Phylogenetic relationships and generic reassessment of *Proustia* and allies (Compositae: Nassauvieae)

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Abstract *Proustia* is a small southern Andean genus of shrubs, vines and small trees, which are characteristic elements of Chilean and Argentine Andean forests, thickets and desert scrubs. Since *Proustia* possesses an unusual and characteristic morphology within the Nassauvieae, its circumscription as well as its phylogenetic placement is decisive in understanding the evolution of the tribe. *Berylsimpsonia*, from the Caribbean, was segregated from *Proustia*, which currently only includes three species. *Lophopappus*, another Andean genus, has been closely related to *Proustia* in sharing style and corolla features that are unusual in the Nassauvieae. The purpose of this work is to evaluate the placement of *Proustia* within the Nassauvieae, its relationships with *Berylsimpsonia* and *Lophopappus*, and the relationships of its species using molecular data and phylogenetic methods. This is the first comprehensive analysis of *Proustia*. We have included in our study the three currently accepted species of *Proustia* and seven of its ten infraspecific taxa. For each taxon of *Proustia*, one to seven accessions were analyzed. Species of *Lophopappus* and *Berylsimpsonia*, as well as 16 species belonging to 13 genera of Nassauvieae were also analyzed. We sequenced the nuclear ribosomal ITS and ETS, and the plastid regions *ndhF*, 5′ *trnK-matK*, *trnL-trnF*, and *trnL(UAG)-rpl32*. Unlike other previous studies, *Proustia* is not sister to the rest but nested in the tribe in our analyses. *Berylsimpsonia vanillosma* and *P. ilicifolia* are distantly related to *P. pyrifolia*, the type of the genus. According to nuclear DNA data, the infraspecific taxa of *P. cuneifolia* were recovered all in a well-supported clade, although based on the plastid data *P. pyrifolia* does not form a lineage separate from *P. cuneifolia*. *Lophopappus* is the genus most closely related to *Proustia*. Regarding the evolution of styles, our results suggest multiple origins of atypical stylar features in the Nassauvieae. As a result of our analyses we propose the segregation of *Proustia ilicifolia* into the new genus *Spinoliva*. Morphological data are consistent with the segregation of *Proustia* and *Lophopappus* as separate genera. Two species of *Proustia*, *P. cuneifolia* and *P. pyrifolia* are recognized. Three lectotypifications, one neotypification, two new combinations and five rank changes are proposed.

Keywords Andes; *Berylsimpsonia*; *Lophopappus*; molecular phylogeny; South America

Supplementary Material DNA sequence alignments are available from https://doi.org/10.12705/671.7.S

■ INTRODUCTION

The Compositae are the largest family of flowering plants and a key element to understand biodiversity globally (Funk & al., 2009; Willis, 2017). *Proustia* Lag. is a small intriguing Andean genus of the Compositae, which belongs to the tribe Nassauvieae within the subfamily Mutisioideae. *Proustia* occurs from Peru to Central Chile and Argentina. Its species are characteristic elements of sclerophyllous Andean forests, thickets and desert scrubs (Cabrera, 1971; Luebert & Pliscoff, 2006). After the Barnadesioideae and Famatinanthoideae, the subfamily Mutisioideae is the sister group to the remaining over 95% of the species of Compositae (Panero & al., 2014). Therefore, understanding the relationships of genera like *Proustia* is crucial in helping to elucidate the early evolution of this family.

While *Proustia* has long been placed in Nassauvieae based on its predominantly bilabiate corollas, tailed anthers and pollen exine stratification (e.g., Crisci, 1974; Cabrera, 1977; Tellería & al., 2003; Katinas & al., 2008a, b), its placement within this tribe is not yet clear. In previous phylogenetic analyses, *Proustia* was recovered as sister to the rest of Nassauvieae (Panero & Funk, 2008), whereas in other studies its placement was variable and even dependent on which molecular markers were employed (Katinas & al., 2008b; Luebert & al., 2009; Simpson & al., 2009; Jara-Arancio & al., 2017). However, these studies did not focus on *Proustia* and each of them included only one of its three species. Because it possesses atypical morphological features, the circumscription of *Proustia*, as well as its phylogenetic placement in the Nassauvieae, are decisive to understand the evolution of the tribe. *Proustia* differs from the core of the tribe by its apically rounded style branches (Crisci,

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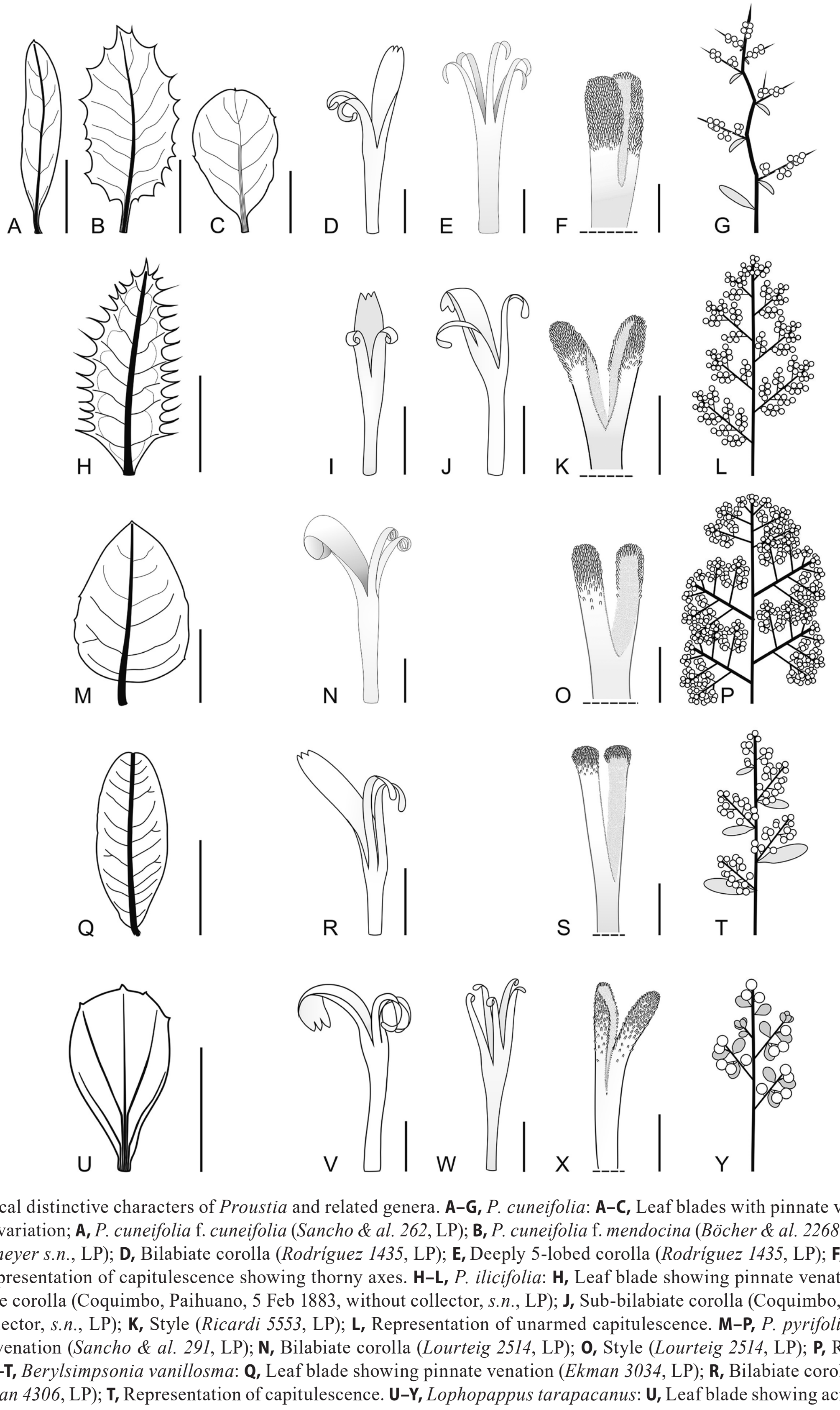


Fig. 1. Morphological distinctive characters of *Proustia* and related genera. **A–G,** *P. cuneifolia*: **A–C,** Leaf blades with pinnate venation showing shape and margin variation; **A,** *P. cuneifolia* f. *cuneifolia* (*Sancho & al. 262*, LP); **B,** *P. cuneifolia* f. *mendocina* (*Böcher & al. 2268*, LP); **C,** *P. cuneifolia* f. *tipia* (*Niemeyer s.n.*, LP); **D,** Bilabiate corolla (*Rodríguez 1435*, LP); **E,** Deeply 5-lobed corolla (*Rodríguez 1435*, LP); **F,** Style (*Ferreyra 13939*, LP); **G,** Representation of capitulescence showing thorny axes. **H–L,** *P. ilicifolia*: **H,** Leaf blade showing pinnate venation (*Wedermann 423*, F); **I,** Bilabiate corolla (Coquimbo, Paihuano, 5 Feb 1883, without collector, *s.n.*, LP); **J,** Sub-bilabiate corolla (Coquimbo, Paihuano, 5 Feb 1883, without collector, *s.n.*, LP); **K,** Style (*Ricardi 5553*, LP); **L,** Representation of unarmed capitulescence. **M–P,** *P. pyrifolia*: **M,** Leaf blade showing pinnate venation (*Sancho & al. 291*, LP); **N,** Bilabiate corolla (*Lourteig 2514*, LP); **O,** Style (*Lourteig 2514*, LP); **P,** Representation of capitulescence. **Q–T,** *Berylsimpsonia vanillosma*: **Q,** Leaf blade showing pinnate venation (*Ekman 3034*, LP); **R,** Bilabiate corolla (*Ekman 4306*, LP); **S,** Style (*Ekman 4306*, LP); **T,** Representation of capitulescence. **U–Y,** *Lophopappus tarapacanus*: **U,** Leaf blade showing acrodromous venation (*Tovar 1386*, LP); **V,** Bilabiate corolla (*Heins 231*, LP); **W,** Deeply 5-lobed corolla (*Tovar 1386*, LP); **X,** Style (*Heins 231*, LP); **Y,** Representation of capitulescence and stem branches with brachyblasts. — White circles represent capitula, grey structures represent leaves. Scale bars: A, B, H, M & Q = 2 cm; C = 6 mm; D, E, I, J, N, R, V & W = 2 mm; F, K, O, S & X = 0.5 mm; U = 1 cm. Note: *Proustia pyrifolia* occasionally has corollas deeply 5-lobed (Crisci, 1974). However, we failed to find this type of corolla. Drawn by Gisela Sancho.

1974; Sancho & al., 2014), dorsally covered by collecting hairs on the distal half (vs. usually apically truncate style branches with an apical tuft of collecting hairs in most Nassauvieae). In addition, the corollas of *Proustia* are sometimes deeply 5-lobed (Fig. 1) instead of the typical bilabiate corollas of Nassauvieae.

Intergeneric relationships of *Proustia* are no less problematic. *Lophopappus* Rusby, an Andean genus of five species (Katinas & al., 2013) has been closely related to *Proustia*, mainly by sharing style, corolla, and pollen features (Crisci, 1974; Tellería & al., 2003; Katinas & al., 2013; Sancho & al., 2014). Figures 1A–P and U–Y show some of their distinctive features. *Lophopappus* and *Proustia* were synonymized by Ferreyra (1995) and have been recovered as sister groups in previous phylogenetic analyses (Panero & Funk, 2008). *Proustia* was considered to be composed of four species by Fabris (1968). Based on differences of morphological characters, one of the species included in *Proustia* by Fabris (1968), *P. vanillosma* C.Wright from the Caribbean, was transferred first to *Acourtia* D.Don (Crisci, 1974) and then to the new genus *Berylsimpsonia* B.L.Turner (Turner, 1993) (Fig. 1Q–T), bringing *Proustia* to its present circumscription with three species. Until now, the phylogenetic relationships between *Berylsimpsonia*, *Lophopappus* and *Proustia* remained unstudied with molecular data.

Although it only includes three species (Fabris, 1968; Sancho & al., 2014) (Table 1), *Proustia* shows a highly variable morphology, and this variation led Fabris (1968) to recognize several forms under each species. Besides, recent morphological phylogenetic analyses have challenged its monophyly (Sancho & al., 2014). If *Proustia* is confirmed as non-monophyletic, the systematic and evolutionary significance of its unusual morphological characters mentioned above would need to be re-evaluated within Nassauvieae.

A non-monophyletic *Proustia* could explain its variable morphology despite the low number of species (Sancho & al., 2014). Indeed, homology of some of the traditional diagnostic features of *Proustia* is under debate. Sancho & al. (2014) indicated that the spiny structures usually referred to as diagnostic of *Proustia* are not homologous and probably adaptations to particular habitats. Spiny structures and secondary inflorescences (capitulescences) support independent lineages each representing one species of *Proustia* (Table 1; Figs. 1, 2). These findings seem to agree with previous authors who subdivided *Proustia* into different sections, with one species each (Don, 1830; Candolle, 1838; Fabris, 1968). The monophyly of *Proustia* and the interspecific relationships have not yet been addressed in molecular phylogenetic studies.

The purpose of this work was to evaluate the placement of *Proustia* within the Nassauvieae, its relationships with *Berylsimpsonia* and *Lophopappus*, and the relationships of its species using molecular data and phylogenetic methods. Since our results confirmed the non-monophyly of *Proustia*, we offer a reinterpretation of homology of morphological characters traditionally used to define *Proustia* and provide generic rearrangements necessary to accommodate monophyletic groups into taxonomic entities.

■ MATERIALS AND METHODS

Taxon sampling. — We included the three accepted species of *Proustia* and seven of its ten infraspecific taxa recognized by Fabris (1968) (Appendix 1). Under the synonymy proposed herein (Table 1), all the infraspecific taxa were sampled. For each taxon of *Proustia*, one to seven accessions were analyzed. Three species (of five) of *Lophopappus* and one (of two) species of *Berylsimpsonia* were included as closely related taxa of *Proustia* (e.g., Fabris, 1968; Crisci, 1974; Luebert & al., 2009). Nassauvieae possesses 25 genera and around 320 species (Katinas & al., 2008a). Twenty-two species belonging to 13 genera of Nassauvieae (*Acourtia*, *Calopappus* Meyen, *Dolichlasium* Lag., *Holocheilus* Cass., *Jungia* L.f., *Leucheria* Lag., *Moscharia* Ruiz & Pav., *Nassauvia* Comm. & Juss., *Oxyphyllum* Phil.,

Table 1. *Proustia* sections, species and forms (according to Fabris, 1968 and Cabrera, 1977), diagnostic characters of sections.

Section	Diagnostic characters	Species	Infraspecific taxa	Taxonomy proposed here
Baccharoides DC.	Erect shrubs; branches unarmed (without thorns or spines); leaf margin spiny; capitula arranged in thyrses	*P. ilicifolia* Hook. & Arn.	f. *baccharoides* (Hook. & Arn.) Fabris	≡ *Spinoliva ilicifolia* subsp. *baccharoides* (D.Don) G.Sancho
			f. *ilicifolia*	*Spinoliva ilicifolia* (Hook. & Arn.) G.Sancho subsp. *ilicifolia*
Harmodia D.Don	Erect shrubs; inflorescence axes distally spiny (thorns); capitula arranged in racemes of spikes or glomerules	*P. cuneifolia* D.Don	f. *angustifolia* (Wedd.) Fabris	= subsp. *mollis* (Kuntze) Katinas
			f. *cinerea* (Phil.) Fabris	≡ subsp. *cinerea* (Phil.) Luebert
			f. *cuneifolia*	subsp. *cunefolia*
			f. *mendocina* (Phil.) Fabris	≡ subsp. *mendocina* (Phil.) Katinas
			var. *mollis* (Kuntze) Cabrera	≡ subsp. *mollis* (Kuntze) Katinas
			f. *oblongifolia* (Wedd.) Fabris	= subsp. *cuneifolia*
			f. *tipia* (Phil.) Fabris	≡ subsp. *tipia* (Phil.) Luebert
Proustia	Scandent shrubs; branches with infrapetiolar spines; capitula arranged in glomerulose thyrses	*P. vanillosma* C.Wright		*Berylsimpsonia vanillosma* (C.Wright) B.L.Turner
		P. pyrifolia DC.	f. *glandulosa* (DC.) Fabris	= *Proustia pyrifolia*
			f. *pyrifolia*	

Proustia vanillosma is currently a species of the genus *Berylsimpsonia* B.L.Turner.

Perezia Lag., *Polyachyrus* Lag., *Triptilion* Ruiz & Pav., *Trixis* P.Browne) were also analyzed. Representatives of Mutisieae (species of *Adenocaulon* Hook., *Chaetanthera* Ruiz & Pav., *Gerbera* L., *Mutisia* L.f., *Pachylaena* Hook.) and Onoserideae (*Gypothamnium* Phil., *Plazia* Ruiz & Pav., *Urmenetea* Phil.) were included as more distantly related outgroups. We rooted the trees with one representative of Barnadesioideae (*Chuquiraga* Juss.), which is sister to all remaining Compositae. Available *Proustia* and outgroup sequences (57) were obtained from GenBank (Appendix 1), provided by Katinas & al. (2008b), Panero & Funk (2008), Luebert & al. (2009), Simpson & al. (2009), Pelser & al. (2010), Panero & al. (2014) and Chacón & al. (2017).

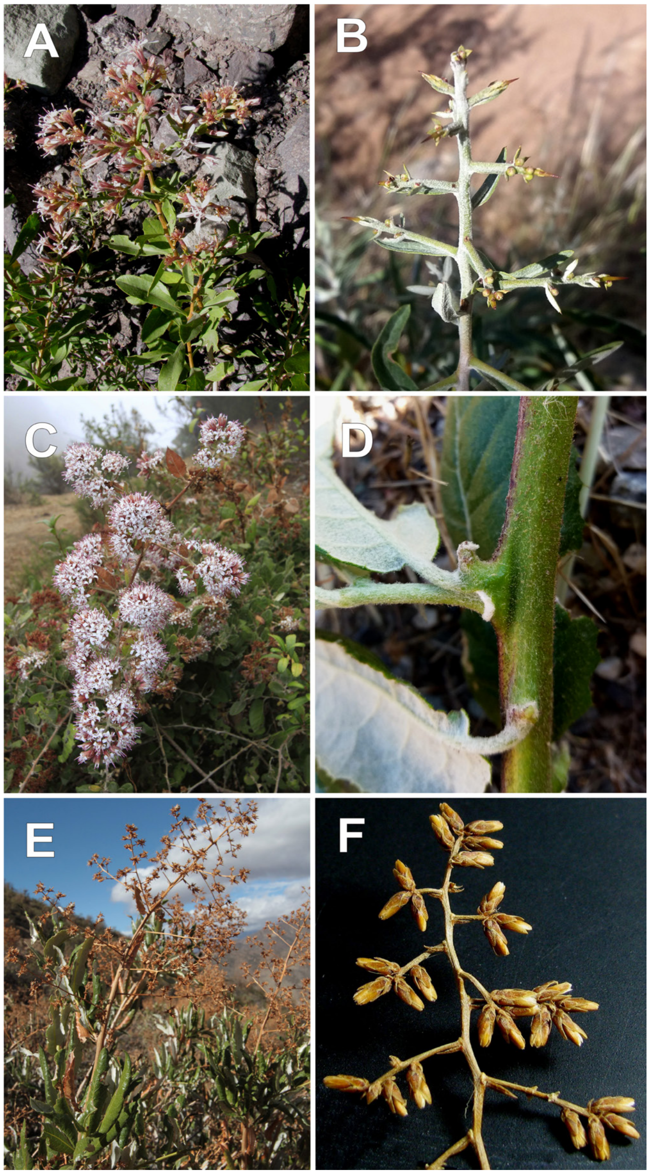


Fig. 2. A & B, *Proustia cuneifolia* f. *cuneifolia*: **A,** Capitulescence; **B,** Detail of capitulescence showing thorns. **C & D,** *P. pyrifolia*: **C,** Capitulescence; **D,** Infrapetiolar spine. **E & F,** *P. ilicifolia*: **E,** Capitulescence; **F,** Detail of unarmed capitulescence. — Photos: A, B, D & F, G. Sancho; C & E, A. Moreira-Muñoz.

DNA isolation, amplification, and sequencing. — Genomic DNA was extracted from leaf material (dried in silica gel or from herbarium specimens) using a modified CTAB method (Doyle & Dickson, 1987), DNeasy plant mini kit (Qiagen, Valencia, California, U.S.A.) or Nucleospin Plant II Kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. We sequenced the nuclear ribosomal ITS and ETS, and the plastid regions *trnL-trnF*, 5′ *trnK-matK*, *trnL(UAG)-rpl32* and *ndhF*. PCR reaction mixes for all markers were 12–13 µl ddH_2O, 1 µl DNA, 2.5 µl type 10× buffer, 2.5 µl 25 mM dNTPs, 1.5–2.5 µl of each 10 µM primer, 1.5 µl 25 mM $MgCl_2$, and 0.2 or 0.4 µl of Invitrogen (Life Technologies, São Paulo, Brazil) *Taq* polymerase. All 25-µl PCR reactions were performed in a Gene Prothermal cycler (Bioer Technology, Hangzhou, Japan-China) or Trio-Thermoblock thermal cycler (Biometra, Göttingen, Germany). Amplification primers for ITS were those of White & al. (1990) and cycling conditions followed Katinas & al. (2008a). The ETS was amplified with primers ETS1 and 18S-IGS (Baldwin & Markos, 1998 and Bayer & al., 2002, respectively) and cycling conditions followed Sancho & al. (2015). Amplification primers c and f for *trnL-trnF* were those of Taberlet & al. (1991) and cycling conditions followed Katinas & al. (2008a). The 5′ *trnK-matK* was amplified with primers 3914F (Johnson & Soltis, 1994) and 1240R (Bayer & al., 2002) and cycling conditions followed Sancho & al. (2015). The *trnL(UAG)-rpl32* region was amplified with primers rpl32 and trnL (Shaw & al., 2007) with cycling conditions following Baird & al. (2010). Amplification primers for *ndhF* were 1F, 1318R, 972F (Olmstead & Sweere, 1994) and +607 (Kim & Jansen, 1995) and cycling conditions followed Kim & Jansen (1995). The PCR products were purified using a QIAquick purification kit (Quiagen) or GeneJET PCR Purification Kit (Thermo Fisher Scientific Biosciences, St. Leon-Rot, Germany) following the manufacturer's instructions. Cycle sequencing was performed with BigDye Terminator v.3.1 and sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, California, U.S.A.). Contig building of resulting sequences was carried out using Geneious Pro v.5.6.5 (Biomatters, Auckland, New Zealand) and aligned using the software MAFFT v.6.603 (Katoh & al., 2002) followed by manual adjustments using PhyDE v.0.9971 (available from http://www.phyde.de/, accessed 17 Apr 2014) and BioEdit v.7.2.6 (Ibis Biosciences, Carlsbad, California, U.S.A.). The 233 newly generated sequences were deposited in GenBank (Appendix 1).

Phylogenetic analyses. — Nuclear (ETS, ITS) and plastid data (*ndhF*, 5′ *trnK*, *trnL-trnF*, *trnL(UAG)-rpl32*) were analyzed separately. The topologies of separate ITS and ETS analyses were checked for congruence before combining them. The nuclear dataset contained 58 accessions and 28% missing data, mostly because we were unable to amplify ITS in several *Proustia* species or because only one of the markers was available from the literature. The plastid dataset contained 56 accessions and 9% missing data (see Appendix 1).

Maximum likelihood (ML; Felsenstein, 1981) and Bayesian (BA; Mau & al., 1999) analyses were carried out for each data matrix on the CIPRES Science Gateway (Miller & al., 2010). ML analyses were conducted in RAxML v.8.2.10 (Stamatakis

& al., 2008) and BA was performed using MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003). Partitions were unlinked in both analyses. The software jModelTest v.2.1.2 (Darriba & al., 2012), under the Akaike information criterion (AIC), was used to determine the substitution model that best fitted sequence data of each data partition.

Bootstrap support (BS) was calculated in the ML based on 1000 replicates. For BA, analyses were conducted in 4 independent runs for 2 million generations sampling every 1000 generations. The first 500 trees (25%) were discarded as burn-in after checking for convergence in Tracer v.1.5 (available at http://tree.bio.ed.ac.uk/software/tracer/, accessed 15 Oct 2012), that ESS values of all parameters lay above 200. Posterior probabilities (PP) at nodes correspond to the 50% majority-rule consensus tree calculated from of the posterior distribution of the BA.

Topology tests. — We conducted topology tests in order to assess the plausibility of alternative topologies given our datasets. The original topology retrieved from the ML analyses of the nuclear and plastid datasets were modified using TreeGraph v.2.7.0-557-beta (Stöver & Müller, 2010) to obtain the following alternative topologies: (1) *Proustia ilicifolia* sister to *Lophopappus* + *P. cuneifolia* + *P. pyrifolia*, (2) *Proustia ilicifolia* sister to *P. cuneifolia* + *P. pyrifolia*, (3) *Proustia ilicifolia* sister to *P. cuneifolia*, (4) *Proustia pyrifolia* sister to *P. cuneifolia*. The approximately unbiased test (AU-test; Shimodaira, 2002) was employed to test all alternative topologies against the respective original topology. We used the IQ-TREE software v.1.5.5-beta (Nguyen & al., 2015) and analyses were run with 10,000 bootstrap replicates.

Morphological observations. — For the taxonomic treatment, the specimens studied are those indicated in Sancho & al. (2014). Additionally, specimens housed at B, K, P, S and SGO were analyzed. Type specimens were examined during research visits to herbaria or from herbarium websites. Pollen terminology follows Punt & al. (1994).

■ RESULTS

Phylogenetic analyses. — Our nuclear dataset had a total of 1321 aligned positions (ETS: 570, ITS: 751) and 995 distinct alignment patterns (ETS: 446, ITS: 549). Substitution model GTR+Γ was selected for ETS and GTR+I+Γ for ITS. ML and BA analyses yielded identical trees, with differences only in the support of some branches. Figure 3A shows the topology of the ML analysis using nrDNA data.

Our plastid dataset had a total of 5402 aligned positions (*ndhF*: 2172, 5′ *trnK-matK*: 1133, *trnL-trnF*: 978, *trnL(UAG)-rpl32*: 1119) and 1473 distinct alignment patterns (*ndhF*: 438, 5′ *trnK-matK*: 275, *trnL-trnF*: 325, *trnL(UAG)-rpl32*: 435). Substitution model HKY+Γ was selected for *trnL-trnF* and GTR+Γ for *ndhF*, 5′ *trnK-matK* and *trnL(UAG)-rpl32*. ML and BA analyses yielded similar trees, with differences only in the support of some branches. Figure 3B shows the topology of the BA analysis using plastid data.

The results obtained from the nuclear and plastid data both suggest that *Berylsimpsonia*, *Trixis*, *Dolichlasium* and *Jungia* form a monophyletic group. Moreover, *Proustia ilicifolia*, *P. pyrifolia* and *Lophopappus* were each retrieved as well-supported monophyletic groups in both nuclear and plastid trees. Furthermore, the results of both nuclear and plastid datasets suggest that *Lophopappus*, *Proustia cuneifolia* and *P. pyrifolia* form a well-supported monophyletic group. Conversely, the topologies obtained from nuclear and plastid data differ in two major aspects. First, in the nuclear tree *P. pyrifolia* was retrieved as sister to *Lophopappus* (PP = 1, BS = 88), while in the plastid tree *P. pyrifolia* was recovered as sister to *P. cuneifolia* f. *mendocina* (PP = 1, BS = 99). Second, *P. ilicifolia* was recovered as sister to all remaining Nassauvieae except *Leucheria*, *Moscharia*, *Oxyphyllum* and *Polyachyrus* in the nuclear tree, whereas in the plastid tree *P. ilicifolia* is sister to the latter four genera. However, the relationships of *P. ilicifolia* are only weakly supported in both analyses. Given the lack of topological congruence between nuclear and plastid trees, we did not combine these datasets.

Topology tests. — The results of the AU-test suggest that all alternative topologies should be rejected (Fig. 4). Neither *Proustia ilicifolia* sister to *Lophopappus*+*P. cuneifolia*+*P. pyrifolia* nor sister to *P. cuneifolia* or to *P. cuneifolia*+*P. pyrifolia* are as good explanations of the datasets as the original topologies obtained both from the nuclear and plastid datasets. The only exception occurs when *P. pyrifolia* is made sister to *P. cuneifolia*, which is rejected only as an explanation of the plastid dataset, but not of the nuclear dataset.

■ DISCUSSION

Phylogenetic relationships of *Proustia* in Nassauvieae. — This is the first comprehensive analysis of *Proustia*, a small but highly variable genus of Nassauvieae with unusual morphology. Unlike previous studies, in our analyses with nuclear data *Proustia* is not sister to the rest but nested in the tribe, albeit with low support. This contrasts with the results of Panero & Funk (2008) and Panero & al. (2014), but agrees with other studies (Katinas & al., 2008b; Luebert & al., 2009).

Two species formerly included in *Proustia*, *P. ilicifolia* and *P. vanillosma* (the latter currently *Berylsimpsonia vanillosma*) are distantly related to *P. pyrifolia*, the type of the genus. However, the placement of *P. ilicifolia* was uncertain in our phylogenetic trees and varied depending on whether plastid or nuclear data were taken into account. Only one previous phylogenetic study had included *P. ilicifolia* (Jara-Arancio & al., 2017), retrieving it as sister to *Macrachaenium* Hook.f. We were not able to include *Macrachaenium* in our analysis. The placement of *Macrachaenium* remains uncertain within the Mutisioideae and necessitates further analyses. *Berylsimpsonia* was retrieved as closely related to *Trixis*, as suggested by Turner (1993). However, these relationships were not recovered in previous phylogenetic analyses based on morphological data (Crisci, 1974; Sancho & al., 2014).

According to our nuclear DNA data, the infraspecific taxa of *P. cuneifolia* are all included in a well-supported clade in agreement with classifications of previous authors (Cabrera,

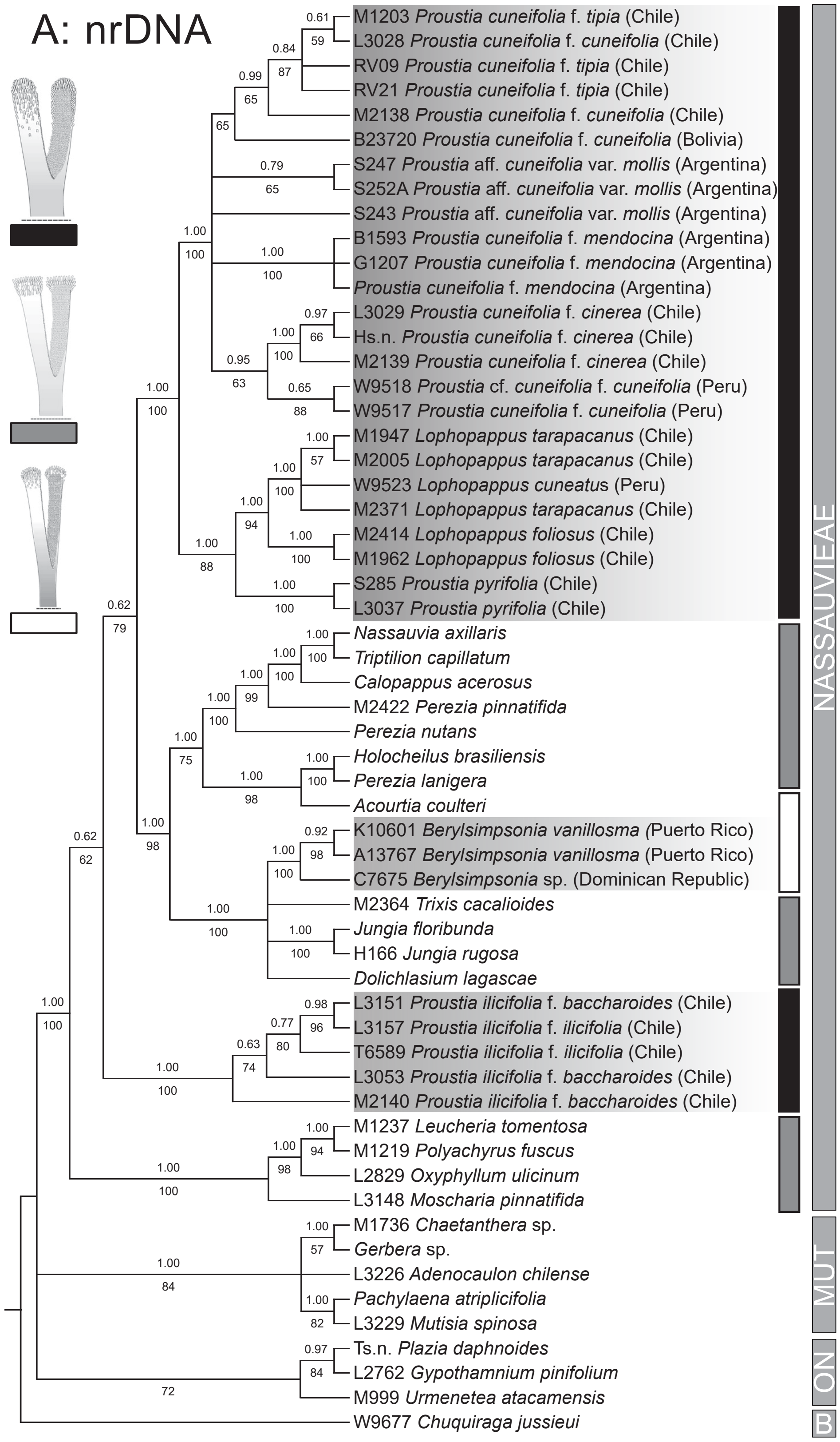
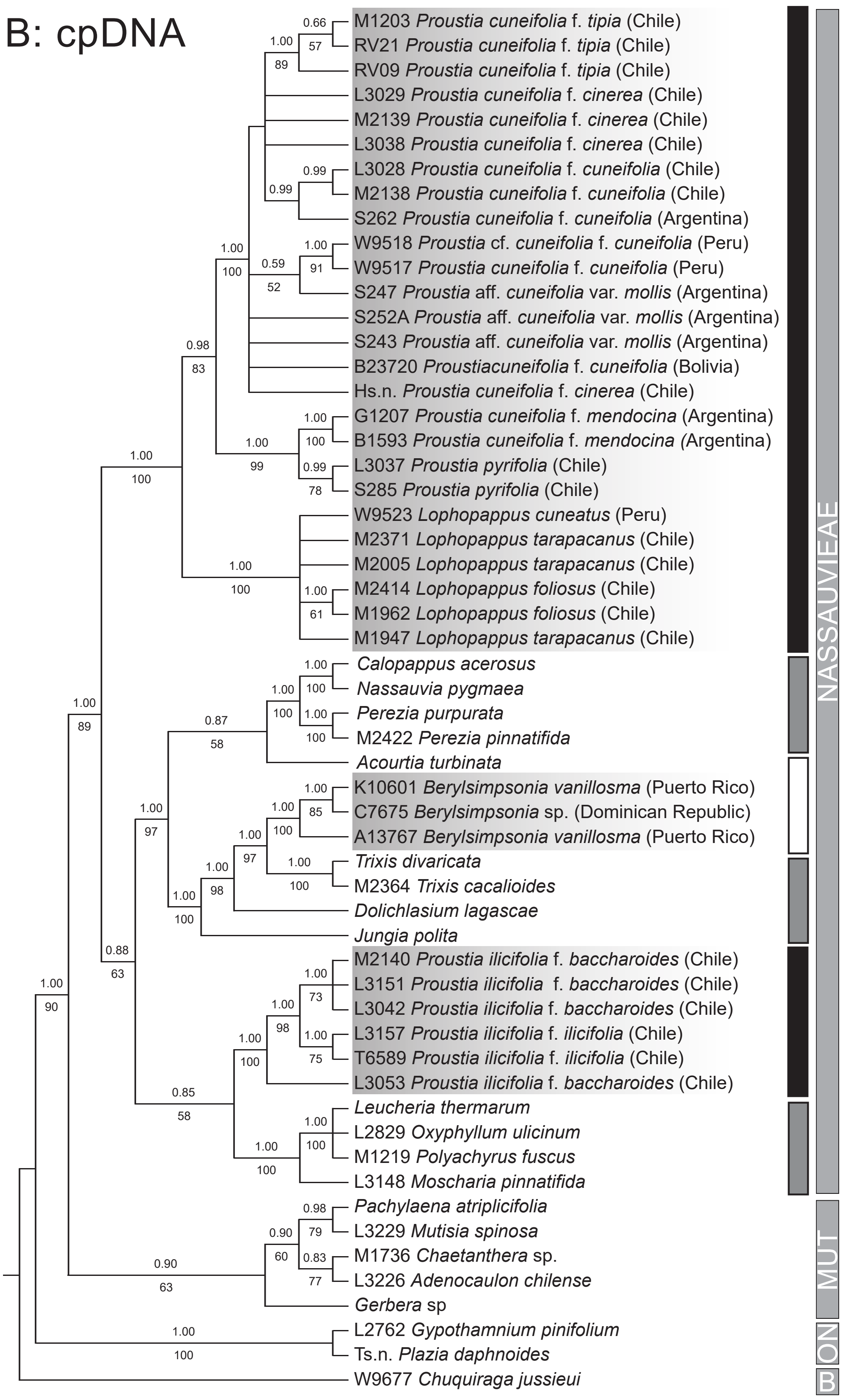


Fig. 3. ML trees obtained in the analyses with Bayesian posterior probabilities >0.5 above branches and ML bootstrap values >50 below branches. Nodes with <50% ML bootstrap support were collapsed to polytomies. Highlighted clades correspond to the genera *Proustia*, *Lophopappus* and *Berylsimpsonia*. Before taxon names of accessions sequenced in this study are the initial of the senior collector's last name and the collection number as indicated in Appendix 1. **A,** Tree obtained from nrDNA data; **B,** Tree obtained from plastid DNA data. — Major clades are indicated: ►



► MUT, Mutisieae; ON; Onoserideae; B, Barnadesioideae (outgroup). Drawings and bars besides taxon names represent the types of styles of Nassauvieae; black bar: style bilobed, branches relatively short, apically rounded and dorsally hairy in distal half, atypical of Nassauvieae; white bar: style bifid, with flattened and truncate branches, dorsally glabrous and crowned with a tuft of elongate collecting hairs, typical of Nassauvieae; grey bar: style bifid, branches relatively long, apically rounded and slightly expanded, dorsally hairy in distal fifth, atypical of Nassauvieae.

1953; Fabris, 1968). However, our plastid data suggest *P. pyrifolia* is sister to *P. cuneifolia* f. *mendocina*. Although a placement of *P. pyrifolia* as sister to *P. cuneifolia* cannot be rejected as a good explanation of our nuclear data (Fig. 4), a hybrid origin of *P. pyrifolia* as a cause for its discordant position in nuclear and plastid trees cannot be ruled out (Degnan & Rosenberg, 2009). This possibility should be further explored. However, the current distributions of *P. pyrifolia*, *P. cuneifolia* f. *mendocina* and *Lophopappus* do not overlap and no morphological characters known to us seem to support an hybrid origin.

Lophopappus is recovered as the most closely related taxon to *Proustia* (excl. *P. ilicifolia*) in all analyses, a result that confirms previous analyses with morphological (Crisci, 1974, 1980; Katinas, 1994) and molecular data (Panero & Funk, 2008; Luebert & al., 2009). These studies highlight that the most important morphological characters are those of the style and the corolla.

The unusual styles and corollas of *Proustia* in the context of Nassauvieae. — In Compositae, style morphology is not only crucial in the context of secondary pollen presentation mechanisms, but contributed important characters to tribal circumscription (Erbar & Leins, 2015; Katinas & al., 2016).

Typically defined as bifid, with flattened and truncate branches, dorsally glabrous and crowned with a tuft of elongate collecting hairs (style type 1; Crisci, 1974), the styles of Nassauvieae are distinctive for the tribe (Fig. 3). Indeed, a deeper analysis of stylar characteristics led Erbar & Leins (2015) to identify two sub-types of styles within the usually regarded as "typical styles" of Nassauvieae.

Some genera, however, depart from these typical characteristics. For instance, styles with relatively short branches, apically rounded and dorsally hairy in distal half are atypical for the tribe and are found only in *Proustia* (*P. cuneifolia*, *P. ilicifolia*, *P. pyrifolia*; Fig. 1F, K, O), *Cephalopappus* Nees & Mart., *Lophopappus* (Fig. 1X) and *Macrachaenium* (style type 2 according to Crisci, 1974; *Proustia* type according to Erbar & Leins, 2015). Another type of style with long, apically expanded branches, dorsally hairy on distal fifth, occurs in *Acourtia*, *Leunisia* Phil., *Berylsimpsonia* (Fig. 1S) and two species of *Perezia* (style type 3; Crisci, 1974). Neither *Leunisia* nor the two species of *Perezia* were included in our phylogenetic analyses.

This stylar morphological variability in Nassauvieae is unusual. Among the early-branching groups of Compositae, just Barnadesioideae and Nassauvieae have several style types (Erbar & Leins, 2015).

Although we did not include all genera of Nassauvieae in our analyses, some observations about the evolution of the styles in this tribe can be outlined. As indicated above, stylar similarities shared by *Berylsimpsonia* and *Acourtia* were highlighted by Crisci (1974). In fact, *Berylsimpsonia vanillosma* (previously *Proustia vanillosma*; Fabris, 1968) was first transferred to *Acourtia* because of its style and pollen characteristics (Crisci, 1974). However, our results show that these two genera are not closely related, and their similar style features appear to have evolved twice independently (Fig. 3).

The same can be said about the styles of *Proustia* and the other genera mentioned above with branches relatively short, apically rounded and dorsally hairy on distal half. However, our analyses show that *P. ilicifolia* is distantly related to the other species of *Proustia* and *Lophopappus* despite all having similar stylar characteristics (Fig. 3). In consequence, our results suggest multiple origins of atypical stylar features in the Nassauvieae (Fig. 3), in agreement with Sancho & al. (2014). The evolution of the unusual disk corollas of *Proustia* within Nassauvieae may have taken similar paths to those of style evolution. Disk corollas of Nassauvieae are typically bilabiate usually with a shallowly 3-lobed external lip and a deeply 2-lobed internal lip (Crisci, 1974; Katinas & al., 2008a) (Fig. 1D, I, N, R, V). As with styles, exceptions to the typical pattern

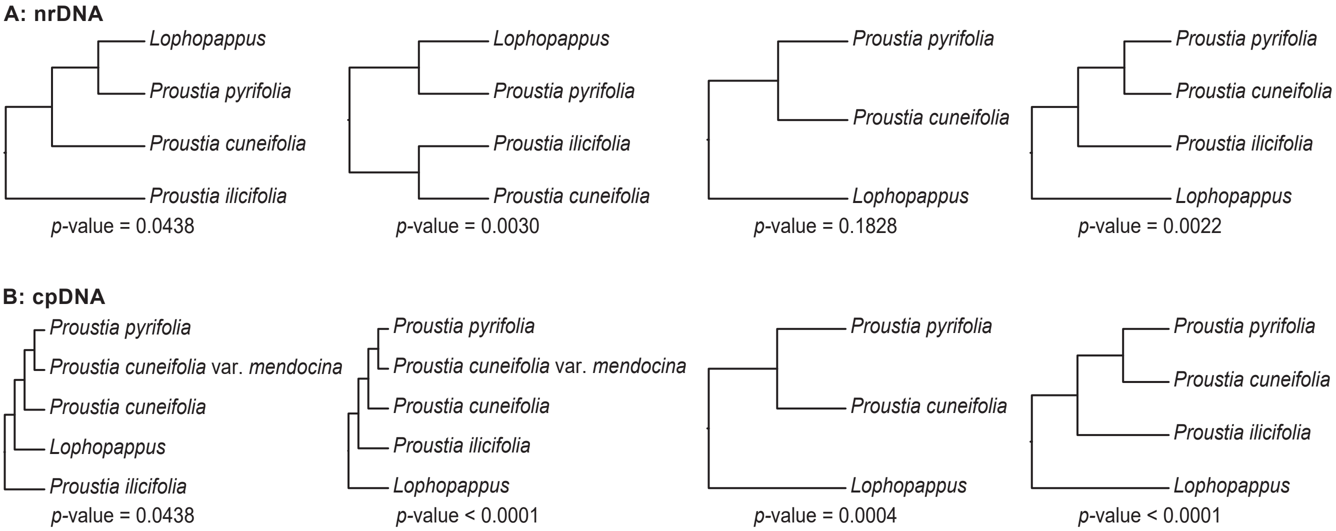


Fig. 4. Results obtained from the topology tests. Shown are simplified alternative topologies tested against nrDNA data **(A)** and plastid DNA data **(B)**, with the respective *p-values* of the AU-test below.

of corollas are found in *Proustia*, *Lophopappus* and *Acourtia*. Deeply 5-lobed disk corollas (instead of bilabiate ones) are present in *Acourtia collina* (S.Watson) Crisci or in some specimens of *Lophopappus peruvianus*, *L. tarapacanus* (Phil.) Cabrera (Fig. 1W), *Proustia pyrifolia* and *P. cuneifolia* (Fig. 1E) (Fabris, 1968; Crisci, 1974; Katinas & al., 2013; Sancho & al., 2014). In *Proustia* and *Lophopappus*, these unusual corollas are not always symmetrically 5-lobed, with all the lobes similar in length, but irregularly 5-lobed, showing different stages and forming a continuum between bilabiate and 5-lobed corollas (Crisci, 1974; Katinas & al., 2013; Sancho & al. 2014) (Fig. 1J, W).

Deeply 5-lobed corollas may also appear in the other tribes of Mutisioideae, for example in *Adenocaulon* and *Eriachaenium* Sch.Bip. of Mutisieae (Funk & al., 2016) and in *Plazia*, *Gypothamnium* and *Aphyllocladus* Wedd. of Onoserideae (Katinas & al., 2008a). It is widely accepted that the ancestral corolla in Compositae was 5-lobed (Bremer, 1994; Stuessy & al., 1996; Stuessy & Urtubey, 2006). Stuessy & Urtubey (2006) proposed that 5-lobed corollas of Barnadesioideae evolved in double split corollas (subbilabiate), from which bilabiate ones originated. If this evolutionary model is accepted, the irregularly 5-lobed corollas found in *Proustia* and *Lophopappus* would represent intermediate states between bilabiate and actinomorphic corollas.

Re-definition of *Proustia*. — According to our results, a re-definition of *Proustia* from its present concept is needed in order to render monophyletic taxa. In the treatment presented below, we have also thoroughly considered the criterion of ease of morphological recognition of the taxa and, at the same time, attempted to maximize nomenclatural stability (Backlund & Bremer, 1998; Vences & al., 2013).

The Caribbean *Berylsimpsonia* is confirmed as distantly related to *Proustia* within the Nassauvieae. The infrapetiolar spines of *Berylsimpsonia vanillosma* are not homologous with those of *Proustia pyrifolia* (Table 1; Fig. 2D). This is an important outcome since spiny structures (Fig. 2B, D) were interpreted as diagnostic features of *Proustia* (Fabris, 1968). Our phylogenetic analyses confirm the results of Sancho & al. (2014), who regarded the spiny structures of *Proustia* as having multiple origins. These authors also suggested different ecological roles for these structures in *Proustia*. *Berylsimpsonia* has been recognized at generic level in all major recent taxonomic treatments (e.g., Hind, 2007; Katinas & al., 2008a), a criterion maintained here. Our results indicate that *Proustia ilicifolia* does not form a monophyletic group with the other two species of the genus and is distantly related to them. This also agrees with the morphological analysis of Sancho & al. (2014), who pointed out that spiny structure type, habit and type of capitulescences (Table 1; Fig. 1G, L, P) support differentiation of each species of *Proustia*. We therefore propose the segregation of *P. ilicifolia* to a new genus (see below). The remaining species of *Proustia*, *P. cuneifolia* and *P. pyrifolia* (hereafter *Proustia* s.str.) were recovered in a well-supported clade together with *Lophopappus*, with *P. pyrifolia* sister to *Lophopappus* in the analysis with nuclear data, but nested in *P. cuneifolia* in the analysis with plastid data.

Once *P. ilicifolia* is segregated to a new genus, several possibilities can be envisioned in order to taxonomically account for the other phylogenetic results: (1) to lump *Lophopappus* in *Proustia* s.str. as was proposed by Ferreyra (1995), an option agreeing with both nuclear and plastid data; (2) to segregate *Proustia* to its type *P. pyrifolia*, include *Lophopappus* in *Proustia*, and create a new genus for *P. cuneifolia*, which is in agreement with our nuclear data, but not with the plastid data; (3) to consider three independent genera: *Lophopappus*, the monospecific *Proustia* (with solely *P. pyrifolia*), and a new genus for *P. cuneifolia*; (4) to consider *Lophopappus* as an independent genus, sister to a reduced *Proustia* (*Proustia* s.str.) which would include *P. pyrifolia* and *P. cuneifolia*, as supported only by our plastid data.

From a morphological point of view, *P. cuneifolia*, *P. pyrifolia* and *Lophopappus* share important features, such as similar styles (atypical within Nassauvieae) and transitional corollas between actinomorphic to bilabiate (Fig. 1), which, as previously pointed out, have evolved more than once in the tribe. With respect to pollen features, which have been regarded as important in defining genera within Nassauvieae (Crisci, 1974), it is not possible to establish a sharp differentiation among these taxa. Indeed, *P. cuneifolia* and *P. pyrifolia* have *Trixis* exine type whereas some species of *Lophopappus* have *Proustia* exine type and some others *Trixis* exine type.

The solitary or few capitula (2–4) of *Lophopappus* (Fig. 1Y), however, contrast with the capitulescences of numerous capitula in *P. cuneifolia* and *P. pyrifolia* (Figs. 1G, P, 2A, C). Some differences concerning leaf venation can also be stressed (Hickey, 1979). The species of *Lophopappus* have a typical acrodromous (i.e., with two or more primary or strongly developed secondary veins toward the leaf apex; Fig. 1U) or actinodromous venation (i.e., three or more primary veins diverging radially from a single point; Katinas & al., 2013) whereas in *P. pyrifolia* and *P. cuneifolia* dominates pinnate venation (i.e., with a single primary vein serving as the origin for the higher order venation; Fig. 1A–C, H, M).

In summary, morphological data are consistent with the segregation of *Proustia* and *Lophopappus* as separate genera, but not with the segregation of *P. cuneifolia* as a new generic entity. The only study known to us in which *Lophopappus* and *Proustia* have been reunited is the treatment of Ferreyra (1995) for the “Flora of Peru”. This option has the advantage of being consistent with the phylogenetic trees obtained from both nuclear and plastid data. Conversely, these genera have been considered as separate units in all recent taxonomic treatments (Fabris, 1968; Cabrera, 1977; Hind, 2007; Katinas & al., 2008a, 2013), floristic checklists (e.g., Zuloaga & al., 2008; Moreira & al., 2012) and phylogenetic studies (e.g., Katinas & al., 2008b; Panero & Funk, 2008; Luebert & al., 2009; Simpson & al., 2009; Panero & al., 2014), but is only consistent with our phylogenetic results derived from plastid data. However, our topology test failed to reject *Lophopappus* and *Proustia* as reciprocally monophyletic with the nuclear data (Fig. 4). Given the topological uncertainty of the phylogenetic analyses in this region of the tree, we take a morphologically consistent and nomenclaturally conservative approach and do not modify the circumscription of *Lophopappus* and *Proustia* (other than segregating *P. ilicifolia*) thus following the recent taxonomic literature.

In agreement with our arguments presented above, we recognize two species in *Proustia*, namely *P. cuneifolia* and *P. pyrifolia* (the type). *Proustia cuneifolia* f. *mendocina* varied its placement in the phylogenetic trees (it was recovered sister to *P. pyrifolia* in the plastid tree and nested within *P. cuneifolia* in the nuclear tree). From all the subordinate taxa of *P. cuneifolia* the form *mendocina* is the most distinctive by its spinose-dentate leaf blades (Fig. 1B). However, *P. cuneifolia* f. *mendocina* shares important morphological features with the other subordinate taxa of *P. cuneifolia*, especially those concerning to the thorny capitulescense, a very distinguishing feature of this species. Furthermore, *P. cuneifolia* f. *mendocina* has a marginal geographical distribution within *P. cuneifolia* (Fig. 5). In the light of this evidence, we propose to maintain *P. cuneifoli*a. f. *mendocin*a as a subordinate taxon of *P. cuneifolia*, although assigning it a new rank of subspecies. For the same reasons, we also propose to raise *P. cuneifolia* f. *cinerea*, *P. cuneifolia* var. *mollis* and *P. cuneifolia* f. *tipia*, to the rank of subspecies. All these taxa are geographical and morphological variants within *P. cuneifolia*. With respect to the other genera once related to *Proustia*, the taxonomy of *Lophopappus* has been fully revised by Katinas & al. (2012) and a treatment of *Berylsimpsonia* has been provided by Turner (1993).

■ TAXONOMIC TREATMENT

I. ***Proustia*** Lag., Amen. Nat. Españ. 1: 33. 1811 – Type: *P. pyrifolia* DC.

A South American genus comprising two species, *P. cuneifolia* and *P. pyrifolia*. Distinguishing characters of these species are provided in Table 1. The morphology of *Proustia* was thoroughly described by Fabris (1968) and Sancho & al. (2014).

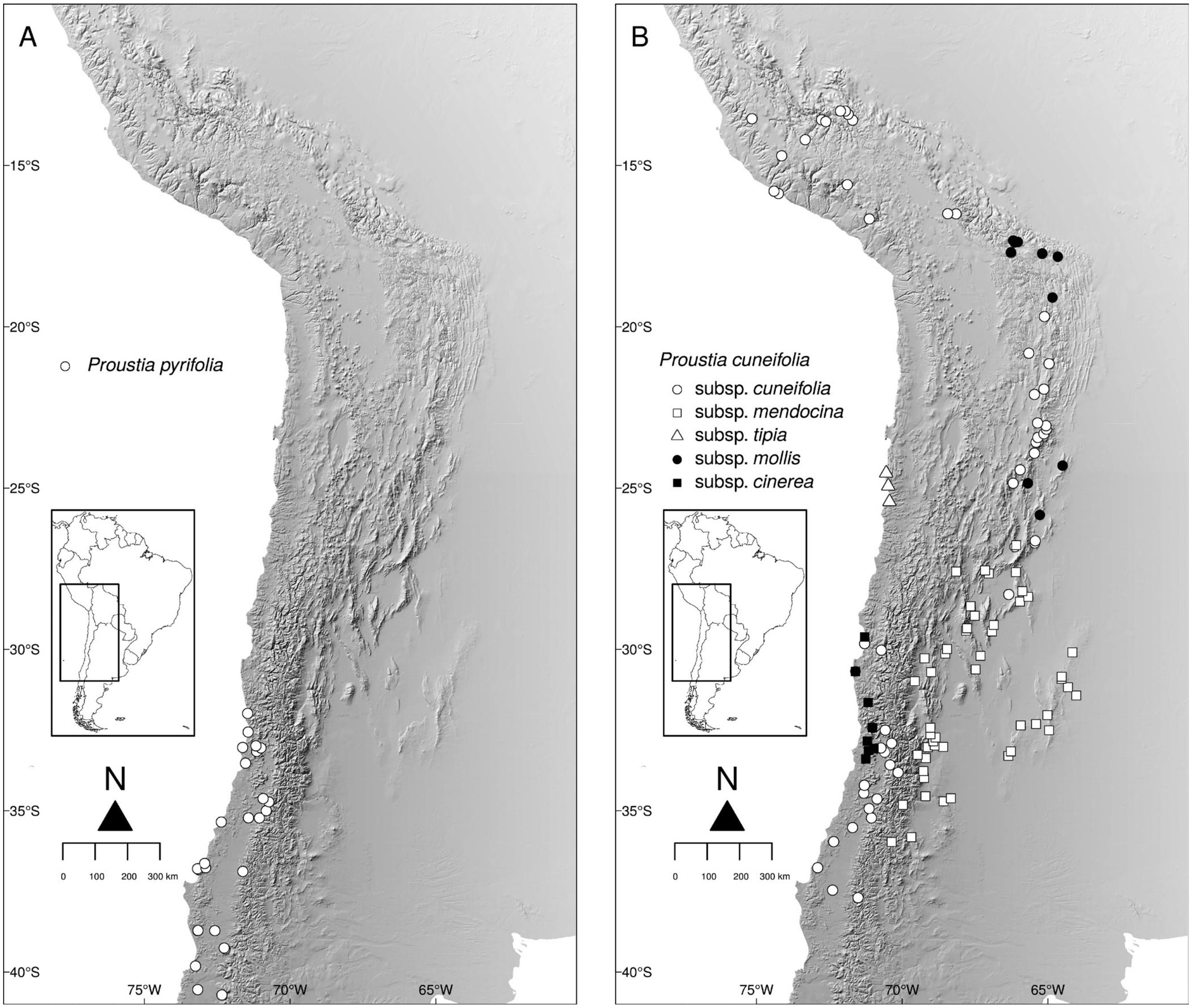


Fig. 5. Distribution map of *Proustia*. **A,** *P. pyrifolia*; **B,** *P. cuneifolia*.

We follow Fabris (1968) regarding the synonymy of most species. Therefore, we only provide the basionyms of each taxon and the synonymy changes proposed herein. In order to hold a nomenclaturally conservative approach, we maintain two of the sections included by Fabris (1968) for the treatment of *Proustia*: sect. *Proustia* (including the type, *P. pyrifolia*) and sect. *Harmodia* D.Don (including *P. cuneifolia*) (Table 1).

Ia. ***Proustia*** sect. ***Proustia***

1. ***Proustia pyrifolia*** DC. in Ann. Mus. Natl. Hist. Nat. 19: 70, pl. 4. 1812 – Holotype: CHILE. "Prope Talcahuano", without collector, *s.n.* (herb. Lagasca y Segura) (G-DC barcode G00318288, photo!).

= *Proustia pyrifolia* f. *glandulosa* (DC.) Fabris in Revista Mus. La Plata, Secc. Bot. 11: 30. 1968 ≡ *P. glandulosa* DC., Prodr. 7: 27. 1838 – Holotype: CHILE. "Valparaiso", *Gaudichaud 145* (G-DC barcode G00318276, photo!; isotypes: G-DC barcode G00358907, photo!, P barcode P03733626, photo!; possible isotype: *Gaudichaud 147*, P barcode P00724943, photo!).

Distribution. – *Proustia pyrifolia* is endemic to Chile (Fig. 5A). This species of vines inhabits sclerophyllous Mediterranean and lauriphyllous temperate forests.

Ib. ***Proustia*** sect. ***Harmodia*** D.Don in Trans. Linn. Soc. London 16: 202. 1830 – Type: *Proustia cuneifolia* D.Don

1. ***Proustia cuneifolia*** D.Don in Trans. Linn. Soc. London 16: 202. 1830 – Holotype: CHILE. Coquimbo, *Caldcleugh s.n.*, ex herb. Lambert (G?, fide Miller, 1970).

Distribution. – *Proustia cuneifolia* ranges from Peru to Central Chile and Argentina (Fig. 5B). This species of shrubs with thorny capitulescences inhabits sclerophyllous forests, thickets and desert scrub.

Key to subspecies of *Proustia cuneifolia*

1\. Leaf blade margins entire, denticulate or spinose-dentate ………… 2
1\. Leaf blade margins strongly spinose .. subsp. ***mendocina***
2\. Leaf blade oblong, elliptic, narrowly elliptic or narrowly obovate ………… 3
2\. Leaf blade widely elliptic to orbiculate …… subsp. ***tipia***
3\. Leaves glabrous or puberulent abaxially ………… subsp. ***cuneifolia***
3\. Leaves tomentose abaxially ………… 4
4\. Leaves papery; central Chile ………… subsp. ***cinerea***
4\. Leaves coriaceous; Bolivia and NW Argentina ………… subsp. ***mollis***

1a. ***Proustia cuneifolia*** subsp. ***cinerea*** (Phil.) Luebert, **stat. nov.** ≡ *P. cinerea* Phil. in Linnea 29: 109. 1858 ≡ *P. cuneifolia* f. *cinerea* (Phil.) Fabris in Revista Mus. La Plata, Secc. Bot. 11: 48. 1968 – **Lectotype (designated here):** CHILE. "In colli S. Cristóval, urbi Santiago proximo", *Philippi s.n.* (SGO No. 44594, photo!; isotypes: B destroyed, F photo neg. 15903!, HAL barcode HAL0113112, photo!, NY barcode 00232662, photo!, P barcode P00732724, photo!, S No. S-R-5277!, SGO No. 60865, photo!; possible isotype: K barcode K000504353, photo!).

1b. ***Proustia cuneifolia*** D.Don subsp. ***cuneifolia***

= *Proustia pungens* var. *oblongifolia* Wedd., Chlor. Andina 1: 23. 1855 ≡ *P. cuneifolia* f. *oblongifolia* (Wedd.) Fabris in Revista Mus. La Plata, Secc. Bot. 11: 43. 1968 – **Lectotype (designated here):** BOLIVIA: "1839", *Pentland 29* (P barcode P03733601, photo!; isolectotype: P barcode P03733604, photo!).

Nomenclatural notes. – The isolectotype of *Proustia pungens* β *oblongifolia* Wedd. at P, barcode P03733604, includes the following information: "Valleés à l'E de la Paz. alt. 12 à 8000 pds angl." In the original publication it is not clearly stated which specimens are assigned to the variety *oblongifolia.* In addition, none of the specimens at P were annotated by Weddell. Then, the lectotype was chosen among those specimens cited in the protologue on the basis of Weddell's description.

1c. ***Proustia cuneifolia*** subsp. ***mendocina*** (Phil.) Katinas, **stat. nov.** ≡ *P. mendocina* Phil. in Anales Univ. Chile 36: 176. 1870 ≡ *P. cuneifolia* f. *mendocina* (Phil.) Fabris in Revista Mus. La Plata, Secc. Bot. 11: 44. 1968 ≡ *P. cuneifolia* var. *mendocina* (Phil.) Ariza in Darwiniana 33: 371. 1995 – **Lectotype (designated here):** ARGENTINA. Mendoza, *Philippi s.n.* (SGO No. 60866, photo!; isotypes: CORD barcode CORD00004731, photo!, GOET barcode GOET001921, photo!, SGO No. 60867, photo!).

1d. ***Proustia cuneifolia*** subsp. ***mollis*** (Kuntze) G.Sancho, **stat. nov.** ≡ *P. angustifolia* var. *mollis* Kuntze, Revis. Gen. Pl. 3(3): 168. 1898 ≡ *P. cuneifolia* var. *mollis* (Kuntze) Cabrera, Fl. Jujuy 10: 615. 1978 – Holotype: BOLIVIA. "Bei Cochabamba, 26 Mar 1892, 3000 m", *Kuntze s.n.* (NY barcode 00232661, photo!; isotype: US barcode 00119620, photo!).

= *Proustia cuneifolia* f. *angustifolia* (Wedd.) Fabris in Revista Mus. La Plata, Secc. Bot. 11: 48. 1968 ≡ *P. angustifolia* Wedd., Chlor. Andina 1: 24. 1855 – Holotype: BOLIVIA. Chuquisaca, "sommet de la côte de Cachimayo", *d'Orbigny 1271* (P barcode P00732723, photo!).

Remarks. – The morphology of the type of *P. angustifolia* var. *mollis* clearly coincides with that of the type of *P. angustifolia*. However, the concept of *Proustia angustifolia* var. *mollis* sensu Cabrera (1978) disagrees with Kuntze's in that the specimens from Jujuy and Salta are unarmed (vs. capitulescence strongly thorny in Kuntze's type specimen). The specimens of *P. cuneifolia* var. *mollis* included in our analyses are unarmed and were collected in Salta and Jujuy. We preliminary consider them as *P. cuneifolia* var. *mollis* until the status of these individuals is clarified.

1e. ***Proustia cuneifolia*** subsp. ***tipia*** (Phil.) Luebert, **stat. nov.** ≡ *P. tipia* Phil., Fl. Atacam.: 28. 1860 ≡ *P. cuneifolia* f. *tipia* (Phil.) Fabris in Revista Mus. La Plata, Secc. Bot. 11: 47.

1968 – Holotype: CHILE. Atacama, “ad Hueso Parado et Paposo”, *Philippi s.n.* (SGO No. 60869!).

II. ***Spinoliva*** G.Sancho, Luebert & Katinas, **gen. nov.** – Type: *S. ilicifolia* (Hook. & Arn.) G.Sancho (≡ *Proustia ilicifolia* Hook. & Arn.).

Description. – Shrubs or small trees, young branches unarmed, subglabrous, glandular with minute glands, older branches glabrous. Leaves alternate, sessile, blades obovate to elliptic, coriaceous, margin spiny, glabrous or tomentose below. Capitula in thyrses, those of secondary axes in spiciform arrangements; capitula sessile, homogamous, discoid. Involucre cylindrical, phyllaries 4-seriate, imbricate, coriaceous. Receptacle epaleate, flat or convex, fimbrillate to setiferous. Florets (2)3–5, isomorphic, bisexual, corolla white to pinkish, bilabiate, outer lip distinctly 3-lobed, inner lip deeply regularly or irregularly divided. Anthers caudate, apical appendages acute. Style bilobed, lobes apically rounded, externally hairy on distal half, hair apices more or less rounded, internally completely covered by stigmatic papillae, base swollen, surrounded by a nectariferous ring. Cypselae nearly prismatic, subglabrous near base, sericeous with twin hairs distally, 4-ribbed. Pappus of 2 series of white, semi-caducous bristles, flat and scabrid at base, apex somewhat barbellate-plumose, bristles of outer series slightly shorter and thinner.

Etymology. – The name refers to the Latin word *spina* because of the spiny leaf margins and the local name “olivillo”, given to this plant by its leaves similar to those of olive tree (*Olea europea* L.).

Distribution. – *Spinoliva* is endemic to Central Chile.

Species. – *Spinoliva* is a monospecific genus with the single species *S. ilicifolia* and two subspecies.

1. ***Spinoliva ilicifolia*** (Hook. & Arn.) G.Sancho, **comb. nov.** ≡ *Proustia ilicifolia* Hook & Arn., Bot. Beechey Voy.: 28. 1830 (“1841”) ≡ *P. pungens* var. *ilicifolia* (Hook. & Arn.) DC., Prodr. 7: 28. 1838 – **Neotype (designated here):** CHILE. Coquimbo, Prov. Elqui, “road from Huanta to

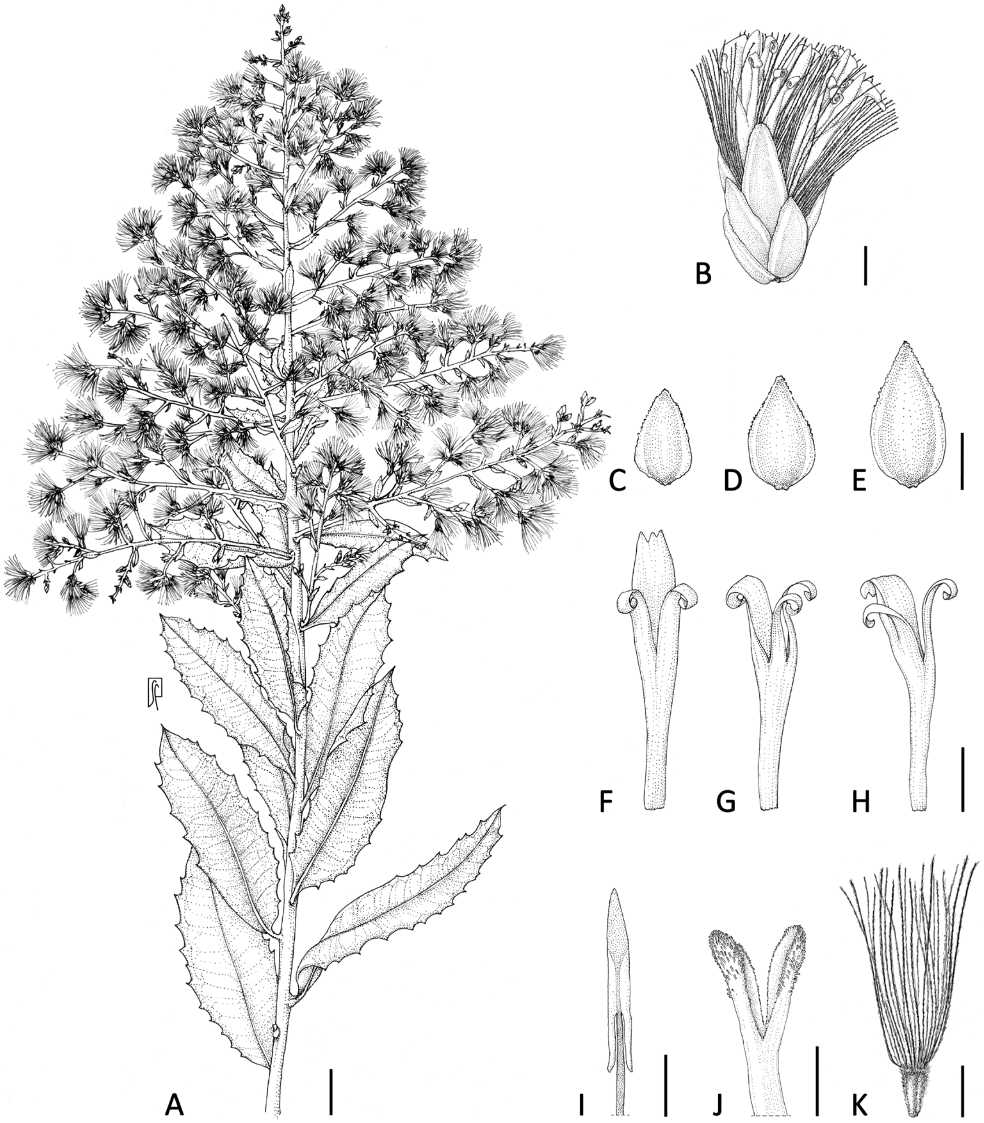


Fig. 6. *Spinoliva ilicifolia.* **A,** General aspect; **B,** Capitulum; **C,** Outter phyllary; **D,** Middle phyllary; **E,** Inner phyllary; **F,** Bilabiate corolla, ventral view; **G,** Bilabiate corolla, lateral view; **H,** Sub-bilabiate corolla showing asymmetrical lobes; **I,** Stamen; **J,** Style; **K,** Cypsela with pappus. — Scales: A = 1 cm; B–H & K = 1 mm; I = 0.5 mm; J = 0.25 mm. Drawn from Chile, Coquimbo, Paihuano, 5 Feb 1883, without collector *s.n.* (LP). Drawn by Samanta Faiad.

Juntas del Toro, 29.96540° S, 70.13243° W, 1950 m, 1 April 2017", *Luebert & Böhnert 3717* (SGO No. 168351!; isoneotypes: B barcode B 100680765!, BONN!, CONC No. 184772!, E barcode E00822517!, EIF No. 12270!, LP barcode LP000172!).

= *Proustia reticulata* Phil. in Anales Univ. Chile 18: 50. 1861, nom. illeg., non Don 1830 ≡ *Acourtia reticulata* (Lag. ex D.Don) Reveal & R.M.King in Phytologia 27(4): 231. 1973 ≡ *Proustia olivillo* Phil. in Anales Univ. Chile 85: 838. 1894 (replacement name for *P. reticulata* Phil.) – Holotype: CHILE. Atacama, "Agua de Antibianco en 2.400 metros de elevación", *Volckmann s.n.* (SGO n.v.).

Description. – Shrubs or small trees, up to 4 m tall, young branches unarmed, somewhat striate, subglabrous, glandular with minute glands, older branches glabrous. Leaves alternate, sessile, blades 4–7.5 × 1–2.8 cm, obovate to elliptic, coriaceous, venation camptodromous, glandular, glabrous or tightly, white tomentose beneath, margins spiny. Capitula in thyrses, those of secondary axes in spiciform arrangements; capitula sessile, homogamous, discoid. Involucre 2.5–4 mm high, cylindrical, phyllaries 4-seriate, imbricate, inner progressively longer, outermost 0.8–1.8 × 0.7–1 mm, innermost 2.8–3.4 × 1.1–1.8 mm, coriaceous, commonly centrally shiny and thicker, margins scarious, glandular. Receptacle epaleate, flat or convex, fimbrillate to setiferous. Florets (2)3–5, isomorphic, bisexual, corolla 4.6–5.5 mm long, bilabiate, outer lip ca. 2.5 mm long, hairy with unicellular acute trichomes, distinctly 3-lobed, lobes ca. 1 mm long, regularly or rarely irregularly incised, inner lip deeply divided, lobes 2.5–3 mm long, coiled. Anthers 2.3–2.5 mm long, caudate, tails 0.5–0.8 mm long, glabrous or with a few projections, apical appendages acute. Style bilobed, branches ca. 0.5 mm long, apex rounded, externally hairy on distal half, internally completely covered by stigmatic papillae, base swollen surrounded by a nectariferous ring with many stomata. Cypselae ca. 1.4 mm long, nearly prismatic, subglabrous near base, sericeous distally with twin hairs, 4-ribbed, short carpopodium at base. Pappus of 2 series of white, semi-caducous bristles, 3.7–4.2 mm long, flat and scabrid at base, apex somewhat barbellate-plumose, bristles of outer series slightly shorter and thinner. Figs. 1H–L, 2E, F, 6, 7A.

Pollen. – The pollen grains are radially symmetrical and isopolar; subprolate in equatorial view, circular in polar view. The polar diameter is 28–33 µm and the equatorial diameter 19–29 µm. The grains are tricolporate, and the colpi long with a microgranulate membrane. The endoaperture is lalongate. As a general description, the exine is tectate, microechinate and 2–4.5 µm thick. Two types of exine structure are found: *Trixis* type (ectosexine thinner than endosexine, both sublayers columellate) or *Proustia* type (ectosexine of same thickness as endosexine, both sublayers columellate). The sexine is about 2 or 3 times as thick as nexine. The internal tectum is parallel to the nexine. The nexine is thickened towards the apertures forming costae (Tellería & al., 2003).

Leaf anatomy. – The blades of *Spinoliva ilicifolia* are covered by scattered glands (vesicular filiform trichome; Ramayya, 1962) and oblique-septate-flagellate trichomes. The blades are amphistomatic. In cross section, the blades show a thick cuticle and a single-layered epidermis of polygonal cells, with straight tangential walls. The mesophyll is dorsiventral with one palisade layer and spongy tissue. Two or more, commonly three, vascular bundles are found at the midrib level. The vascular bundles are completely encircled by sclerenchyma. Below the vascular bundles, inflated cells with refractive content are arranged as if they have secretory products.

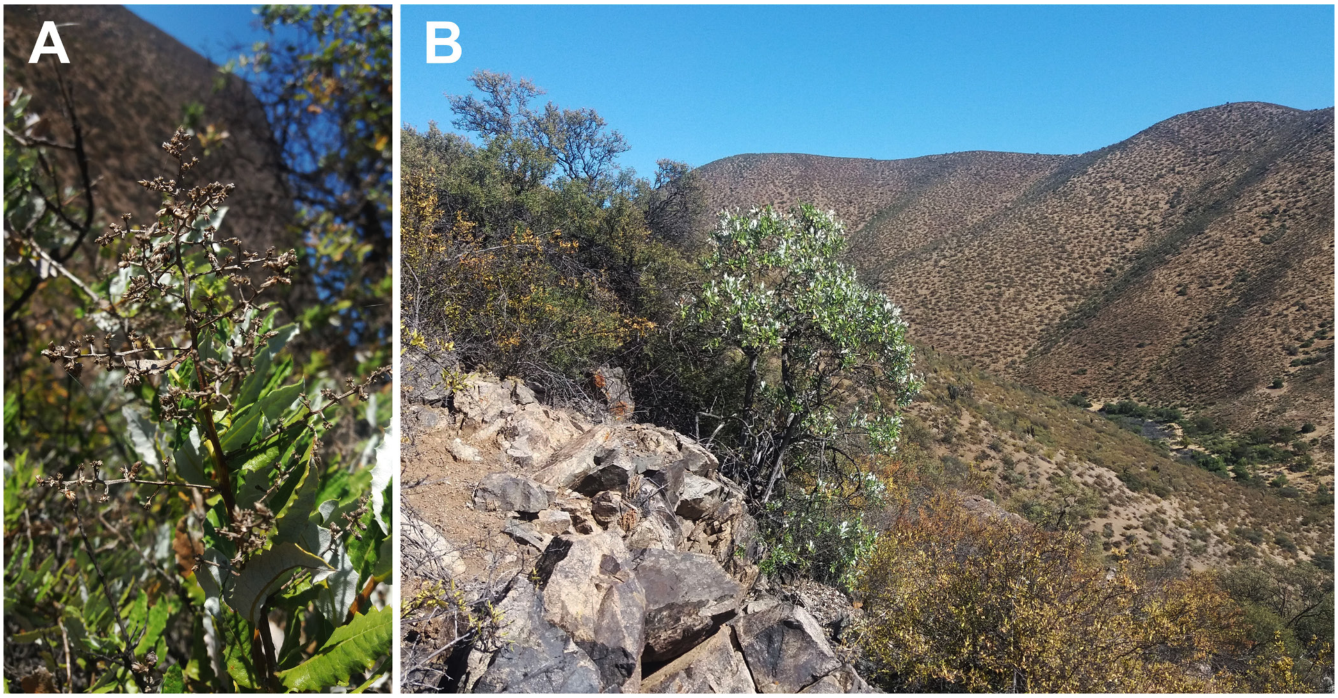


Fig. 7. *Spinoliva ilicifolia.* **A,** Detail of branch; **B,** Specimen in native hábitat. — Photos: G. Sancho.

Distribution and habitat. – *Spinoliva ilicifolia* is endemic to Chile between the Regions of Atacama and Santiago (Fig. 8). This species inhabits desert, thorny and sclerophyllous Mediterranean scrubs dominated by species such as *Adesmia argentea* Meyen and *Bulnesia chilensis* Gay between 300 to 1800 m or *Flourensia thurifera* (Molina) DC. and *Colliguaja odorifera* Molina between 300 and 2300 m (Luebert & Pliscoff, 2006) (Fig. 7B).

Nomenclatural notes. – Hooker & Arnott (1830: 28) described *Proustia ilicifolia* based on a specimen from Coquimbo, Chile. This specimen was collected during the expedition commanded by Captain Beechey, probably in May 1828 (“Hab. Coquimbo”). We failed to find original material at E and K. Indeed, Noltie (2010) indicated that no original material can be assigned to this species in E or K. Fabris (1968), on the other hand, did not cite type material for this species. A neotype is selected above to preserve the usage established by previous applications of the name in agreement to the Art. 9.16 (McNeill & al., 2012). The specimen selected here was collected in Coquimbo, the same area where the specimen designated by Hooker and Arnott was originally gathered.

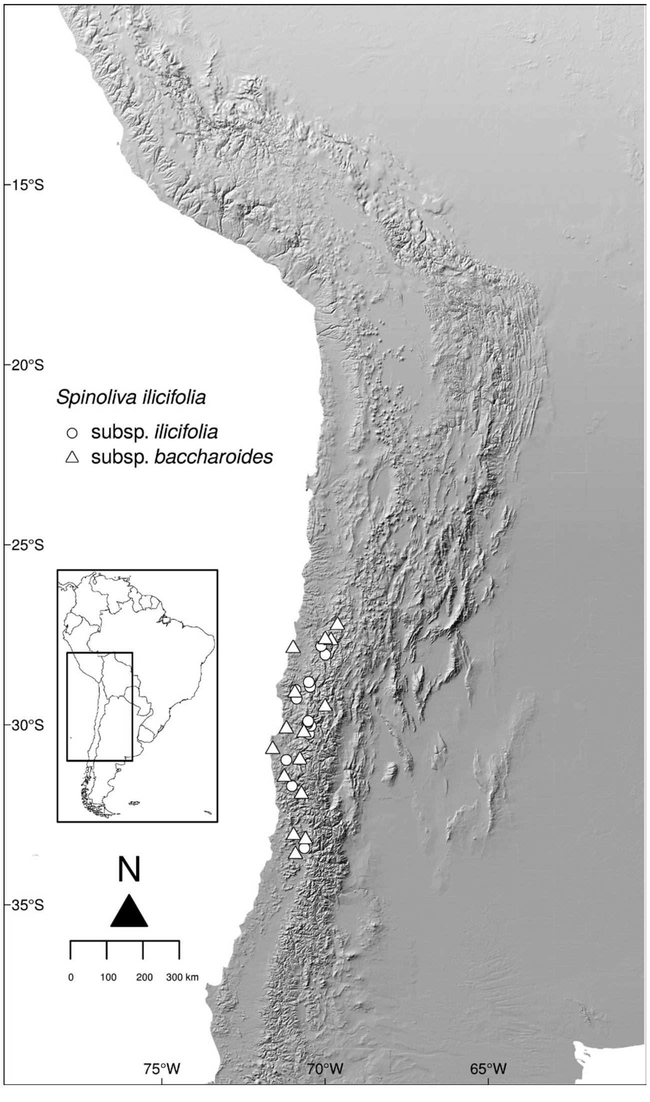


Fig. 8. Distribution map of *Spinoliva ilicifolia*.

Key to subspecies of *Spinoliva ilicifolia*

1\. Leaf blades glabrous on both surfaces .... subsp. ***ilicifolia***

1\. Leaf blades tightly, white-tomentose beneath .... subsp. ***baccharoides***

1a. ***Spinoliva ilicifolia*** subsp. ***ilicifolia***

1b. ***Spinoliva ilicifolia*** subsp. ***baccharoides*** (D.Don ex Hook. & Arn.) G.Sancho, **comb. & stat. nov.** ≡ *Proustia baccharoides* D.Don ex Hook. & Arn. in Compan. Bot. Mag. 1: 106. 1835 ≡ *P. ilicifolia* f. *baccharoides* (D.Don. ex Hook. & Arn.) Fabris in Revista Mus. La Plata, Secc. Bot. 11: 37. 1968 – Holotype: CHILE. “Coquimbo”, without collector, *s.n.* (K barcode K001092242!).

Nomenclatural notes. – Fabris (1968: 37, 38) designated the specimen collected in Coquimbo by Macrae in 1825 (at K) as the lectotype of *Proustia baccharoides*: K (K000504352 ex Herb. Benthamianum!). However, there is another specimen at K (K001092242 ex Herb. Hookerianum) annotated by Hooker indicating: “Coquimbo, *Proustia*”. This specimen was labeled by D.J.N. Hind as “Holotype” of *P. baccharoides*. With this evidence, we consider this last specimen the holotype of *P. baccharoides*. In this new frame, Fabris’s lectotype should be superseded as indicated by Art. 9.19 (McNeill & al., 2012).

■ AUTHOR CONTRIBUTIONS

GS, LK and FL designed the study; all authors contributed materials; GS, JNVB and FL conducted analyses; GS, LK and FL prepared the taxonomic treatment; GS and FL led the writing. All authors approved the final version of the manuscript. — FL, https://orcid.org/0000-0003-2251-4056; GS, sancho@fcnym.unlp.edu.ar

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Appendix 1. Voucher information and GenBank accession numbers for taxa used in the present study.

Species, country, voucher specimen and/or reference and GenBank accession numbers for ETS, ITS, *trnK-matK*, *ndhF*, *trnL-rpl*32, *trnL-trnF*, respectively. Newly generated sequences are indicated by an asterisk (*) and missing sequences are indicated by a dash (–).

Acourtia coulteri (A.Gray) Reveal & R.M.King: Mexico, Tamaulipas, *Iltis 30748* (TEX), Simpson & al. (2009), –, FJ979680, –, –, –, –. ***Acourtia turbinata*** (La Llave & Lex.) DC.: Mexico, *Panero 2891* (TEX), Panero & Funk (2008), –, –, EU385317, EU385126, –, EU385032. ***Adenocaulon chilense*** Less.: Chile, Reg. Aysén, *Luebert & Daniłowicz-Luebert 3226* (BONN), MG553831*, MG553794*, MG553765*, MG553685*, MG553876*, MG553725*. ***Berylsimpsonia* sp.:** República Dominicana, P. Escondido, *Clase 7675* (B), MG553825*, MG553791*, MG553759*, MG553679*, MG553870*, MG553719*. ***Berylsimpsonia vanillosma*** (C.Wright) B.L.Turner: Puerto Rico, Adjuntas, *Axelrod & Turquist 13767* (UPRRP), –, MG553775*, –, MG553663*, MG553852*, MG553703*; Puerto Rico, *King & Proctor 10601* (US), –, MG553774*, MG553736*, –, –, MG553696*. ***Calopappus acerosus*** Meyen: Chile, Los Andes, *Panero & Crozier 8457* (TEX), Panero & al. (2014), –, FJ979685, KM192112, KM192101, FJ979735, KM191902. ***Chaetanthera* sp.:** Chile, Reg. Atacama, *Moreira 1736* (SGO), MG553835*, MG553798*, MG553768*, MG553688*, MG553880*, MG553729*. ***Chuquiraga jussieui*** J.F.Gmel.: Peru, Lambayeque, *Weigend & al. 9677* (BONN), MG553840*, MG553801*, MG553773*, MG553693*, MG553885*, KY458442. ***Dolichlasium lagascae*** D.Don: Argentina, Mendoza, *Bonifacino 1635* (LP), *Simon 811* (US), Panero & Funk (2008), Katinas & al. (2008), –, EF530259, EU385347, EU385155, –, EU385062. ***Gerbera* sp.:** *Vincent 13223* (MU), Pelser & al. (2010), GU818171, GU818551, GU817629+GU817463, GU817862, –, GU817955+GU817941. ***Gypothamnium pinifolium*** Phil.: Chile, Reg. Antofagasta, *Luebert & García 2762/1156* (SGO), –, EU729342, MG553744*, EU729346, MG553853*, EU729338. ***Holocheilus brasiliensis*** (L.) Cabrera: Uruguay, Maldonado, *Bonifacino 1203* (MVFA), Katinas & al. (2008), –, EF530247, –, –, –, –. ***Jungia floribunda*** Less.: Uruguay, Cerro Largo, *Bonifacino 1306* (MVFA), Katinas & al. (2008), –, EF530233, –, –, –, –. ***Jungia rugosa*** Less.: Peru, Cajamarca, *Henning & Schneider 166* (B), –, MG553778*, –, –, –, –. ***Jungia polita*** Griseb.: Argentina, *Simon 292* (US), Panero & Funk (2008), –, –, EU385370, EU385178, –, EU385084. ***Leucheria tomentosa*** (Less.) Crisci: Chile, Reg. Valparaíso, *Moreira & Luebert 1237* (SGO), MG553813*, MG553776*, –, –, –, –. ***Leucheria thermarum*** (Phil.) Phil.: Chile, *Simon 383* (US), Panero & Funk (2008), –, –, EU385371, EU385179, –, EU385085. ***Lophopappus cuneatus*** R.E.Fr.; Peru, Moquegua, *Weigend & al. 9523* (BONN), MG553838*, –, MG553771*, MG553691*, MG553883*, MG553732*. ***Lophopappus foliosus*** Rusby: Chile, Reg. Arica y Parinacota, *Moreira 1962* (SGO), MG553823*, MG553790*, MG553757*, MG553677*, MG553868*, MG553717*; Chile, Reg. Arica y Parinacota, *Moreira & Luebert 2414* (SGO), MG553839*, MG553800*, MG553772*, MG553692*, MG553884*, MG553733*. ***Lophopappus tarapacanus*** (Phil.) Cabrera: Chile, Reg. Arica y Parinacota, *Moreira 1947* (SGO), MG553824*, –, MG553758*, MG553678*, MG553869*, MG553718*; Chile, Reg. Arica y Parinacota, *Moreira & Luebert 2371* (BONN), MG553837*, –, MG553770*, MG553690*, MG553882*, MG553731*; Chile, *Moreira & al. 2005* (SGO), MG553803*, –, MG553734*, MG553653*, MG553842*, MG553694*. ***Moscharia pinnatifida*** Ruiz & Pav.: Chile, Reg. Coquimbo, *Luebert & Hilger 3148* (BONN), MG553832*, MG553795*, –, –, MG553877*, MG553726*. ***Mutisia spinosa*** Ruiz & Pav.: Chile, Reg. Aysén, *Luebert & Daniłowicz-Luebert 3229* (BONN), MG553836*, MG553799*, MG553769*, MG553689*, MG553881*, MG553730*. ***Nassauvia axillaris*** (Lag. ex Spreng.) D.Don, Argentina, Mendoza, *Bonifacino 1610* (LP), Katinas & al. (2008), –, EF530232, –, –, –, –. ***Nassauvia pygmaea*** (Cass.) Hook.f.: Argentina, *Bonifacino 179* (US), Panero & Funk (2008), –, –, EU385377, EU385186, –, EU385092. ***Oxyphyllum ulicinum*** Phil.: Chile, Reg. Atacama, *Luebert & García 2829/1223* (SGO), MG553812*, EU729344, MG553745*, EU729348, MG553854*, EU729340. ***Pachylaena atriplicifolia*** D.Don ex Hook. & Arn.: Argentina, *Bonifacino 1602* (LP), *Simon 684* (US), Panero & Funk (2008), Katinas & al. (2008), –, EF530250, EU385383, EU385192, –, EU385098. ***Perezia lanigera*** Hook. & Arn.: Argentina, Santa Cruz, *Albert 8-XI-2006-2* (TEX), Simpson & al. (2009), –, FJ979678, –, –, –, –. ***Perezia nutans*** Less.: Chile, *Wen 7472* (F), Simpson & al. (2009), –, FJ979671, –, –, –, –. ***Perezia pinnatifida*** (Bonpl.) Wedd.: Chile, Reg. Arica y Parinacota, *Moreira & Luebert AM2422* (SGO), MG553834*, MG553797*, MG553767*, MG553687*, MG553879*, MG553728*. ***Perezia purpurata*** Wedd.: *Beck 31111* (LPB), *Simon 594* (US), Panero & Funk (2008); Simpson & al. (2009), –, –, EU385385, EU385194, FJ979693, EU385100. ***Plazia daphnoides*** Wedd.: Chile, Reg. Arica y Parinacota, *Trivelli s.n.* (SGO), MG553822*, MG553789*, MG553756*, MG553676*, MG553867*, MG553716*. ***Polyachyrus fuscus*** (Meyen) Walp.: Chile, Reg. Antofagasta, *Moreira & Luebert 1219* (SGO), MG553814*, MG553777*, MG553746*, MG553664*, MG553855*, MG553704*. ***Proustia cuneifolia*** f. ***cinerea*** (Phil.) Fabris: Chile, Reg. Santiago, *Luebert & Moreira 3038* (SGO), –, –, –, MG553666*, MG553857*, MG553706*; Chile, Reg. Valparaíso, *Hichins s.n.* (SGO), MG553820*, MG553787*, MG553755*, MG553675*, MG553866*, MG553715*; Chile, Reg. Valparaíso, *Luebert & Daniłowicz 3029* (SGO), MG553816*, MG553780*, MG553748*, MG553668*, MG553859*, MG553708*; Chile, *Moreira 2139* (SGO), MG553804*, –, MG553735*, MG553654*, MG553843*, MG553695*. ***Proustia cuneifolia*** f. ***cuneifolia*** D.Don: Bolivia, Tarija, *Beck & al. 23720* (S), MG553830*, –, MG553764*, MG553684*, MG553875*, MG553724*; Chile, Reg. Santiago, *Sancho & al. 262* (LP), –, –, MG553743*, MG553662*, MG553851*, MG553702*; Chile, Reg. Valparaíso, *Luebert & Daniłowicz 3028* (SGO), MG553817*, MG553781*, MG553749*, MG553669*, MG553860*, MG553709*; Chile, *Moreira 2138* (SGO), MG553809*, –, MG553740*, MG553659*, MG553848*, –; Peru, Moquegua, *Weigend & al. 9517* (BONN), MG553828*, –, MG553762*, MG553682*, MG553873*, MG553722*; Peru, Moquegua, *Weigend & al. 9518* (BONN), MG553829*, –, MG553763*, MG553683*, MG553874*, MG553723*. ***Proustia cuneifolia*** f. ***mendocina*** (Phil.) Fabris: Argentina, Córdoba, *Gutiérrez & al. 1207* (LP), MG553805*, –, –, MG553655*, MG553844*, MG553697*; Argentina, La Rioja, *Bonifacino 1547* (LP), Katinas & al. (2008), –, EF530244, –, –, –, –; Argentina, San Juan, *Bonifacino 1593* (LP), Katinas & al. (2008), MG553802*, EF530251, –, –, MG553841*, EF530297. ***Proustia cuneifolia*** var. ***mollis*** (Kuntze) Cabrera: Argentina, Jujuy, *Sancho & Viera 252A* (LP), MG553808*, –, MG553739*, MG553658*, MG553847*, MG553699*; Argentina, Salta, *Sancho & al. 243* (LP), MG553806*, –, MG553737*, MG553656*, MG553845*, MG553698*; Argentina, Salta, *Sancho & al. 247* (LP), MG553807*, –, MG553738*, MG553657*, MG553846*, –. ***Proustia cuneifolia*** f. ***tipia*** (Phil.) Fabris: Chile, Reg. Antofagasta, *Moreira & Luebert 1203* (SGO), –, MG553784*, MG553752*, MG553672*, MG553863*, MG553712*; Chile, Reg. Antofagasta, *Romero & Medina V21* (EIF), –, MG553785*, MG553753*, MG553673*, MG553864*, MG553713*; Chile, Reg. Antofagasta, *Romero & Medina V09* (EIF), MG553819*, MG553786*, MG553754*, MG553674*, MG553865*, MG553714*. ***Proustia ilicifolia*** f. ***ilicifolia*** Hook. & Arn.: Chile, Reg. Coquimbo, *Luebert & Hilger 3157* (BONN), MG553826*, MG553792*, MG553760*, MG553680*, MG553871*, MG553720*; Chile, Reg. Coquimbo, *Teillier 6589* (BONN), –, MG553782*, MG553750*, MG553670*, MG553861*, MG553710*. ***Proustia ilicifolia*** f. ***baccharoides*** (D.Don) Fabris: Chile, Reg. Coquimbo, *Luebert & Hilger 3151* (BONN), MG553827*, MG553793*, MG553761*, MG553681*, MG553872*, MG553721*; Chile, Reg. Santiago, *Luebert & Moreira 3042* (SGO), –, –, –, MG553665*, MG553856*, MG553705*; Chile, *Luebert 3053* (SGO), MG553818*, MG553783*, MG553751*, MG553671*, MG553862*, MG553711*; Chile, *Moreira 2140* (SGO), MG553810*, –, MG553741*, MG553660*, MG553849*, MG553700*. ***Proustia pyrifolia*** DC.: Chile, Reg. O'Higgins, *Sancho & al. 285* (LP), MG553811*, –, MG553742*, MG553661*, MG553850*, MG553701*; Chile, Reg. Santiago, *Luebert & Moreira 3037* (SGO), MG553815*, MG553779*, MG553747*, MG553667*, MG553858*, MG553707*. ***Triptilion capillatum*** Hook. & Arn.: Chile, Reg. Metropolitana, *Bonifacino 1336* (LP), Katinas & al. (2008), –, EF530222, –, –, –, –. ***Trixis cacalioides*** (Kunth) D.Don: Chile, Reg. Arica y Parinacota, *Moreira & Luebert 2364* (SGO), MG553833*, MG553796*, MG553766*, MG553686*, MG553878*, MG553727*. ***Trixis divaricata*** (Kunth) Spreng.: Brazil, *Santos 2659* (TEX), Panero & Funk (2008), –, –, EU385405, EU385214, –, EU385120. ***Urmenetea atacamensis*** Phil.: Chile, Reg. Antofagasta, *Medina 999* (SGO), MG553821*, MG553788*, –, –, –, –.