Molecular Phylogeny of *Chuquiraga* (Asteraceae-Barnadesioideae): Infrageneric Classification and Generic Affinities

Ariana L. Padin,[1] Carolina I. Calviño,[1] and Cecilia Ezcurra[1,2]

[1]INIBIOMA, Conicet-Universidad Nacional del Comahue, Centro Regional Universitario Bariloche,
Quintral 1250, 8400 Bariloche, Argentina.
[2]Author for correspondence (ezcurrac@comahue-conicet.gob.ar)

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Abstract—*Chuquiraga* is a genus of evergreen shrubs endemic to the arid and semiarid regions of the Andes and southern South America. The genus has been classified into two sections based on its variation in leaf morphology: *Chuquiraga* and *Acanthophylla*. Within section *Chuquiraga,* two series, *Chuquiraga* and *Parviflorae,* have been recognized based on variation in size of flower heads. The objectives of this study were to test this classification and to assess the monophyly of *Chuquiraga* and its intergeneric relationships with the closely related genera *Doniophyton* and *Duseniella.* The phylogenetic relationships of 24 of the 27 species and/or subspecies of *Chuquiraga* (19 of its 22 recognized species), plus 14 species representing seven of the remaining eight genera of Barnadesioideae, and two species of non-barnadesioid Asteraceae were inferred using sequence data from the chloroplast DNA *psbA-trnH*, *rps16-trnK*, *trnL-rpl32,* and/or nuclear ribosomal DNA ITS regions. The plastid and nuclear data sets were analyzed individually and combined, using maximum parsimony, maximum likelihood, and Bayesian inference methods. The phylogenetic results show that *Chuquiraga, Duseniella,* and *Doniophyton* form a well-supported monophyletic group, but the relationships among these genera and the monophyly of *Chuquiraga* are still uncertain. The phylogenies obtained support the monophyly of the sections and reject the monophyly of the series. Section *Chuquiraga* is divided into two subclades: one includes all species of *Chuquiraga* series *Parviflorae*, plus *Chuquiraga calchaquina* and *C. longiflora* (of *Chuquiraga* series *Chuquiraga*), and the other subclade includes all remaining species of *Chuquiraga* series *Chuquiraga*.

Keywords—Chloroplast DNA, *Doniophyton, Duseniella,* nrDNA ITS, systematics.

The phylogeny and classification of the Asteraceae, the largest family of flowering plants, has been thoroughly studied in the last 20 yr (reviewed in Funk et al. 2009a). Based on molecular analyses, the Asteraceae are currently classified into 12 subfamilies (Panero and Funk 2008; Funk et al. 2009b). Among these, the subfamily Barnadesioideae K. Bremer & R. K. Jansen is a small group of Asteraceae endemic to South America that was traditionally placed as a subtribe within the tribe Mutisieae and characterized by several morphological characters (Cabrera 1977). Since 1987, it has attracted much scientific attention because it was discovered that the Barnadesioideae lack two DNA chloroplast inversions that are unique to the rest of the Asteraceae (Jansen and Palmer 1987; Bremer and Jansen 1992; Kim et al. 2005).

Barnadesioideae are therefore considered a subfamily that is the sister group to all other members of the family. The early-diverging Barnadesioideae have thereafter been regarded as a key group in the evolutionary reconstruction of Asteraceae origin and diversification, and because of this renewed interest, they have been subject to several studies (reviewed in Stuessy et al. 2009). The first phylogenies of the group were based on morphological data (Bremer 1994; Stuessy et al. 1996; Urtubey and Stuessy 2001), but later, molecular data were also included (Gustafsson et al. 2001; Gruenstaeudl et al. 2009). All these studies mostly aimed to clarify phylogenetic relationships among genera of the subfamily, and therefore did not include extensive species sampling within genera. As a result, phylogenies at the infrageneric level have not been treated in detail in Barnadesioideae yet.

Chuquiraga Juss. is the second largest genus of the subfamily Barnadesioideae, the most latitudinally extended, and the most diverse morphologically. The genus consists of 27 species and/or subspecies of spiny evergreen shrubs that grow in the Andes and Patagonia from more than 4,000 m in Colombia, to sea level in central Chile and Argentina (Ezcurra 1985; Harling 1991; Sagástegui Alva and Sánchez Vega 1991; Ferreyra 1995; Ezcurra 2002). Most of these species are found in temperate deserts and semideserts such as the Patagonian steppe, the Puna and the high Andes, or in warmer dry areas such as the Pacific desert, the Chilean matorral, the Prepuna, and the Monte (Ezcurra 1985). The species are conspicuous elements in the arid and semiarid environments they inhabit. The wide morphological diversity of their leaves (acicular, boat shaped, and flat) and the great diversity in size and color of their flower-heads (large and red to small and yellow) have attracted scientific attention (e.g. Böcher 1979; Ezcurra 1985; Ezcurra et al. 1997; Ezcurra 2002).

Traditionally, *Chuquiraga* species were classified into three to five groups or sections based on their variation in leaf morphology (Candolle 1838; Gaspar 1945). More recently, a new classification of all the species into two sections was proposed (Ezcurra 1985; Table 1). Section *Chuquiraga* comprised 13 species, and was characterized by the presence of axillary spines and flat leaves. Section *Acanthophylla* was composed of nine species, lacking axillary spines and presenting boat-shaped to acicular leaves (Ezcurra 1985; Ezcurra and Crisci 1987). Within section *Chuquiraga*, two series were recognized: *Chuquiraga* (eight species) and *Parviflorae* (five species), which differed in that series *Chuquiraga* presented larger flower heads than series *Parviflorae* (Ezcurra 1985; Fig. 1).

The validity of sections *Chuquiraga* and *Acanthophylla* was generally corroborated through phenetic (Ezcurra and Crisci 1987) and phylogenetic studies of the genus from morphological (Stuessy et al. 1996; Urtubey and Stuessy 2001; Ezcurra 2002) and molecular characters (Gustafsson et al. 2001). However, the most recent molecular phylogenetic study of subfamily Barnadesioideae (Gruenstaeudl et al. 2009) indicates that the sections and series of *Chuquiraga* (except *Chuquiraga* series *Chuquiraga*) are not monophyletic, casting doubts on the robustness of the current classification of the genus (Ezcurra 1985). Because these molecular studies (Gustafsson et al. 2001; Gruenstaeudl et al. 2009) were focused on intergeneric relationships of subfamily Barnadesioideae, they only sampled up to 11 of the 27 species and/or subspecies of *Chuquiraga*,

TABLE 1. Infrageneric classification of *Chuquiraga* sensu Ezcurra (1985).

Section *Acanthophylla* C.Ezcurra
    Chuquiraga acanthophylla Wedd.
    Chuquiraga atacamensis Kuntze
    Chuquiraga aurea Skottsb.
    Chuquiraga echegarayi Hieron.
    Chuquiraga erinacea subsp. *erinacea* D. Don
    Chuquiraga erinacea subsp. *hystrix* (D.Don) C.Ezcurra
    Chuquiraga kuschelii Acevedo
    Chuquiraga rosulata Gaspar
    Chuquiraga ruscifolia D.Don
    Chuquiraga ulicina subsp. *ulicina* Hook.
    Chuquiraga ulicina subsp. *acicularis* (D.Don) C.Ezcurra
Section *Chuquiraga*
  Series *Chuquiraga*
    Chuquiraga arcuata Harling
    Chuquiraga calchaquina Cabrera
    Chuquiraga jussieui J.F.Gmel.
    Chuquiraga longiflora (Griseb.) Hieron
    Chuquiraga oblongifolia Sagást. & Sánchez Vega
    Chuquiraga raimondiana A.Granda
    Chuquiraga spinosa subsp. *spinosa* D.Don
    Chuquiraga spinosa subsp. *australis* C.Ezcurra
    Chuquiraga spinosa subsp. *huamanpinta* C.Ezcurra
    Chuquiraga spinosa subsp. *rotundifolia* (Wedd.) C.Ezcurra
    Chuquiraga weberbaueri Tovar
  Series *Parviflorae* C.Ezcurra
    Chuquiraga avellanedae Lorentz
    Chuquiraga morenonis (Kuntze) C.Ezcurra
    Chuquiraga oppositifolia D.Don
    Chuquiraga parviflora (Griseb.) Hieron
    Chuquiraga straminea Sandwith

and only some of the molecular markers they used have adequate variability to resolve interspecific relationships in this group. Therefore, phylogenetic hypotheses obtained to date for *Chuquiraga* are from morphological characters (Stuessy et al. 1996; Urtubey and Stuessy 2001; Ezcurra 2002), or from molecular characters but considering only a few species (Gustafsson et al. 2001; Gruenstaeudl et al. 2009). Thus it is desirable at this point to test previous conflicting hypotheses from morphological and molecular phylogenetic analyses with an exhaustive taxon sampling, using adequate molecular markers.

Another important question to address is the monophyly of the genus *Chuquiraga*. Two small genera from southern South America have traditionally been considered closely related to *Chuquiraga*: *Doniophyton* (e.g. Candolle 1838; Cabrera 1977) and *Duseniella* (e.g. Bremer 1994). The three genera form a well-supported evolutionary lineage from morphological and molecular evidence (e.g. Stuessy et al. 1996; Gustafsson et al. 2001; Ezcurra 2002; Gruenstaeudl et al. 2009), but their phylogenetic position in some analyses renders *Chuquiraga* paraphyletic (e.g. Ezcurra 2002; Gruenstaeudl et al. 2009). To reconstruct the evolution of *Chuquiraga*, it is very important to assess the phylogenetic relationships among these three genera of Andean-Patagonian Barnadesioideae and to include all the species of this large and morphologically diverse genus.

The main objectives of this study are, therefore, to: (1) estimate phylogenetic relationships of all species and subspecies of *Chuquiraga* using sequence data from the chloroplast DNA (cpDNA) markers *psbA-trnH*, *rps16-trnK*, *trnL-rpl32,* and nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS); (2) test the currently accepted classification of the genus with this phylogeny; and (3) assess the monophyly of

Chuquiraga and its intergeneric relationships regarding the closely related *Doniophyton* and *Duseniella*.

MATERIALS AND METHODS

Taxa and Outgroup Selection—In total, 46 accessions of Barnadesioideae were examined for cpDNA markers *psbA(GUG)-trnH*, *rps16-trnK(UUU)*, *trnL(UAG)-rpl32,* and/or nrDNA ITS sequence variation. These accessions included 24 of the 27 species and/or subspecies of *Chuquiraga* (i.e. of 19 of its 22 recognized species), plus 14 species representing seven of the remaining eight genera of Barnadesioideae, and two species of non-barnadesioid Asteraceae. DNA sequences for 34 of these accessions were specifically obtained for this study (Appendix 1); data for the remaining 12 accessions were obtained from Gruenstaeudl et al. (2009; Appendix 2). The only species of *Chuquiraga* not included in this study are *C. arcuata, C. oblongifolia,* and *C. raimondiana,* but they are only known from their type collections which could not be obtained on loan for DNA extraction. These species are sympatric and morphologically closely related to *C. jussieui* and *C. weberbaueri* (Harling 1991; Sagástegui Alva and Sánchez Vega 1991; Ferreyra 1995), which have been included in this study.

All phylogenetic trees were rooted with *Perezia carthamoides* and *Mutisia decurrens*, both members of subfamily Mutisioideae, which is one of the first diverging lineages of the sister clade of subfamily Barnadesioideae (Funk et al. 2009b).

DNA Extraction, Amplification and Sequencing—Leaf material for DNA extraction was obtained from herbarium specimens or from new collections in the field (Appendix 1). Total genomic DNA was obtained from about 20 mg of dried leaf tissue using a Wizard SV Genomic DNA Kit (Promega, Madison, United States). For some accessions, extraction with this kit was not efficient, so we used instead a Purelink Plant Total DNA Purification Kit (Invitrogen, California, United States).

The chloroplast intergenic spacers *psbA-trnH*, *rps16-trnK*, and *trnL-rpl32* were PCR-amplified using the primer pairs presented elsewhere (Shaw et al. 2005, 2007; Calviño and Downie 2007). These regions presented the highest numbers of parsimony-informative characters among the seven or 34 regions of cpDNA evaluated for Barnadesioideae or angiosperms by Gruenstaeudl et al. (2009) and/or Shaw et al. (2005, 2007), respectively. Each PCR reaction was performed in a total volume of 25 μL using the following reaction components: 2.5 μL of 10 X Taq polymerase reaction buffer; 200 μM of each dNTP; 2.8 mM of MgCl₂; 1.25 units of Taq polymerase (Invitrogen, California, United States); 0.4 μM of each primer; and a 1 μL aliquot of genomic DNA. The PCR reaction cycles are according to Downie and Katz-Downie (1996), but the annealing temperature was set to 55°C for the *psbA-trnH* and *trnL-rpl32* regions. The strategies used to obtain the ITS sequence data are presented elsewhere (Calviño et al. 2008). All sequencing was done using an ABI (Applied Biosystems, Foster City, California, United States) 23 3730XL high-throughput DNA capillary sequencer at Macrogen Inc. (Seoul, Korea). Simultaneous consideration of both DNA strands across the entire cpDNA regions for most taxa allowed unambiguous base determination. All newly obtained cpDNA and ITS sequences have been submitted to GenBank (Appendix 1).

Sequence Comparisons and Phylogenetic Analyses—DNA sequences were edited, assembled, and aligned manually using BioEdit version 6.0.7 (Hall 1999). Gaps were positioned to minimize nucleotide mismatches. A matrix of binary-coded indels was constructed for each locus to incorporate length-mutational information into the phylogenetic analysis. Gap coding was according to Calviño and Downie (2007); for several regions, gap coding was problematic because of homopolymers or indirect duplications of adjacent elements in two or more taxa. These gaps were not scored and these ambiguous regions were excluded from subsequent analysis.

Some regions of the alignments were scored as missing. Data for portions of the ITS or the *rps16-trnK* regions could not be obtained for *Chuquiraga acanthophylla*, *C. calchaquina*, *C. jussieui*, *C. longiflora*, *Doniophyton weddellii,* and *Duseniella patagonica*. The *trnL-rpl32* intergenic spacer in *C. calchaquina*, *C. spinosa* subsp. *spinosa*, and *C. weberbaueri* could not be PCR-amplified (Appendix 1). Similarly, for some of the species whose sequences were obtained from GenBank, *trnL-rpl32* sequences were not available (Appendix 2). Overall, missing data represent 0.6% of the ITS and 12% of the cpDNA matrices. The aligned combined DNA matrix was submitted to TreeBASE (study number 15813).

Sequence boundaries of the *psbA-trnH*, *rps16-trnK,* and *trnL-rpl32* intergenic spacer were determined by comparison of these DNA sequences to the chloroplast genome of *Lactuca sativa* (GenBank DQ383816). Boundaries of nrDNA genes 18S, 5.8S, and 26S were determined by comparison of these DNA sequences to corresponding boundaries in *Daucus carota*
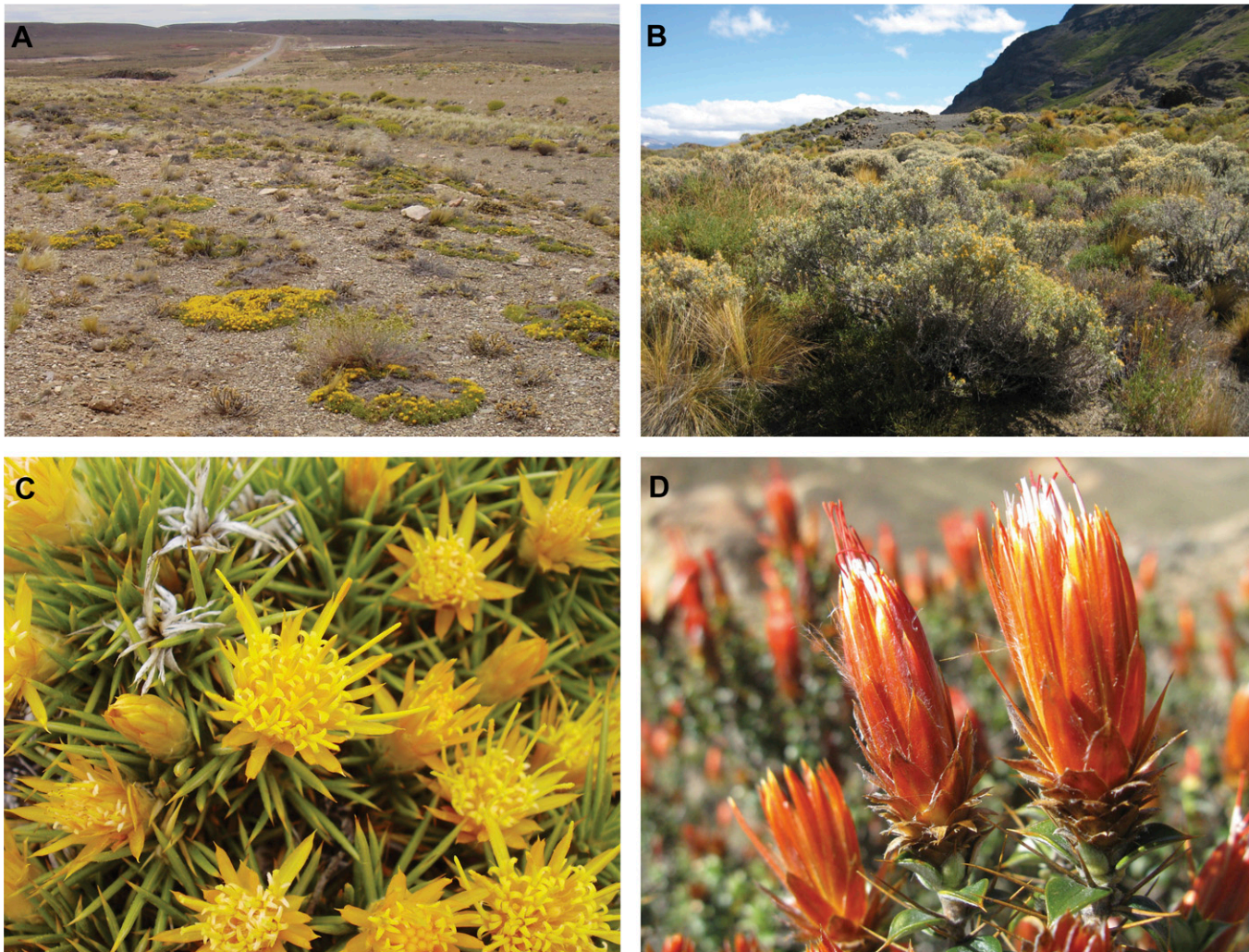
FIG. 1. Morphological diversity of *Chuquiraga*. A. Cushions of *C. aurea* in Patagonia. B. Shrubs of *C. oppositifolia* in the southern Andes. C. Flower heads and leaves of *C. aurea*. D. Flower heads and leaves of *C. spinosa* subsp. *rotundifolia*. Photographs by: Ariana L. Padin (A, C), Carolina I. Calviño (B), Andrés Moreira-Muñoz (D).

(Yokota et al. 1989). Characterization of the regions studied was facilitated using BioEdit version 6.0.7 (Hall 1999) and PAUP version 4.0b10 (Swofford 2002). Uncorrected pairwise nucleotide distances of unambiguously aligned positions were determined using the distance matrix option of PAUP*.

The cpDNA (i.e. the combined *psbA-trnH, rps16-trnK, trnL-rpl32* regions) and ITS data matrices (with and without their corresponding scored indels) were analyzed, separately and combined, using maximum parsimony (MP) as implemented by PAUP*. The heuristic search strategies employed by Calviño et al. (2006) were followed. Bootstrap values were calculated from 100,000 replicate analyses using "fast" stepwise-addition of taxa and only those values compatible with the majority-rule consensus tree were recorded. To examine the extent of conflict between the cpDNA and ITS data sets, we assessed whether there was well-supported (e.g., >78% BS and >98% PP) conflict across topologies, an approach described by Mason-Gamer and Kellogg (1996) and Seelanan et al. (1997).

The cpDNA and ITS matrices were also analyzed separately and combined (excluding indels) using the methods of Bayesian inference (BI) and maximum likelihood (ML), as implemented by MrBayes version 3.2.0 (Huelsenbeck and Ronquist 2001) and RAxML version 7.7.6 (Stamatakis 2006), respectively. Prior to analysis, MrModeltest version 2.3 (Posada and Crandall 1998) was used to select an evolutionary model of nucleotide substitution that best fits each of the four non-coding cpDNA data partitions, as selected by the Akaike information criterion estimator (Posada and Buckley 2004). The best-fit models selected were GTR+I+G for the *trnL-rpl32* region, and GTR+G for the ITS, *psbA-trnH,* and *rps16-trnK* regions. In the Bayesian analysis for each data matrix, two independent analyses were run from different random starting trees, for five

million generations; in some instances the analyses were stopped earlier when the average standard deviation of the split frequencies between the runs dropped to less than 0.01 using a relative burn-in of 25% (indicating convergence in topology between the runs). Trees were saved every 100 generations. For the cpDNA and combined cpDNA and ITS matrices the overall mutation rate was allowed to vary among partitions. Variation in likelihood scores to determine stationarity was examined graphically for each independent run using the program Tracer version v1.5 (A. Rambaut and A. Drummond, University of Oxford, unpublished data).The states of the chain that were sampled before stationarity were discarded, and the posterior probability values (expressed as percentages) for each bipartition of the phylogeny were determined from the remaining trees. To summarize and compare the samples from each analysis, the sump and sumt commands of MrBayes were used. MCMC convergence was also explored by examining the potential scale reduction factor (PSRF) convergence diagnostics for all parameters in the model (provided by the sump and sumt commands). For the ML analyses, tree searches were performed under the GTR+G model, and the bootstrap values were calculated from 1,000 replicate analyses using the fast option search of the program RAxML version 7.7.6 (Stamatakis 2006). Only those values compatible with the majority-rule consensus tree were recorded.

Evaluating Competing Hypotheses—The significance of alternative phylogenetic relationships between *Doniophyton*, *Duseniella,* and *Chuquiraga*, or the monophyly of the latter, were evaluated using the Approximately Unbiased (AU; Shimodaira 2002), the Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa 1999), and the weighted Shimodaira-Hasegawa tests (WSH; Shimodaira and Hasegawa 1999), as implemented in CONSEL version 0.1i (Shimodaira and Hasegawa 2001). Four independent ML

analyses with the following constraints were run based on the combined cpDNA and ITS matrix: 1- ((*Chuquiraga*, *Duseniella*), *Doniophyton*); 2- ((*Chuquiraga*, *Doniophyton*), *Duseniella*); 3- (((Sect. *Acanthophylla*, *Doniophyton*), Sect. *Chuquiraga*), *Duseniella*); 4- (((Sect. *Chuquiraga*, *Duseniella*), Sect. *Acanthophylla*), *Doniophyton*). The resulting ML best trees were used to calculate a matrix of log-likelihoods per site, using the program RAxML version 7.7.6 (Stamatakis 2006).

Results

Chloroplast DNA Sequence Comparisons and Phylogenetic Analyses—Sequence characteristics of the cpDNA *psbA-trnH*, *rps16-trnK,* and *trnL-rpl32* regions, separated and combined, are presented in Table 2. Among the three regions compared, the *psbA-trnH* intergenic spacer is the shortest, whereas the *rps16-trnK* and *trnL-rpl32* intergenic spacers are similar in length (but see caption notes in Table 2). Alignment of these sequences resulted in a matrix of 2,168 positions. Of these, 365 were excluded from subsequent analysis because of alignment ambiguities. The remaining 1,803 aligned positions yielded 167 parsimony-informative characters. In addition, 89 unambiguous alignment gaps were inferred, of which 26 were parsimony-informative. Of the latter, eleven, ten, and five occurred within the *psbA-trnH*, *rps16-trnK*, and *trnL-rpl32* intergenic spacers, respectively (Table 2). Informative indels ranged in size from one to 34 bp, being mostly 10 bp in length or shorter. The largest indel was a deletion of 34 bp that occurred in the *rps16-trnK* region in *Chuquiraga calchaquina*, *C. longiflora*, and all the species belonging to *Chuquiraga* section *Chuquiraga* series *Parviflorae*. The region with the highest number of parsimony-informative characters (substitutions plus indels) is the *rps16-trnK* intergenic spacer (Table 2). The three intergenic spacers had high levels of pairwise sequence divergence estimates among all taxonomic levels considered, with maximum divergence values of 11.4% among all taxa (*trnL-rpl32*), and 4.3% among *Chuquiraga* species (*psbA-trnH* and *rps16-trnK*; Table 2).

MP analysis of the cpDNA plus indels data matrix resulted in the preset maximum tree limit of 20,000 trees, each of 559 steps (consistency indices, CIs = 0.8408 and 0.7101, with and without uninformative characters, respectively; retention index, RI = 0.8934). Repeating the MP analysis without the scored gaps also resulted in the preset limit of 20,000 trees, each of 523 steps (CIs = 0.8489 and 0.7085, with and without uninformative characters, respectively; RI = 0.8907). The topology of this strict consensus tree (not shown) was almost identical to that when gaps were included, but the support

values of some clades were lower, with the only topological difference being a trichotomy that included *Schlechtendalia luzulifolia*, *Chuquiraga* plus *Doniophyton* plus *Duseniella*, and the rest of the Barnadesoideae when gaps were excluded, whereas *Schlechtendalia luzulifolia* was sister to *Chuquiraga* plus *Doniophyton* plus *Duseniella* when gaps were included (trees not shown). The two independent Bayesian analyses were stopped after 880,000 generations and, after discarding the burn-in, a majority-rule consensus tree that summarizes topology and branch length information was calculated based upon the remaining 13,200 trees (not shown).

The phylogenies estimated using MP, BI, and ML analyses of cpDNA data were largely identical with one another. The ML tree, with MP and ML bootstrap support values (MPB and MLB, respectively) and the posterior probability values (PP) of the clades, is presented in Fig. 2A, with topological differences between the analyses denoted by dotted lines. The MP strict consensus tree is slightly less resolved at the tips than the BI and ML trees. *Schlechtendalia luzulifolia* is placed as sister to all other Barnadesioideae in the BI and ML trees (62% MLB and 57% PP), whereas in the MP strict consensus tree it is placed as sister to *Chuquiraga*, *Doniophyton*, and *Duseniella* (<50% MPB). In all cpDNA derived trees, *Chuquiraga*, *Doniophyton,* and *Duseniella* form a well-supported monophyletic group (100% MPB, MLB, PP) with *Duseniella patagonica* sister to *Doniophyton* plus *Chuquiraga* (<50% MPB and MLB, 83% PP). Within this latter clade of *Doniophyton* plus *Chuquiraga,* two major lineages are recognized: one composed of all species traditionally grouped into *Chuquiraga* section *Acanthophylla* plus *Doniophyton*, and the other one comprising all species of *Chuquiraga* section *Chuquiraga* (Fig. 2A). Section *Acanthophylla* is highly supported in all analyses (91% MPB, 95% MLB, 100% PP), whereas section *Chuquiraga* is only highly supported in the BI analysis (<50% MPB, 61% MLB, 99% PP). *Doniophyton* species form a clade (100% MPB, MLB, PP) that is sister to section *Acanthophylla* (<50% MPB, 63% MLB, 98% PP). Section *Chuquiraga* is divided into two subclades: one includes all species of *Chuquiraga* series *Parviflorae* (white squares in Fig. 2A), plus *Chuquiraga calchaquina* and *C. longiflora* of *Chuquiraga* series *Chuquiraga* (94% MPB, 93% MLB 100% PP); the other subclade includes all remaining species of *Chuquiraga* series *Chuquiraga* (black squares in Fig. 2A; 70% MPB, 88% MLB, 89% PP). Of the species with subspecies, *C. ulicina* is monophyletic (96% MPB, 95% MLB, 100% PP), but the monophyly of *C. erinacea* and *C. spinosa* is unresolved. The two accessions of *C. aurea*

TABLE 2.   Sequence characteristics of the cpDNA *psbA-trnH*, *rps16-trnK,* and *trnL-rpl32* and nrDNA ITS regions, separated and combined, for 46 accessions of Asteraceae subfamily Barnadesioideae. [a] Number of parsimony-informative nucleotide substitutions plus number of parsimony-informative gaps. [b] Partial sequences, missing 68 bp of the 3′ end. [c] Partial sequences, missing 161 bp of the 3′ end.

| Sequence characteristic	*psbA-trnH*	*rps16-trnK* [b]	*trnL-rpL32* [c]	cpDNA	ITS	Total evidence
Length variation (range in bp)	295–407	668–781	702–758	1731–1864	628–645	2360–2540
No. aligned positions	490	844	834	2168	672	2840
No. positions eliminated	121	121	123	365	42	407
No. positions not variable	279	531	593	1403	292	1695
No. positions autapomorphic	48	112	73	233	97	330
No. positions parsimony-informative	42	80	45	167	241	408
No. unambiguous alignment gaps	30	38	21	89	33	122
No. unambiguous alignment gaps parsimony-Informative	11	10	5	26	13	39
Sequence divergence (range in %)						
All taxa included	0–10.2	0–10.9	0–11.4	0–10.7	0–27.1	0.1–16.8
Within *Chuquiraga*	0–4.3	0–4.3	0–3.5	0–3.5	0–7.0	0.1–5.9
Total no. parsimony-informative characters [a]	53	90	50	193	254	447
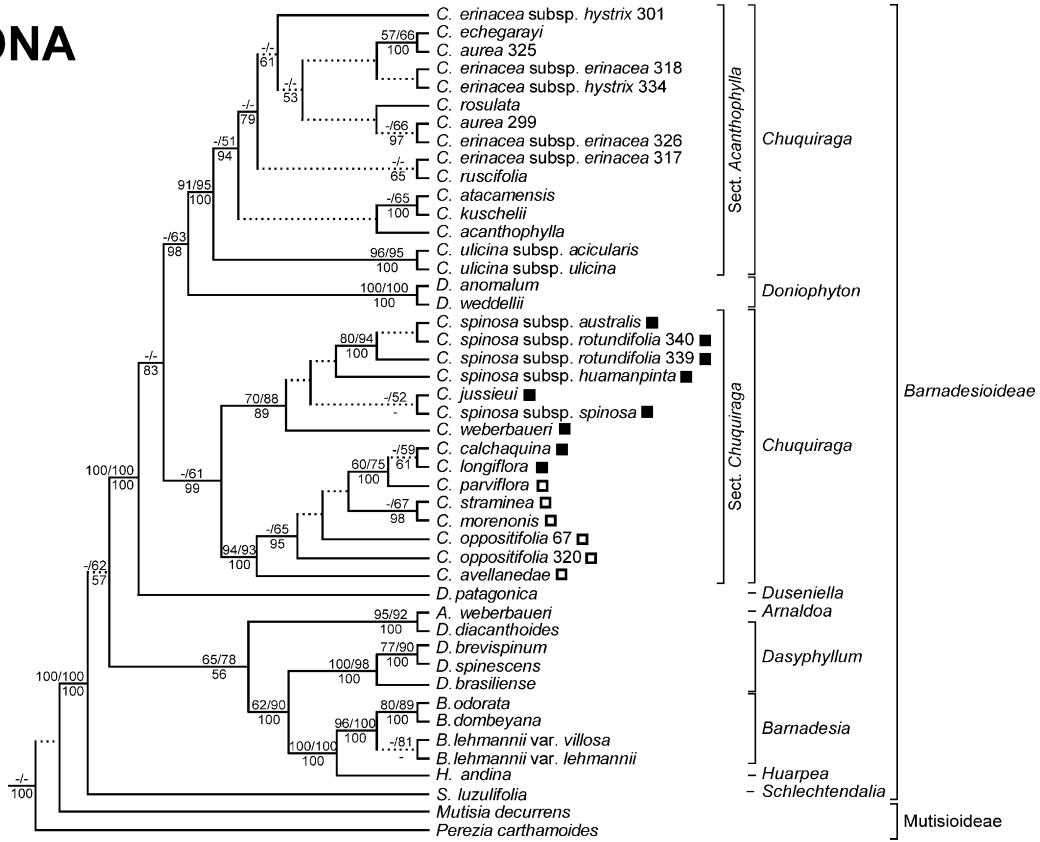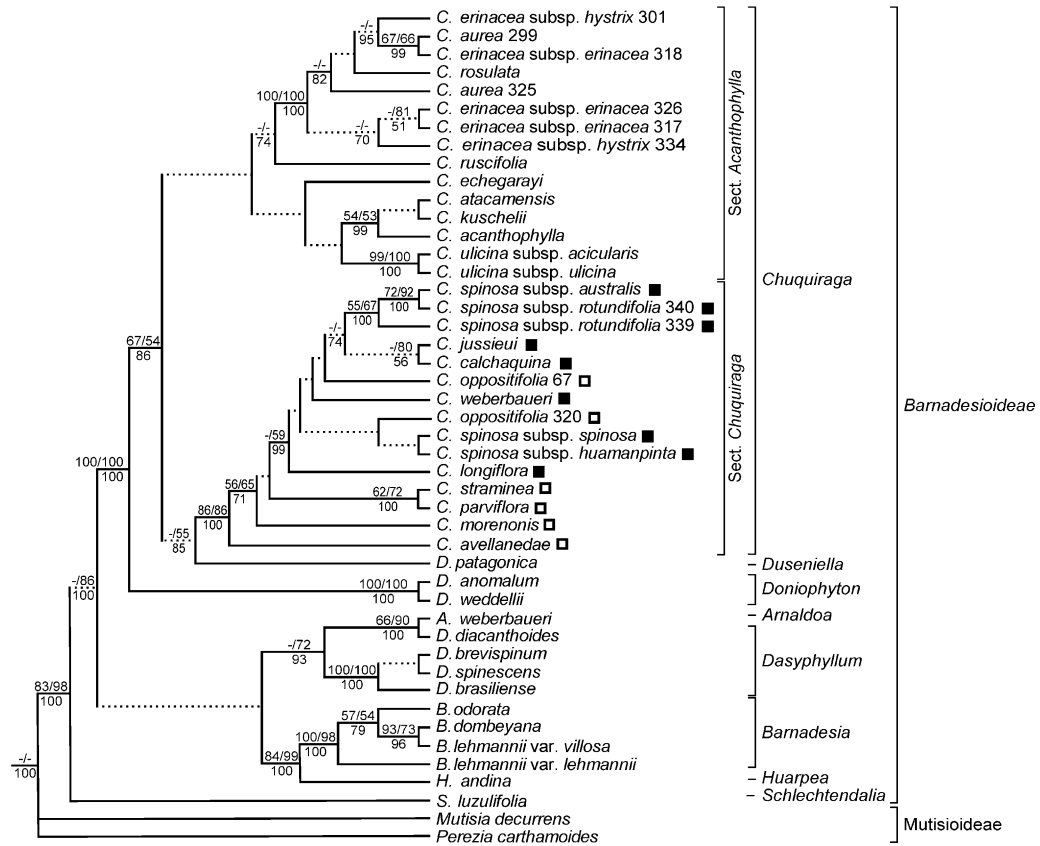
FIG. 2. A. Tree derived from maximum likelihood analysis of cpDNA *psbA-trnH*, *rps16-trnK*, and *trnL-rpl32* sequences. B. Tree derived from maximum likelihood analysis of nrDNA ITS sequences. Numbers above branches correspond to maximum parsimony and maximum likelihood bootstrap values (left and right, respectively); numbers below branches represent posterior probability values (in percentage). Dotted lines represent branches that are absent in the maximum parsimony strict consensus tree and/or in the Bayesian inference majority-rule consensus tree. Species of *Chuquiraga* section *Chuquiraga* series *Chuquiraga* are indicated with black squares and those of series *Parviflorae*, with white squares.

do not form a monophyletic group, and the accession *C. aurea* 325 is sister to *C. echegarayi* (57% MPB, 66% MLB, 100% PP).

Nuclear rDNA ITS Sequence Comparisons and Phylogenetic Analyses—Sequence characteristics of the nrDNA ITS region are presented in Table 2. Among the 46 sequences compared, the ITS region varied in size from 628 (*Chuquiraga spinosa* subsp. *huamanpinta*) to 645 bp (*Mutisia decurrens*). Alignment of these sequences resulted in a matrix of 672 positions. Of these, 42 were excluded from subsequent analyses because of alignment ambiguities. The remaining 630 aligned positions yielded 241 parsimony-informative characters. In addition, 33 unambiguous alignment gaps were inferred, of which 13 were parsimony-informative. Informative indels ranged in size from one to three bp. Pairwise sequence divergence estimates ranged from 0–7.0% of nucleotides within *Chuquiraga*, while across all taxa maximum sequence divergence was 27.1% (Table 2).

MP analysis of the ITS plus indels data matrix resulted in 1,323 trees, each of 884 steps (consistency indices, CIs = 0.6290 and 0.5707, with and without uninformative characters, respectively; retention index, RI = 0.7818). Repeating the MP analysis without the scored gaps also resulted in 1,323 trees, each of 871 steps (CIs = 0.6234 and 0.5632, with and without uninformative characters, respectively; RI = 0.7727). The topology of this strict consensus tree (not shown) was identical to that obtained when gaps were included. The two independent Bayesian analyses were stopped after 430,000 generations and after discarding the burn-in, a majority-rule consensus tree that summarizes topology and branch length information was calculated based upon the remaining 6,450 trees (not shown).

The phylogenies estimated using MP, BI, and ML analyses are congruent with one another. The ML tree with MPB, MLB, and PP values of the clades is presented in Fig. 2B, with topological differences between the analyses denoted by dotted lines. The ML tree is completely dichotomized whereas the MP and BI trees show several polytomies. In all ITS derived trees, *Chuquiraga*, *Doniophyton*, and *Duseniella* form a well-supported monophyletic group (100% MPB, MLB, PP). *Doniophyton* is monophyletic (100% MPB, MLB, PP), and sister to *Chuquiraga* plus *Duseniella* (67% MPB, 54% MLB, 86% PP). The monophyly of section *Acanthophylla* is not resolved in the BI trees and MP trees, whereas in the ML tree the section is resolved as monophyletic, albeit with <50% MLB support. The species that traditionally comprise section *Chuquiraga* form a monophyletic group with moderate to high support values (86% MPB and MLB, 100% PP). *Duseniella* is sister to section *Chuquiraga* (<50% MPB, 55% MLB, 85% PP) in the ML and BI trees, but this relationship is ambiguous in the MP trees (81.5% of the MP trees show *Duseniella* sister to a monophyletic *Chuquiraga*, and the remaining 18.5% of the trees show the same relationship as the ML and BI trees). *Chuquiraga* series *Parviflorae* and series *Chuquiraga* are not resolved as monophyletic in any of the analyses, but resolution within the section is poor. Of the species with subspecies, *C. ulicina* is monophyletic (99% MPB, 100% MLB and PP), but *C. spinosa* and *C. erinacea* are not resolved as monophyletic in any of the analyses. The two accessions of *C. aurea* do not form a monophyletic group, and the accession *C. aurea* 299 is sister to *C. erinacea* subsp. *erinacea* 318 (67% MPB, 66% MLB, 99% PP).

Comparison of cpDNA and nrDNA Phylogenies and Total Evidence Analysis—A visual comparison of plastid- and nuclear-derived trees indicates that the phylogenies do not strongly contradict one another. However, there are clades that in the cpDNA phylogenies received lower support values than in the ITS phylogenies and vice versa. For example, the clade that includes all species of *Chuquiraga* section *Chuquiraga* is poorly supported in the cpDNA trees (<50–61% bootstrap), whereas in the ITS trees it is strongly supported (86% bootstrap). In contrast, the clade of *Chuquiraga* section *Acanthophylla* is highly supported in the cpDNA trees (91–95% bootstrap), whereas in the ITS trees, it has no support (ML analysis) or it is not resolved (MP and BI analyses). Given the strengths and weaknesses of each data set, it was desirable to combine chloroplast and nuclear data for a "total evidence" analysis.

Alignment of the cpDNA *psbA-trnH*, *rps16-trnK*, and *trnL-rpl32* and nrDNA ITS sequences resulted in a matrix of 2,840 positions. Of these, 407 were excluded from subsequent analysis because of alignment ambiguities. The remaining 2,433 aligned positions yielded 408 parsimony-informative characters. In addition, 39 parsimony-informative indels were inferred (Table 2).

MP analysis of the cpDNA and ITS plus indels data matrix resulted in 554 trees, each of 1,466 steps (consistency indices, CIs = 0.6999 and 0.5978, with and without uninformative characters, respectively; retention index, RI = 0.8118). Repeating the MP analysis without the scored gaps resulted in 4,635 trees, each of 1,417 steps (CIs = 0.6965 and 0.5885, with and without uninformative characters, respectively; RI = 0.8015). The topology of this strict consensus tree (not shown) was similar to that when gaps were included, but the support values of many clades were weaker, and topologically it was slightly less resolved. The two independent Bayesian analyses were stopped after 650,000 generations and after discarding the burn-in, a majority-rule consensus tree that summarizes topology and branch length information was calculated based upon the remaining 9,750 trees (not shown).

The phylogenies estimated using MP, BI, and ML analyses are consistent with each other, with most of the differences generally weakly supported. The ML tree, with MPB, MLB, and PP values of the clades, is presented in Fig. 3B, with topological differences between the analyses denoted by dotted lines. In all trees obtained from the combined cpDNA and ITS data set, *Chuquiraga*, *Doniophyton*, and *Duseniella* form a well-supported monophyletic group (100% MPB, MLB, PP). *Doniophyton* is monophyletic (100% MPB, MLB, PP), and sister to *Chuquiraga* plus *Duseniella* in the MP (73% MPB), whereas *Duseniella* is sister to *Chuquiraga* plus *Doniophyton* in the ML and BI trees (<50% MLB, 64% PP). Each of the sections *Acanthophylla* and *Chuquiraga* forms a monophyletic group with high support (section *Acanthophylla* 86% MPB, 97% MLB, 100% PP; section *Chuquiraga* 92% MPB, 95% MLB, 100% PP; Fig. 3B). The two sections are sister clades only in the MP analyses, but with no support (<50% MPB); in the ML and BI analyses *Doniophyton* is sister to section *Acanthophylla* (65% MLB, 98% PP). The lineage that comprises section *Chuquiraga* is divided into two subclades: one includes all species of *Chuquiraga* series *Parviflorae* (white squares in Fig. 3B) plus *Chuquiraga calchaquina* and *C. longiflora* of *Chuquiraga* series *Chuquiraga* (83% MPB, 84% MLB 100% PP); the other subclade includes all remaining species of *Chuquiraga* series *Chuquiraga* (black squares in Fig. 3B; 75% MPB, 84% MLB, 77% PP). Of the species with subspecies, *C. ulicina* is monophyletic (100% MPB, MLB, PP), but *C. spinosa* and *C. erinacea* are not resolved as monophyletic in any of the analyses. The two accessions of *C. aurea* do not
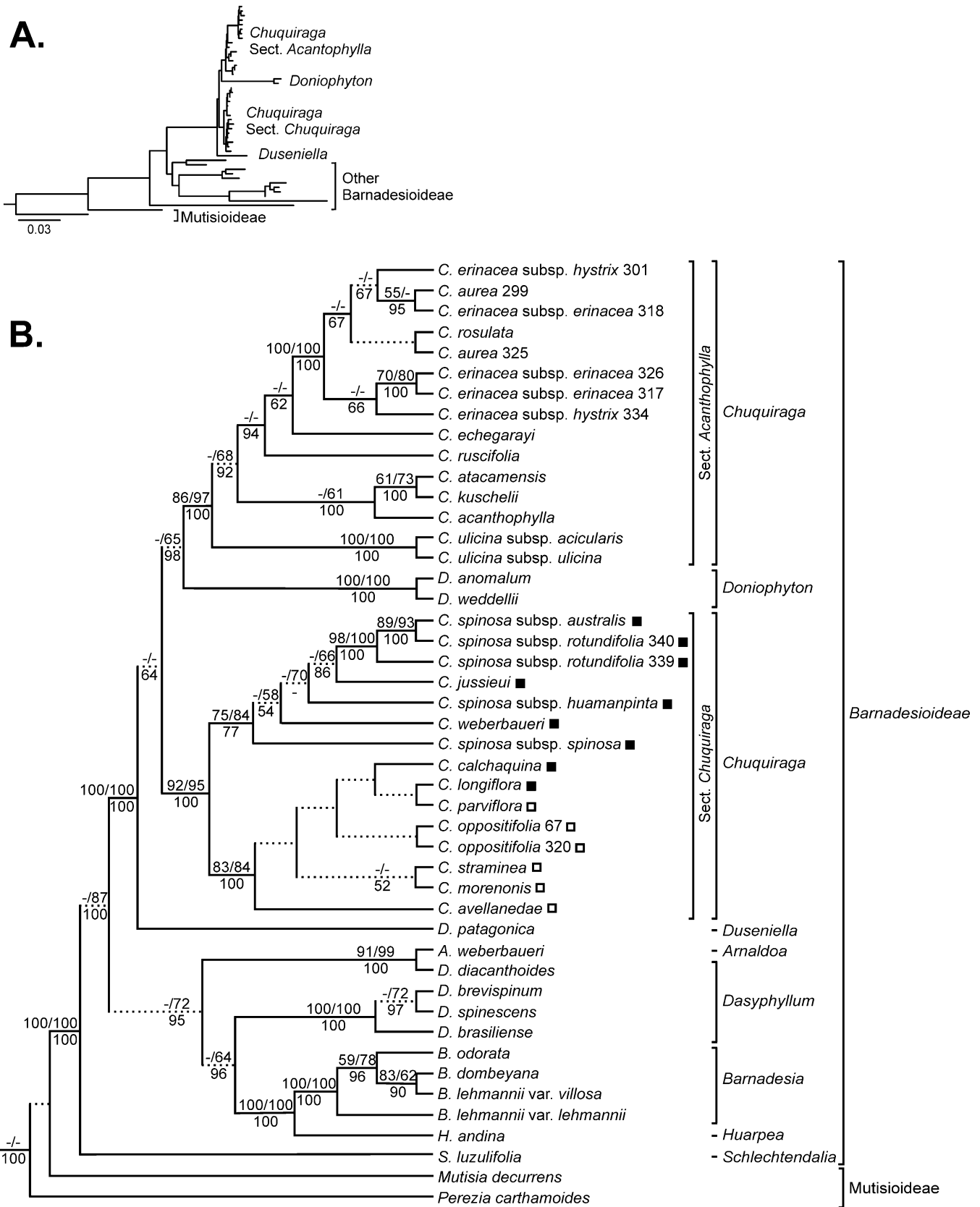
Fig. 3.   Tree derived from maximum likelihood analysis of the cpDNA *psbA-trnH, rps16-trnK, trnL-rpl32,* and nrDNA ITS sequences. A. Tree showing branch lengths proportional to nucleotide substitutions per site. B. Tree showing topological pattern of relationships. Numbers above branches correspond to the maximum parsimony and maximum likelihood bootstrap values (left and right, respectively); numbers below branches represent posterior probability values (in percentage). Dotted lines represent branches that are absent in the maximum parsimony strict consensus tree and/or in the Bayesian inference majority-rule consensus tree. Species of *Chuquiraga* section *Chuquiraga* series *Chuquiraga* are indicated with black squares and those of series *Parviflorae,* with white squares.

Table 3. Significance values of alternative phylogenetic relationships evaluated using AU, SH, and WSH tests, as described in the text. Hypotheses are rejected at $p \leq 0.05$.

Hypothesis	p-values		
	AU	SH	WSH
(*Chuquiraga*)	0.113	0.221	0.334
(*Chuquiraga* + *Doniophyton*)	0.745	0.798	0.800
(*Chuquiraga* + *Duseniella*)	0.255	0.324	0.402
(Sect. *Acanthophylla* + *Doniophyton*)	0.824	0.842	0.835
(Sect. *Chuquiraga* + *Duseniella*)	0.359	0.337	0.496

form a monophyletic group, and the accession *C. aurea* 299 is sister to *C. erinacea* subsp. *erinacea* 318 (55% MPB, <50% MLB, 95% PP).

Competing Hypotheses Tests—The AU, SH, and WSH tests indicated that none of the hypotheses evaluated, i.e. alternative relationships between *Doniophyton*, *Duseniella,* and *Chuquiraga*, or the monophyly of the latter, could be rejected at a significance level of $p < 0.05$ (Table 3).

DISCUSSION

This study is the first to estimate phylogenetic relationships of nearly all *Chuquiraga* species and subspecies based on molecular characters of the chloroplast and nuclear genomes. Previous molecular phylogenetic studies that included *Chuquiraga* species (Gustafsson et al. 2001; Gruenstaeudl et al. 2009) focused on intergeneric relationships and included only 30% of the species and subspecies of the genus. Here this sampling was tripled, analyzing ca. 90% of the species and subspecies currently recognized in *Chuquiraga*. The results of this work are important in relation to the revision of the classification of the genus, and are also necessary for the study of key morphological characters associated with important selective forces in the evolutionary history of the genus and related genera (Padin et al. submitted).

Monophyly of Chuquiraga and Intergeneric Relationships— Phylogenetic analyses performed in this study show that *Chuquiraga*, *Doniophyton,* and *Duseniella* form a well-supported monophyletic group (100% MPB, MLB, PP), but the relationships among these genera and the monophyly of *Chuquiraga* are still uncertain. Most results of our work show that *Doniophyton* arises within a paraphyletic *Chuquiraga* (all cpDNA analyses and ML and BI total evidence analyses). However, all ITS analyses show that *Duseniella* arises within a paraphyletic *Chuquiraga*. Only in the MP analysis from the total evidence data set (and some of the trees of the MP ITS analysis) is *Chuquiraga* monophyletic, with *Doniophyton* sister to *Chuquiraga* plus *Duseniella*. These ambiguous and poorly supported relationships may be the result of too few informative characters at those portions of the trees (i.e. among these genera). In fact, by looking at the length of branches in the BI or ML trees (e.g. Figure 3A), it is evident that branches that support the relationships between *Duseniella*, *Doniophyton,* and *Chuquiraga* are relatively short, especially in relation to the branch that leads to the ancestor of the three genera. These observations lead to the question if this lack of informative characters reflects artifacts of the methods and/or data used, or evolutionary processes that are not congruent with a bifurcating pattern of species diversification (Calviño et al. 2008). Branches may be short because of insufficient data (i.e. by using molec-

ular or morphological data that are not variable enough at the taxonomic level considered, or not having enough data to solve the problem), or because of a hard multifurcation (i.e. simultaneous or rapid splitting of several lineages). These two scenarios are sometimes difficult to resolve. Rapid radiation will tend to defy resolution using most types of data. In contrast, if the polytomy is not caused by truly short times between divergences, relationships should ultimately be resolvable using data sources with appropriate levels of variation for the target age of divergence (Whitfield and Lockhart 2007).

Previous phylogenetic analyses of the group also failed to resolve the relationships between *Chuquiraga*, *Doniophyton*, and *Duseniella* with high support, irrespective of the type of data used: i.e. morphology, or up to 10 DNA regions with different levels of sequence divergence (Stuessy et al. 1996; Urtubey and Stuessy 2001; Ezcurra 2002; Gruenstaeudl et al. 2009). Moreover, alternative relationships between *Doniophyton*, *Duseniella,* and *Chuquiraga*, or the monophyly of the latter, are statistically equally good explanations of the data, as estimated by the AU, SH or WSH tests in this work. *Doniophyton* is morphologically close to *Chuquiraga* (Cabrera 1977), to the point that one of its two species was originally described as *Chuquiraga anomala* D. Don and *C. patagonica* Phil. (Candolle 1838; Katinas and Stuessy 1997). *Duseniella*, on the other hand, is less similar morphologically (Urtubey and Ezcurra 1996), so much as not to be initially included in the Barnadesioideae group (Cabrera 1977). Nevertheless, phylogenetic analyses based on morphology also place this genus sister to *Doniophyton*, sister to *Chuquiraga* plus *Doniophyton*, or within a paraphyletic *Chuquiraga* (Stuessy et al. 1996; Urtubey and Stuessy 2001; Ezcurra 2002, respectively). So, even with the array of phylogenetic analyses already attempted, the phylogenetic relationships between these three genera seem very difficult to eventually resolve. Therefore, a rapid radiation in the lineage that gave rise to *Chuquiraga*, *Doniophyton,* and *Duseniella* seems the most likely conclusion with current evidence. This highly supported lineage also appeared as monophyletic in phylogenetic analyses based on morphology (e.g. Stuessy et al. 1996; Urtubey and Stuessy 2001; Ezcurra 2002) and sequence data (Gustafsson et al. 2001; Gruenstaeudl et al. 2009). Moreover, the ancestor of *Chuquiraga*, *Doniophyton,* and *Duseniella* originated in the extremely dry Monte region (Gruenstaeudl et al. 2009). It would be interesting to study whether the origin of the lineage coincides with a shift of niche to new, more xeric habitats that offered an ecological opportunity (Schluter 2000) that could explain the rapid radiation observed.

In the light of these results and in order to have a classification of the family that reflects monophyletic groups, it could be desirable to reduce *Doniophyton* and *Duseniella* to the synonymy of *Chuquiraga*. By doing so, the newly circumscribed genus would reflect an important lineage of the Barnadesioideae that originated in xeric habitats, and that is highly supported by molecular synapomorphies. However, the only putative morphological synapomorphies for the group described so far are long-tailed anthers, pollen without intercolpal depressions, and pollen walls with columellate-granular ectexine (Stuessy et al. 1996; Urtubey and Stuessy 2001; Stuessy et al. 2009). Further studies are in order to investigate if other morphological synapomorphies exist that support a new delimitation of the genus *Chuquiraga* based on the molecular phylogenies now available.

Classification of Chuquiraga—The molecular phylogenies obtained in the present study support the monophyly of both sections *Chuquiraga* and *Acanthophylla* in which the genus is currently classified (Ezcurra 1985). These results provide new and independent evidence for the same groupings that resulted from some of the previous phenetic (Ezcurra and Crisci 1987) and phylogenetic analyses based on morphological characters (e.g. Urtubey and Stuessy 2001). Both sections are characterized by differences in leaf morphology (Ezcurra 1985). Section *Chuquiraga* presents flat, hypostomatic, or amphistomatic leaves with or without adaxial pubescence, with a prominent midvein and axillary spines. Section *Acanthophylla* is supported by the following morphological synapomorphies: presence of boat-shaped or acicular, epistomatic leaves with adaxial pubescence, absence of a prominent midvein and axillary spines, lignified abaxial epidermis, and continuous hypodermic sclerenchyma (Padin et al. in press). Even though some of the previous morphological and molecular phylogenies had given equivocal support to these groups (Gustafsson et al. 2001; Ezcurra 2002; Gruenstaeudl et al. 2009), the results of our work, with nearly complete taxon sampling of the genus, clearly confirm the division into two sections of the current classification of *Chuquiraga* based on leaf morphology (Ezcurra 1985). They also support the idea that the environmental selection of different leaf types has been an important evolutionary force in the diversification of the genus (Ezcurra 2002).

In section *Acanthophylla*, two groups of species can be recognized in the phylogeny: a well supported clade of species with acicular leaves (*Chuquiraga erinacea*, *C. rosulata,* and *C. aurea*), and a basal unresolved grade of species with boat-shaped leaves. Some species of each of these two groups were classified under the invalid names *Unguis-cati* and *Ruscifolia,* respectively, in a partial treatment of *Chuquiraga* from Argentina in which types were not designated (Gaspar 1945). But, as the basal grade of our phylogeny is unresolved or has poor support, our results do not warrant a taxonomic subdivision of Section *Acanthophylla*. On the other hand, in this section, the well-supported clade with acicular leaves (comprised by *C. erinacea* subsp. *hystrix* 301 to *C. erinacea* subsp. *hystrix* 334; Fig. 3B) is not resolved internally, which suggests little genetic differentiation. This agrees with the historical difficulties in morphologically delimiting these closely related, polymorphic, and mostly sympatric species of the Monte and Patagonia (e.g. Weddell 1855; Ezcurra 1985; Ezcurra and Crisci 1987).

Within section *Chuquiraga*, series *Chuquiraga* and *Parviflorae* as defined by Ezcurra (1985) are not monophyletic according to the present study. These series were characterized by differences in sizes of their flower heads. Series *Chuquiraga* was characterized by large, usually red or orange heads, presumably pollinated by hummingbirds, and series *Parviflorae* by smaller, yellow heads that are insect-pollinated (Ezcurra 1985, 2002). The phylogenies obtained from the cpDNA and the total evidence analyses indicate that the lineage that comprises section *Chuquiraga* is divided into two subclades: one, generally more southern, that includes all species of *Chuquiraga* series *Parviflorae* with small flower heads plus *Chuquiraga calchaquina* and *C. longiflora* of *Chuquiraga* series *Chuquiraga*; and the other, generally more northern, that includes all remaining large-headed species of *Chuquiraga* series *Chuquiraga.*

The series *Chuquiraga* and/or *Parviflorae* were also recognized as artificial in several previous studies based on morphological and molecular evidence (Gustafsson et al. 2001;

Urtubey and Stuessy 2001; Ezcurra 2002; Gruenstaeudl et al. 2009). But this is the first time that these relationships are estimated from molecular evidence and with a high number of species and subspecies. Thus, our results suggest that large-headed species could have arisen through selective pressure of hummingbird pollination more than once in the evolution of the genus. Repeated pollinator shifts from insects to hummingbirds have been found in the evolution of many genera from different plant families (reviewed in Tripp and Manos 2008), and at least three times in Barnadesioideae evolution (Gruenstaeudl et al. 2009). Thus, changes in capitula size can be the result of parallel evolution and should not be considered in the delimitation of series within *Chuquiraga* as previously proposed (Ezcurra 1985, 2002). Therefore, the results of this work support the classification of *Chuquiraga* in two sections characterized by differences in leaf morphology, but do not warrant the subdivision of these sections into series.

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APPENDIX 1. New accessions of Asteraceae subfamily Barnadesioideae from which cpDNA *psbA-trnH, rps16-trnK,* and/or *trnL-rpl32,* and/or nuclear rDNA ITS sequences were obtained, with corresponding DNA accession and voucher information and GenBank reference numbers (*psbA-trnH, rps16-trnK, trnL-rpl32,* ITS).

Chuquiraga acanthophylla Wedd. DNA no. 356, ARGENTINA. Jujuy, Dpto. Yavi, 19 February 2011, *Zuloaga 13088* (SI) KJ789784, KJ789817, KJ789719, KJ789750. *Chuquiraga atacamensis* Kuntze. DNA no. 315, ARGENTINA. Jujuy, Dpto. Tumbaya, 15 December 2010, *Ezcurra 3715* (BCRU) KJ789785, KJ789818, KJ789720, KJ789751. *Chuquiraga aurea* Skottsb. DNA no. 299, ARGENTINA. Santa Cruz, Dpto. Corpen Aike, 7 March 2011, *Padin 100* (BCRU) KJ789786, KJ789819, KJ789721, KJ789752; DNA no. 325, ARGENTINA. Mendoza, Dpto. Las Heras, 16 March 2005, *Kiesling, Ezcurra, & Meglioli 10207* (MERL) KJ789787, KJ789820, KJ789722, KJ789753; *Chuquiraga avellanedae* Lorentz. DNA no. 316, ARGENTINA. Neuquén, Dpto. Collón Curá, 6 April 2011, *Ezcurra 3755* (BCRU) KJ789788, KJ789821, KJ789723, KJ789754. *Chuquiraga calchaquina* Cabrera. DNA no. 398, ARGENTINA. Salta, Dpto. Capital, 2001, *Chiarini, Barboza, Matesevach, & Novara 496* (CORD) KJ789789, KJ789822, –, KJ789755. *Chuquiraga echegarayi* Hieron. DNA no. 300, ARGENTINA. San Juan, Dpto. Calingasta, 15 March 2005, *Kiesling, Ezcurra, & Meglioli 10206* (MERL) KJ789790, KJ789823, KJ789724, KJ789756. *Chuquiraga erinacea* subsp. *erinacea* D. Don. DNA no. 317, ARGENTINA. Jujuy, Dpto. Humahuaca, 15 December 2010, *Ezcurra 3716* (BCRU) KJ789791, KJ789824, KJ789725, KJ789757; DNA no. 318, ARGENTINA. La Pampa, Dpto. Puelén, 7 April 2011, *Ezcurra 3757* (BCRU) KJ789792, KJ789825, KJ789726, KJ789758; DNA no. 326, ARGENTINA. San Juan, Dpto. Calingasta, 15 March 2005, *Kiesling, Ezcurra, & Meglioli 10205* (MERL) KJ789793, KJ789826, KJ789727, KJ789759. *Chuquiraga erinacea* subsp. *hystrix* (D.Don) C.Ezcurra. DNA no. 301, ARGENTINA. Neuquén, Dpto. Confluencia, 6 April 2011, *Ezcurra 3756* (BCRU) KJ789794, KJ789827, KJ789728, KJ789760; DNA no. 334, ARGENTINA. San Juan, Dpto. Iglesia, 11 March 2005, *Kiesling, Ezcurra, & Meglioli 10180* (MERL) KJ789795, KJ789828, KJ789729, KJ789761. *Chuquiraga jussieui* J.F.Gmel. DNA no. 358, BOLIVIA. La Paz, Dpto. La Paz, 4 July 2005, *Yanapa 431* (LPB) KJ789796, KJ789829, KJ789730, KJ789762. *Chuquiraga kuschelii* Acevedo. DNA no. 336, CHILE. Región XV, Arica, 3 March 1997, *Eggli 2813* (CONC) KJ789797, KJ789830, KJ789731, KJ789763. *Chuquiraga longiflora* (Griseb.) Hieron. DNA no. 399, ARGENTINA. Catamarca, Dpto. Andagalá, 13 February 2013, *Fernandez 56* (BCRU) KJ789798, KJ789831, KJ789732, KJ789764 *Chuquiraga morenonis* (Kuntze) C.Ezcurra. DNA no. 319, ARGENTINA. Santa Cruz, Dpto. Magallanes, 24 December 2011, *Padin 103* (BCRU) KJ789799, KJ789832, KJ789733, KJ789765. *Chuquiraga oppositifolia* D.Don. DNA no. 67, CHILE. Región V, Valparaíso, 9 December 2010, *Ezcurra 3703* (BCRU) KJ789800, KJ789833, KJ789734, KJ789766; DNA no. 320, ARGENTINA. Neuquén, Dpto. Ñorquín, 15 March 2008, *Calviño 735* (BCRU) KJ789801, KJ789834, KJ789735, KJ789767. *Chuquiraga parviflora* (Griseb.) Hieron. DNA no. 338, ARGENTINA. San Juan, Dpto. Jachal, 10 March 2005, *Kiesling, Ezcurra, & Meglioli 10162* (MERL) KJ789802, KJ789835, KJ789736, KJ789768. *Chuquiraga rosulata* Gaspar. DNA no. 321, ARGENTINA. Neuquén, Dpto. Confluencia,

16 April 2011, *Ezcurra 3758* (BCRU) KJ789803, KJ789836, KJ789737, KJ789769. ***Chuquiraga ruscifolia*** D.Don. DNA no. 322, ARGENTINA. Mendoza, Dpto. Las Heras, 8 December 2010, *Ezcurra 3700* (BCRU) KJ789804, KJ789837, KJ789738, KJ789770; ***Chuquiraga spinosa*** subsp. ***australis*** C.Ezcurra. DNA no. 323, ARGENTINA. Jujuy, Dpto. Humahuaca, 16 December 2010, *Ezcurra 3718* (BCRU) KJ789805, KJ789838, KJ789739, KJ789771. ***Chuquiraga spinosa*** subsp. ***huamanpinta*** C.Ezcurra. DNA no. 327, PERÚ, 12 January 1996, *Sotello 1* (BCRU) KJ789806, KJ789839, KJ789740, KJ789772. ***Chuquiraga spinosa*** subsp. ***rotundifolia*** (Wedd.) C.Ezcurra. DNA no. 339, CHILE Región XV, Arica, Parinocota, 29 January 2004, *Panero 8442* (CONC) KJ789807, KJ789840, KJ789741, KJ789773; DNA no. 340, CHILE. Región XV, Parinocota, Putre, 12 June 2007, *Rosas 4895* (CONC) KJ789808, KJ789841, KJ789742, KJ789774. ***Chuquiraga spinosa*** subsp. ***spinosa*** D.Don. DNA no. 400, PERÚ. Pasco, Dpto. Pasco, 28/30 November 1986, *Reynel & Van Eynde 2266* (UNALM) KJ789809, KJ789842, –, KJ789775. ***Chuquiraga straminea*** Sandwith. DNA no. 328, ARGENTINA. Neuquén, Dpto. Picunches, 14 March 2008, *Calviño 730* (BCRU) KJ789810, KJ789843, KJ789743, KJ789776. ***Chuquiraga ulicina*** subsp. ***acicularis*** (D.Don) C.Ezcurra. DNA no. 302, CHILE. Región III de Atacama, Parque Nacional Llanos de Challe, 12 December 2010, *Ezcurra 3711* (BCRU) KJ789811, KJ789844, KJ789744, KJ789777. ***Chuquiraga ulicina*** subsp. ***ulicina*** Hook. DNA no. 330, CHILE. Región II, Antofagasta, 8 August 2007, *Ezcurra 3594* (BCRU) KJ789812, KJ789845, KJ789745, KJ789778. ***Chuquiraga weberbaueri*** Tovar. DNA no. 401, PERÚ. Sánchez Carrión, Dpto. La Libertad, 22 July 2009, *Glenn 214* (MO), EU841283*, EU547601*,–, KJ789779. ***Dasyphyllum diacanthoides*** (Less.) Cabrera. DNA no. 368, ARGENTINA. Río Negro, Dpto. Bariloche, 2 April 2011, *Padin 101* (BCRU) KJ789813, KJ789846, KJ789746, KJ789780. ***Doniophyton anomalum*** (D.Don) Kurtz. DNA no. 332, ARGENTINA. Río Negro, Dpto. Pilcaniyeu, 1 February 2009, *Ezcurra 3600* (BCRU) KJ789814, KJ789847, KJ789747, KJ789781. ***Doniophyton weddellii***

Katinas & Stuessy. DNA no. 369, ARGENTINA. Neuquén, Dptos. Chos Malal y Pehuenches, 1 December 1999, *Quiroga 2542* (BCRU) KJ789815, KJ789848, KJ789748, KJ789782. ***Duseniella patagonica*** Pilg. & Ulbr. DNA no. 333, ARGENTINA. Río Negro, Dpto. General Roca, 16 October 2009, *Ezcurra 3601* (BCRU) KJ789816, KJ789849, KJ789749, KJ789783.

*Sequences from Gruenstaeudl et al. 2009, voucher TS 12496 (WU).

APPENDIX 2. Accessions of Asteraceae subfamily Barnadesioideae obtained from GenBank, with corresponding voucher information and Genbank reference numbers (*psbA-trnH, rps16-trnK, ITS*). All sequences were originally published in Gruenstaeudl et al. (2009), except the *trnL-rpl32* sequence of *Perezia carthamoides* (voucher: GH:E. Wall s. n; GenBank no. FJ979692), from Simpson et al. (2009).

Arnaldoa weberbaueri (Muschl.) Ferreyra. *TS 12524* (WU) EU841268, EU547586, EU841139. ***Barnadesia dombeyana*** Less. *TS 12470* (WU) EU841270, EU547587, EU841140. ***Barnadesia lehmannii*** var. ***lehmannii*** Hieron. *TS 12465* (WU) EU841271, EU547588, EU841141. ***Barnadesia lehmannii*** Hieron. ex Sodiro var. ***villosa*** (I.C.Chung) Urtubey. *TS 12699* (WU) EU841142, EU547589, EU841272. ***Barnadesia odorata*** Griseb. *TS 12947* (WU) EU841274, EU547590, EU841144. ***Dasyphyllum brasiliense*** (Spreng.) Cabrera. *Hatschbach 51270* (MU) EU841291, EU547607, EU841159. ***Dasyphyllum brevispinum*** Sagást. & M.O.Dillon. *TS 12689* (WU) EU841290, EU547606, EU841158. ***Dasyphyllum spinescens*** (Less.) Cabrera. *Silva 121* (MU) EU841293, EU547608, EU841161. ***Huarpea andina*** Cabrera. *Dalmasso 10-Dez-04* (LP) EU841299, EU547611, EU841167. ***Mutisia decurrens*** Cav. *TS 12463* (WU) EU841304, EU547613, EU841169. ***Perezia carthamoides*** Hook. & Arn. *TS 12730* (WU) EU841307, EU547614, EU841171. ***Schlechtendalia luzulifolia*** Less. *TS 12820* (WU) EU841300, EU547616, EU841166.