

Warwick, S.I., Al-Shehbaz, I.A., Price, R.A. & Sauder, C.A. 2002. Phylogeny of *Sisymbrium* (Brassicaceae) based on ITS sequences of nuclear ribosomal DNA. *Canad. J. Bot.* 80: 1002–1017. http://dx.doi.org/10.1139/b02-089

Warwick, S.I., Al-Shehbaz, I.A. & Sauder, C.A. 2006. Phylogenetic position of *Arabis arenicola* and generic limits of *Eutrema* and *Aphragmus* (Brassicaceae) based on sequences of nuclear ribosomal DNA. *Canad. J. Bot.* 84: 269–281. http://dx.doi.org/10.1139/b05-161

Warwick, S.I., Sauder, C., Al-Shehbaz, I.A. & Jacquemoud, F. 2007. Phylogenetic relationships in the Brassicaceae tribes Anchonieae, Chorisporeae, Euclidieae, and Hesperideae based on nuclear ribosomal ITS DNA sequences. *Ann. Missouri Bot. Gard.* 94: 56–78. http://dx.doi.org/10.3417/0026-6493(2007)94[56:PRITTA]2.0.CO;2

Warwick, S.I., Sauder, C.A. & Al-Shehbaz, I.A. 2008. Phylogenetic relationships in the tribe Alysseae (Brassicaceae) based on nuclear ribosomal ITS DNA sequences. *Canad. J. Bot.* 86: 315–336. http://dx.doi.org/10.1139/B08-013

Warwick, S.I., Sauder, C.A., Mayer, M.S. & Al-Shehbaz, I.A. 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. http://dx.doi.org/10.1139/B09-051

Warwick, S.I., Mummenhoff, K., Sauder, C.A., Koch, M.A. & Al-Shehbaz, I.A. 2010. Closing the gaps: Phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS. *Pl. Syst. Evol.* 285: 209–232. http://dx.doi.org/10.1007/s00606-010-0271-8

Warwick, S.I., Sauder, C.A. & Al-Shehbaz, I.A. 2011. Systematic position of *Ivania*, *Scoliaxon*, and *Phravenia* (Brassicaceae). *Taxon* 60: 1156–1164.

Appendix 1. List of taxa and GenBank accession numbers for DNA sequences (ITS, *trnL-F*) used in this study. Voucher information are given for specimens sequenced in this study (marked with an asterisk) or in Salariato & al. (2012) (marked with [a]).

OUTGROUP. ***Aethionema grandiflorum*** Boiss. & Hohen., DQ249867, AP009367; ***Arabis alpina*** L., AF137559, AY034180; ***Boechera holboellii*** (Hornem.) Á. Löve & D. Löve, AY457932, DQ013055; ***Brayopsis colombiana*** Al-Shehbaz, EU620283, EU620339; ***Chorispora sabulosa*** Cambess., FN821598, FN677724; ***Clausia podlechii*** Dvořák, FN821595, FN677718; ***Cremolobus chilensis*** (Lag. ex DC.) DC. **(a)** (GQ424530, –), **(b)** PERU: Ancash: Corongo: Road from Huallanca to Yanac., *Weigend 5009* (MO), KF662764*, –; ***Cremolobus peruvianus*** (Lam.) DC., PERU: Pasco: Oxapampa: Distrito Oxapampa, Parque Nacional Yanachaga-Chemillén, *Monteagudo & al. 13630* (MO), KF662763*, KF662808*; ***Cremolobus rhomboideus*** Hook., PERU: La Libertad: Otuzco: Arriba de Piedra Gorda, ruta Salpo-Samne, *Leiva 1705* (MO), KF662762*, KF662807*; ***Eudema nubigenum*** Bonpl., EU620296, EU620352; ***Halimolobos elatus*** (Rollins) Al-Shehbaz & C.D. Bailey, DQ336388, DQ336387; ***Ladakiella klimesii*** (Al-Shehbaz) D.A. German & Al-Shehbaz, EF514609, FN677736; ***Mancoa bracteata*** (S. Watson) Rollins, AF307633, AF307556; ***Mathewsia foliosa*** Hook. & Arn., DQ357563, EU620360; ***Mathewsia peruviana*** O.E. Schulz, EU620303, EU620362; ***Polypsecadium magellanicum*** (Juss. ex Pers.), AF531589, AY958558; ***Schizopetalon biseriatum*** Phil., EU620313, EU620375; ***Schizopetalon rupestre*** (Barnéoud) Reiche, EU620314, EU620376; ***Sisymbrium altissimum*** L., HQ896628, JF298537. **INGROUP.** ***Menonvillea chilensis*** (Turcz.) B.D. Jacks., CHILE: **(a)** II Antofagasta: Taltal: Quebrada San Ramón, 5 km al norte de Taltal, *Hoffmann & Rodríguez 180* (CONC), KF662739*, KF662777*, **(b)** III Atacama: Caldera: Carretera Panamericana entre Caldera y Chañaral, km 18, *Ricardi & al. 1285* (CONC), KF662742*, KF662780*; ***Menonvillea cicatricosa*** (Phil.) Rollins, ARGENTINA: Mendoza: Malargüe: Mirador del Valle Hermoso, **(a)** *Álvarez 2* (SI), KF662747*, KF662786*; **(b)** *Zuloaga & al. 12365* (SI), KF662759*, KF662801*; ***Menonvillea comberi*** Sandwith, ARGENTINA: Río Negro: Bariloche: Parque Nac. Nahuel Huapi, **(a)** Cerro Estratos, ladera Este., *Ferreyra 409* (BCRU), KF662750*, KF662791*; **(b)** Cerros Carbón y Estratos., *Ferreyra 449* (BCRU), KF662751*, KF662793*; ***Menonvillea cuneata*** (Gillies & Hook.) Rollins, ARGENTINA: **(a)** Mendoza: Las Heras: Subida al Cristo Redentor. Manchón de vegetación al costado del camino, poco antes del monumento., *Álvarez 15* (SI), KF662761*, KF662803*, **(b)** San Juan: Iglesia: Ruta Nacional 150, Paso de Agua Negra, pasando Arrequintín hacia límite con Chile, Los Corrales, *Zuloaga & al. 12775* (SI), KF662746*, KF662784*; ***Menonvillea famatinensis*** (Boelcke) Rollins, ARGENTINA: La Rioja: Famatina: **(a)** Cueva de Perez, *Hunziker 1941* (SI) (JX470553[a], KF662806*), **(b)** Real Viejo, *Barboza & al. 3343* (CORD), KF662768*, KF662812*; ***Menonvillea filifolia*** subsp. ***filifolia*** Fisch. & C.A. Mey., CHILE: IV Coquimbo: Ovalle: Corral Quemado, *Jiles 3507* (CONC), KF662756*, KF662798*; ***Menonvillea filifolia*** subsp. ***marticorenae*** Al-Shehbaz, CHILE: IV Coquimbo: Illapel: Carretera Panamericana, 10 km N of Huentelauquén, *Marticorena & al. 1408* (CONC), KF662755*, KF662797*; ***Menonvillea flexuosa*** Phil., CHILE: **(a)** V Valparaíso: Los Andes: 3 km E del Río Blanco, en el lado opuesto Piscicultura, en pendientes paradas al lado Sur del Río Blanco, *Hutchinson 181* (CONC), KF662766*, KF662810*, **(b)** IV Coquimbo: Vicuña: Mil curvas bajando a Juntas, *Arancio 91855* (ULS), KF662771*, KF662815*; ***Menonvillea frigida*** (Phil.) Rollins, CHILE: II Antofagasta: Antofagasta: **(a)** Volcán Llullaillaco, *Arroyo & al. 94006* (CONC), KF662767*, KF662811*; **(b)** faldeos del Volcán Llullaillaco, camino mina Iris, *Muñoz 3798* (SGO), KF662770*, KF662814*; ***Menonvillea hirsuta*** Rollins, ARGENTINA: Río Negro: Bariloche: Parque Nac. Nahuel Huapi, Cerro Ventana, *Ferreyra 624* (BCRU), JX470552[a], KF662792*; ***Menonvillea linearis*** DC., CHILE: VIII Biobío: Ránquil: Ránquil, Fundo El Milagro, *Sparre 10059* (CONC), KF662741*, KF662779*; ***Menonvillea litoralis*** (Barnéoud) Rollins, CHILE: **(a)** III Atacama: Vallenar: Cuesta Pajonales, al N de La Serena, *Garaventa 8005* (CONC), KF662754*, KF662796*; **(b)** IV Coquimbo: Ovalle: Parque Nacional Bosque de Fray Jorge, desembocadura del Rio Limarí, *Zöllner 9930* (CONC), KF662753*, KF662795*; ***Menonvillea minima*** Rollins, CHILE: III Atacama: Freirina: Camino de los Choros a Carrizalillo, *Arancio 91439* (CONC), KF662735*, KF662773*; ***Menonvillea nordenskjoeldii*** (Dusén) Rollins, CHILE: XII Magallanes: Torres del Paine: Senos de Catherine, *Arroyo 92418* (CONC), KF662752*, KF662794*; ***Menonvillea orbiculata*** Phil., CHILE: III Atacama: Caldera: 5 km al N de Travesía, *Rodríguez 2687* (CONC), KF662740*, KF662778*; ***Menonvillea patagonica*** Speg., ARGENTINA: Chubut: Languiñeo: Ruta 12 entre Gualjaina y Paso del Sapo, pasando el río Gualjaina, *Biganzoli & Larsen* 1907 (SI), KF662745*, KF662783*, **(b)** Río Senguer: Ruta Nacional 40, entre Nueva Lubecka y Estancia Laurita, *Zuloaga 13932* (SI), KF662769*, KF662813*; ***Menonvillea pinnatifida*** Barnéoud, CHILE: IV Coquimbo: Río Hurtado: Séron, *Jiles 3300* (CONC), KF662738*, KF662776*; ***Menonvillea purpurea*** (Hastings) Rollins, CHILE: Region Metropolitana de Santiago: San José de Maipo: Cajón

Appendix 1. Continued.

de Morales, entre Baños Morales y las Panimávidas, *Teillier & Márquez 5301* (CONC), KF662734*, KF662772*; ***Menonvillea rigida*** Rollins, ARGENTINA: **(a)** Río Negro: Bariloche: Cerro Ñireco, ladera exposición N, *Cadillo & Posse s.n.* (BCRU), KF662757*, KF662799*, **(b)** Neuquén: Huiliches: Parque Nac. Lanín, ladera N, subiendo al refugio por sendero de mulas, pedrero, *Ezcurra & al. 2979* (BCRU), KF662758*, KF662800*; ***Menonvillea rollinsii*** Al-Shehbaz & Martic., CHILE: II Antofagasta: Calama: **(a)** Camino de Conchi a Chuquicamata, Cerro Platero, *Arroyo & al. 97661* (CONC), KF662736*, KF662774*, **(b)** Lado O de Cerro Bayo, *Arroyo & al. 97673* (CONC), KF662744*, KF662782*, **(c)** San Pedro de Atacama: El Loa, Cordillera de Domeyko, Cerro Quimal, en lado O., *Baumann 148* (CONC), KF662737*, KF662775*; ***Menonvillea scapigera*** subsp. ***longipes*** (Rollins) Prina, ARGENTINA: Mendoza: Malargüe: **(a)** Cerros del Portezuelo de Borbarán, 20 km NW de Agua Escondida, RP180, *Al-Shehbaz 824* (SI), JX470563[a], KF662789*, **(c)** Mirador de Valle Hermoso, *Zuloaga & al. 12364* (SI), JX470554[a], KF662788*, **(b)** CHILE: Región Metropolitana de Santiago: Lo Barnechea: Ruta G-21 entre Farellones y Valle Nevado, *Johnson 10-130* (SI), JX470555[a], KF662785*; ***Menonvillea scapigera*** subsp. ***scapigera*** (Phil.) Rollins, ARGENTINA: Mendoza: **(a)** Malargüe: Banquina rocosa camino de Las Leñas a Valle Hermoso, *Al-Shehbaz 814* (SI), JX470561[a], KF662805*, **(b)** San Rafael: Hotel Termas del Sosneado. Sobre ladera rocosa, *Zuloaga & al. 12380* (SI), JX470564[a], KF662804*; ***Menonvillea spathulata*** (Gillies & Hook.) Rollins, ARGENTINA: Mendoza: **(a)** San Carlos: En las nacientes del río Diamante, *Álvarez 10* (SI), KF662748*, KF662787*, **(b)** San Rafael: Subida desde Ruta Provincial 180 a Cerro El Nevado, *Zuloaga & al. 12258* (SI), KF662760*, KF662802*; ***Menonvillea virens*** (Phil.) Rollins, ARGENTINA: **(a)** La Rioja: Famatina: Cueva de Pérez, camino a la mina La Mejicana, *Donadío & al. 124* (SI), KF662749*, KF662790*, **(b)** Jujuy: Manuel Belgrano: De Refugio Militar al Chañi, *Zanotti & Suescún* 286 (SI), KF662765*, KF662809*, **(c)** CHILE: III Atacama: Diego de Almagro: Ladera Oeste Cerro Los Patitos, *Latorre & al. 204* (CONC), KF662743*, KF662781*.