

A new generic circumscription in tribe Lycieae (Solanaceae)

Rachel A. Levin,¹ Gabriel Bernardello,² Carolyn Whiting¹ & Jill S. Miller¹

¹ Department of Biology, Amherst College, Amherst, Massachusetts 01002, U.S.A.

² Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba-CONICET, C.C. 495, 5000 Córdoba, Argentina

Author for correspondence: Rachel A. Levin, rlevin@amherst.edu

Abstract Tribe Lycieae (Solanaceae) currently includes ca. 92 species in three genera. Two genera account for only a few species, as *Phrodus* is monotypic and *Grabowskia* includes just four species. By contrast, *Lycium* is one of the largest genera in the family, with ca. 88 species and over 95% of the species diversity in tribe Lycieae. Previous molecular studies have suggested that *Lycium* is paraphyletic and that species of *Grabowskia* are nested within *Lycium*. These studies also suggested that the genus *Grabowskia* was monophyletic, but questioned the integrity of species within it. Likewise, although the genus is defined by a unique fruit structure, morphology is of limited use in distinguishing species within *Grabowskia*. Depending on the study, *Phrodus microphyllus* has been placed either as sister to the rest of the tribe or nested within *Lycium*. In the present study we include data from two nuclear regions (granule-bound starch synthase and nitrate reductase) and four plastid spacer regions (*trnH-psbA*, *trnD^{GUC}-trnT^{GGU}*, *rpl32-trnL^{UAG}*, *ndhF-rpl32*) to more clearly resolve evolutionary relationships among genera within the tribe and among species in *Grabowskia*. Results confirm that *Lycium* is paraphyletic and includes a monophyletic *Grabowskia*. However, inclusion of multiple accessions of several *Grabowskia* species does not support the maintenance of distinct species in the genus. In addition, *Phrodus microphyllus* is moderately supported as basal within the tribe. Given these results, which are further strengthened by morphological and cytological data, we synonymize three *Grabowskia* species with *Grabowskia boerhaviifolia* and include this species within *Lycium*, using its basionym, *Lycium boerhaviifolium* L. f. Additionally, we transfer *Phrodus microphyllus* to the genus *Lycium*, proposing a new combination: *Lycium bridgesii* (Miers) Levin, Miller & Bernardello. Tribe Lycieae is now monotypic, including the single genus *Lycium*.

Keywords *Grabowskia*; Lycieae; *Lycium*; *Phrodus*; phylogeny; Solanaceae

■ INTRODUCTION

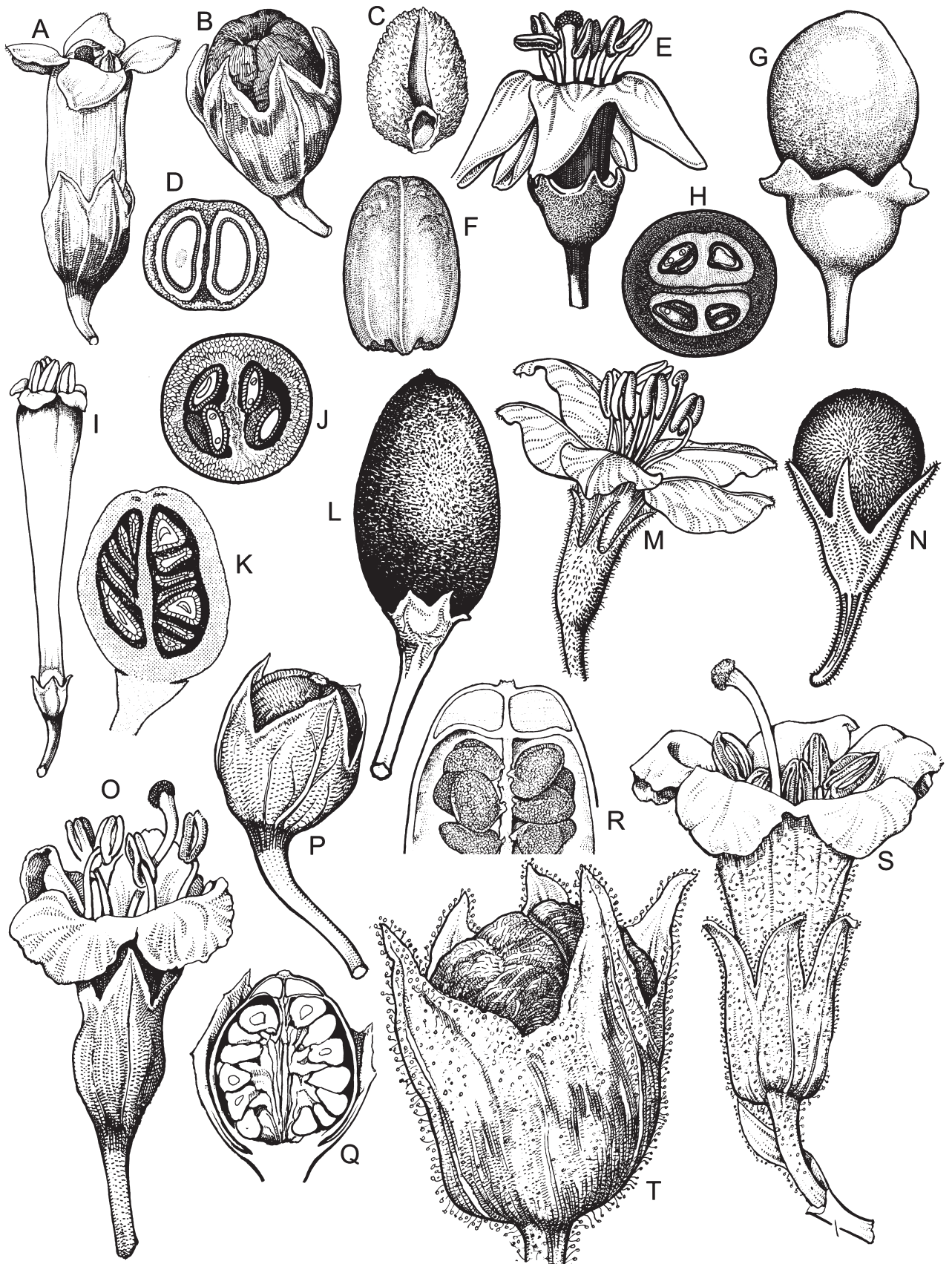
Tribe Lycieae Hunz. is among the largest in Solanaceae, with ca. 92 species included in three genera. In the tribe, the vast majority of species and the greatest geographic diversity are in the large genus *Lycium* L. (ca. 88 species). The remaining two genera include the monotypic *Phrodus microphyllus* (Miers) Miers (Bernardello & Hunziker, 1987) and the small genus (3 or 4 species) *Grabowskia* Schldl. *Lycium* occurs worldwide and has its natural range on all temperate and tropical continents, with centers of diversity in southern South America, southern Africa, and southwestern North America. In contrast, *Phrodus microphyllus* is endemic to the Atacama Desert in northern Chile, and *Grabowskia* species are limited to South America, although one species also occurs in a restricted area of central Mexico. Regardless of their provenance, all species in the three genera are woody shrubs (sometimes treelets) that inhabit arid to semi-arid or coastal environments.

Hunziker (1977) first united *Grabowskia*, *Lycium*, and *Phrodus* in tribe Lycieae based on four characters. These characters include: (1) the shared presence of developing buds with imbricate or imbricate-plicate (including cochlear and quincuncial) corolla aestivation, (2) dorsifixed anthers, (3) bicarpellate gynoecea with either two or four locules, and (4) fruits that are usually multi-seeded berries, or rarely drupaceous having 2–8 seeds (Fig. 1) (see also Hunziker, 2001). Olmstead & al. (2008) found that tribe Lycieae is monophyletic and nested in a clade that also includes *Jaborosa* Juss., *Latua* Phil., *Nolana* L.,

Sclerophylax Miers, and tribe Hyoscyameae. Within this clade, Lycieae is most closely related to *Nolana* and *Sclerophylax* (see also Levin & al., 2007). Interestingly, Hunziker (2001) excluded both *Nolana* and *Sclerophylax* from Solanaceae based on their distinctive gynoeceal and fruit morphologies.

Floral morphology is quite uniform across species in Lycieae and cannot be used to distinguish genera, although specific floral morphological features (or combinations of features) can be diagnostic for species (Fig. 1). Rather, fruit type is the primary character that distinguishes the three genera. In *Lycium*, the majority of species produce a multi-seeded fleshy berry (Fig. 1J, L, N), and some species have berries with various degrees of sclerification (Fig. 1K, Q) (Chiang-Cabrera, 1981; Bernardello, 1983, 1986a; Chiang, 1983; Miller, 2002). In addition, four *Lycium* species have drupaceous fruits with two single-seeded pyrenes (Fig. 1B–D). Like the majority of *Lycium*, the fruit of *Phrodus microphyllus* is a multi-seeded berry, but seeds are mucilaginous, and the berries are distinguished by the presence of two apical sclerifications or sclerosomes (Fig. 1R, T) (Bernardello & Hunziker, 1987; Hunziker, 2001). By contrast, *Grabowskia* species uniformly have drupaceous fruits with two apically septate pyrenes, each containing 2–4 seeds (Fig. 1F–H). Despite these differences in fruit morphology, previous molecular studies of tribe Lycieae (Levin & Miller, 2005; Levin & al., 2007, 2009a) and Solanaceae (Olmstead & al., 2008) suggested that both *Grabowskia* and *Phrodus* may be nested in *Lycium*.

Miers (1849) originally described three species in the genus *Phrodus*, which has subsequently included from two to



four species (reviewed in Bernardello & Hunziker, 1987). Following extensive examination (Filippa & Bernardello, 1987) of various specimens across all named species and types, Bernardello & Hunziker (1987) reduced *Phrodus* to a single species, *P. microphyllus*. Imbricate-plicate corolla aestivation differentiates *Phrodus* from the imbricate aestivation of *Lycium* and *Grabowskia*. Apart from aestivation and the above-mentioned sclerosomes in fruit, there is little to distinguish the genus. Although the vegetation and flowers of *P. microphyllus* are densely glandular pubescent (Fig. 1S–T), several *Lycium* species share these traits. Further, *Phrodus* has showy white flowers (Fig. 1S) that are considerably larger (up to 25 mm long; Hunziker, 2001) than most species in the tribe. However, such flowers are not unique, with similar large flowers occurring in the North American *Lycium pallidum* Miers.

The genus *Lycium* was named by Linnaeus (1753: 191–192), and his son later described the species *Lycium boerhaviifolium* L. f. (Linnaeus f., 1782: 150). Schlechtendal (1832) subsequently segregated *L. boerhaviifolium* in its own genus, *Grabowskia*, with a single species proposed (*G. boerhaviifolia*). Although other species were later defined (as many as 16 in total; reviewed in Hunziker, 1997), Hunziker (1997, 2001) recognized only four: *Grabowskia boerhaviifolia* (L. f.) Schldtl., *G. duplicata* Arn., *G. obtusa* Arn., and *G. megalosperma* Speng. As mentioned above, *Grabowskia* species have unique drupaceous fruits (Fig. 1F–H), and the vegetation is also somewhat distinctive, with all *Grabowskia* species sharing thick, ovate, glaucous leaves (except for the single collection of *G. megalosperma*, which has smaller, lanceolate leaves). Hunziker (1997) noted that although the genus was easily recognized, its species were very difficult to distinguish. Indeed, Hunziker (1997) differentiated the four species based mainly on corolla size and the number of flowers per inflorescence (the ranges of which overlap among species), as well as the presence of flowers on old or new branches.

Consideration of the geographic distribution of *Grabowskia* species does little to elucidate species circumscriptions. One species, *G. megalosperma*, is known only from a single collection in Argentina. The three other taxa (*G. boerhaviifolia*, *G. duplicata*, *G. obtusa*) have overlapping geographic distributions in Argentina. *Grabowskia obtusa* is restricted to Argentina, whereas *G. duplicata* ranges from Argentina into Paraguay, Uruguay, and southern Brazil. *Grabowskia boerhaviifolia* has by far the widest distribution, ranging from Argentina and Chile north to Peru and Bolivia. This species also occurs on the Galapagos Islands and has been collected from several locations near Tehuacán in Puebla, Mexico. The occurrence of these isolated Mexican populations would suggest that *Grabowskia boerhaviifolia* has been introduced to this area;

however, there is no evidence available to support or refute this explanation.

In combination with a lack of defining morphological characteristics, these overlapping geographic distributions suggested that species circumscriptions should be re-evaluated. In particular, the wide range of *G. boerhaviifolia* hinted that perhaps all *Grabowskia* species were in fact simply local variants of a single widespread species. Thus, one goal of this study was to assess the monophyly of the currently defined *Grabowskia* species using DNA sequence data from multiple individuals across the range of the genus. Furthermore, previous studies (Levin & Miller, 2005; Levin & al., 2007, 2009a; Olmstead & al., 2008) have suggested that *Grabowskia* and *Phrodus* are in fact nested within the large genus *Lycium*. We evaluate the results of the present study in light of these previous analyses and make recommendations as to the taxonomy of the three genera within tribe Lycieae.

■ MATERIALS AND METHODS

Taxon sampling. — We included 15 *Grabowskia* accessions spanning the geographic range of the genus and representing all species (except *G. megalosperma*) recognized by Hunziker (2001). Given previous analyses (Levin & Miller, 2005; Levin & al., 2007, 2009a) suggesting that *Grabowskia* was closely related to a distinctive clade of five *Lycium* species (*L. cooperi* A. Gray, *L. macrodon* A. Gray, *L. pallidum* Miers, *L. puberulum* A. Gray, *L. shockleyi* A. Gray), representatives of all of these species were also included. Additionally, we included species representatives from the major lineages within *Lycium* (Levin & al., 2007), as well as a single accession of *Phrodus microphyllus*. *Nolana werdermannii* I.M. Johnston was included as an outgroup, outside of tribe Lycieae (Levin & al., 2007; Olmstead & al., 2008). See Appendix for complete taxon sampling and GenBank accession numbers.

DNA sequence data. — Sequence data from two nuclear regions, granule-bound starch synthase (GBSSI) and the 5' end of nitrate reductase (NIA), were included in this study. These regions were previously useful for inferring relationships among species in *Lycium* (Levin & al., 2007, 2009a). For a subset of taxa, four plastid spacer regions (*trnH-psbA*, *trnD^{GUC}-trnT^{GGU}*, *rpl32-trnL^{UAG}*, *ndhF-rpl32*) were also amplified and sequenced following the protocols of Miller & al. (2009).

For GBSSI, we sequenced exons 2 through 10 (rarely exons 3 through 8), following Levin & Miller (2005) and Levin & al. (2007). For NIA, intron 1, exon 2, and part of intron 2 were amplified using primers NIAF5' and midR (see Fig. 1 in Levin & al., 2009a). Standard PCR conditions were used (Levin &

◀ **Fig. 1.** Flowers and fruits in tribe Lycieae. **A–D**, *Lycium ameghinoi*: **A**, flower, ×6.5; **B**, drupaceous fruit, ×6.5; **C**, pyrene, ×6.5; **D**, fruit cross section showing two unilocular pyrenes, ×6.5. **E–H**, *Grabowskia boerhaviifolia* (= *Lycium boerhaviifolium*): **E**, flower, ×4.5; **F**, pyrene, ×4.5; **G**, drupaceous fruit, ×4.5; **H**, fruit cross section showing two bilocular pyrenes, ×4.5. **I–L**, *L. gilliesianum*: **I**, flower, ×4.5; **J**, fruit cross section, ×4.5; **K**, fruit longisection showing two minute apical sclerosomes, ×4.5; **L**, baccate fruit, ×4.5. **M–N**, *L. ciliatum*: **M**, flower, ×4.5; **N**, baccate fruit, ×4.5. **O–Q**, *L. schreiteri*: **O**, flower, ×4.5; **P**, baccate fruit, ×4.5; **Q**, fruit longisection showing two apical macroscopic sclerosomes, ×4.5. **R–T**, *Phrodus microphyllus* (= *Lycium bridgesii*): **R**, distal part of fruit longisection showing two apical macroscopic sclerosomes, ×2.3; **S**, flower, ×4.5; **T**, baccate fruit, ×4.5.

al., 2009a), with a thermal cycler program of 94° for 4 min; 40 cycles of 94° for 30 s, 52° for 1 min, and 72° for 1 min; ending with 7 min at 72°. PCR products were cleaned using either the MinElute PCR Purification Kit (Qiagen, Valencia, California, U.S.A.) or an ExoSAP procedure with 5 µl PCR product, 1 µl shrimp alkaline phosphatase (1 unit/µl, SAP), 0.5 µl Exonuclease I (10 units/µl), and 1.5 µl 10× PCR buffer. Cleaned PCR products were directly sequenced (using the amplification primers) by the DNA Sequencing Facility of the Biotechnology Resource Center at Cornell University (Ithaca, New York, U.S.A.) or the PennState University Nucleic Acid Facility (University Park, Pennsylvania, U.S.A.).

For four individuals, direct sequences of NIA suggested the presence of two alleles that differed in length (yielding unreadable sequences). Thus, PCR products were cloned prior to sequencing using the Novagen pSTBlue-1 AccepTor Vector Giga Kit (Novagen, EMD Chemicals, Madison, Wisconsin, U.S.A.). Colonies were PCR amplified in 12.5 µl reactions using 2.5 µl of each template and the vector primers R20 and U19 at final concentrations of 0.125 mM. Reactions contained 0.3 units *Taq* polymerase, 1× buffer, 0.25 mM dNTPs, and 1.5 mM MgCl₂. The thermal cycler program had an initial denaturation at 94°C for 5 min; 6 cycles at 94°C for 1 min, 55°C–53°C (decreasing 1° every 2 cycles) for 1 min, 72°C for 2 min; 30 cycles at 94°C for 1 min, 52°C for 1 min, 72°C for 2 min; ending with an extension at 72°C for 5 min. PCR products were cleaned and sequenced as above using the vector primers R20 and U19. Multiple colonies per accession were sequenced in a single direction using the vector primers. As alleles were identified, one colony per allele was sequenced in the opposite direction in order to obtain a complete sequence.

Sequence alignment and phylogenetic analyses. — Sequences within an individual were edited, aligned, and assembled using Sequencher v.4.7/4.8 (Gene Codes Corp., 1991–2007). Consensus sequences from each genomic accession were manually aligned across species using SeAl v.2.0a11 (Rambaut, 2002).

Using PAUP* v.4.0b10 for MacOSX (Swofford, 2002), pairwise distances (uncorrected *P* or dissimilarity) were calculated for the GBSSI data for all 15 *Grabowskia* accessions. For comparison, intraspecific pairwise distances were also calculated for four *Lycium* species (*L. californicum* A. Gray, *L. carolinianum* Walter, *L. chilense* Miers ex Bertero, *L. shawii* Roem. & Schult.) for which GBSSI sequences from multiple individuals across a wide geographic range were available (Yeung & al., 2005; Levin & al., 2007; Levin & Miller, unpub. data).

NIA and GBSSI datasets were analyzed separately using maximum likelihood (ML) as implemented in PAUP* (Swofford, 2002). Substitution model parameters were estimated using the Akaike information criterion in Modeltest v.3.7 (Posada & Crandall, 1998). Best-fit models corresponded to K81uf+G for NIA and TrN+G for GBSSI. ML settings in PAUP* included the heuristic search option, all most-parsimonious trees from an initial parsimony-based heuristic search (1000 random addition sequence replicates, MulTrees disabled) as the starting trees (note that not all of the most-parsimonious starting trees are actually used by PAUP*, depending on their ML scores),

tree bisection reconnection (TBR) branch-swapping, and the MulTrees option in effect. Maximum likelihood nonparametric bootstrap (BS) analyses were conducted for the NIA and GBSSI datasets, using the same model parameters as in the original ML analyses, and 100 full heuristic bootstrap replicates, each with 10 random-addition sequence replicates, TBR branch-swapping, and the MulTrees option in effect. As there were no supported differences (BS > 75) between the topologies inferred from NIA and GBSSI data, the NIA and GBSSI data were concatenated and analyzed using ML, with substitution model parameters estimated as above. The best-fit likelihood model corresponded to K81uf+I+G. An ML nonparametric BS analysis was also conducted for the combined dataset, using the same model parameters as in the original ML analysis, and 500 full heuristic bootstrap replicates, each with 10 random-addition sequence replicates, TBR branch-swapping, and the MulTrees option in effect. Maximum likelihood BS analyses were conducted using PAUP* v.4.0b10 for UNIX (Swofford, 2002) on the Condor (Condor Project, 2005) computer cluster at Amherst College. Bootstrap replicates were parsed for processing using RepMaker (Wilgenbusch, 2003).

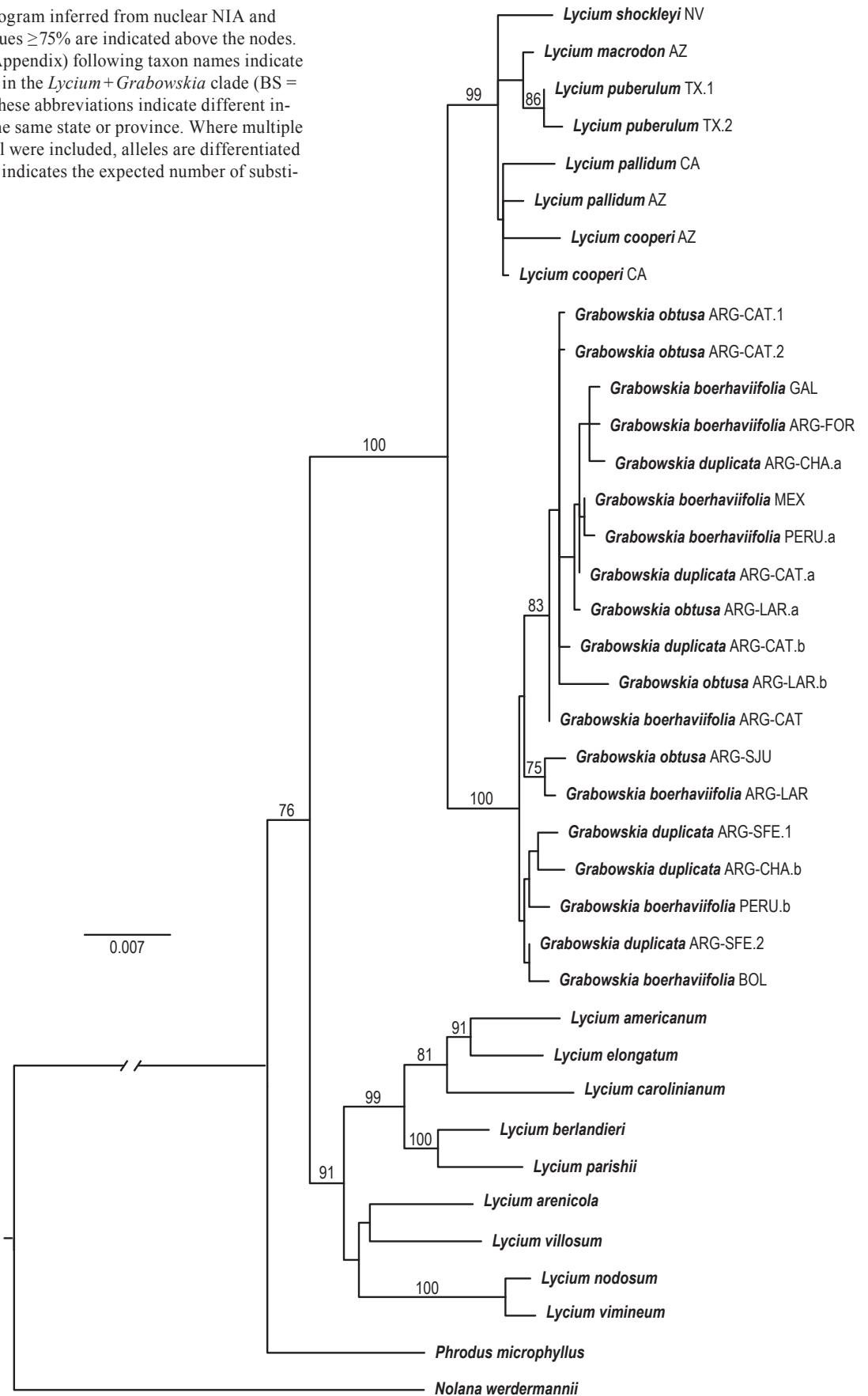
For the 27 taxa for which there were sequence data from GBSSI, NIA, and four plastid spacers, all three datasets (GBSSI, NIA, plastid) were analyzed simultaneously using Bayesian Estimation of Species Trees (BEST v.2.2; Liu, 2008), which estimates a posterior distribution of species trees based on distributions of gene trees. Each locus had its own substitution model, and the analysis included two independent runs, each with four Markov chains, 150 million generations, and a temperature of 0.15. A consensus of the estimated distribution of species trees was constructed in BEST using the `sumt` command and a burn-in of 40% of the trees.

■ RESULTS

Pairwise distances. — Average pairwise distances for GBSSI data across all 15 *Grabowskia* accessions was 0.0013. In contrast, the mean pairwise distance across six individuals of *L. shawii* and six individuals of *L. chilense* was 0.0044 and 0.0040, respectively. The mean pairwise distances for 18 *L. californicum* individuals was 0.0024, and the average pairwise distance was 0.0033 for 12 *Lycium carolinianum* individuals across the range of this species.

Maximum likelihood analysis of GBSSI and NIA data. — A total of 34 individuals were included in this analysis, with a total of 38 terminals due to the inclusion of two different NIA alleles for four *Grabowskia* individuals (Fig. 2). There is moderate support (BS = 76) for a clade of all *Lycium* and *Grabowskia* species. Within this clade, there is a well-supported (BS = 100) group including two sister lineages: five *Lycium* species (*L. cooperi*, *L. macrodon*, *L. pallidum*, *L. puberulum*, *L. shockleyi*) that are strongly supported as monophyletic (BS = 99) and a clade (BS = 100) of all *Grabowskia* individuals from three named species. Sister to these five *Lycium* species and the *Grabowskia* accessions is a well-supported clade (BS = 91) including all other *Lycium* species sampled.

Fig. 2. The single ML phylogram inferred from nuclear NIA and GBSSI data. Bootstrap values $\geq 75\%$ are indicated above the nodes. Letter abbreviations (see Appendix) following taxon names indicate localities for those species in the *Lycium* + *Grabowskia* clade (BS = 100). Numbers following these abbreviations indicate different individuals collected from the same state or province. Where multiple alleles within an individual were included, alleles are differentiated by an “a” or “b”. Scale bar indicates the expected number of substitutions per site.



Within the *Grabowskia* clade there is limited resolution, and individuals within species do not group together as each other's closest relatives. For example, there are only two clades with $BS \geq 75\%$ in the *Grabowskia* lineage, and both of these clades contain *Grabowskia* accessions that are classified as different species (Fig. 2). Further, there does not appear to be any strongly supported geographical clustering among these accessions (see Appendix).

BEST analysis of GBSSI, NIA, and plastid data. — This 27 taxa analysis yielded a strongly supported tribe Lycieae, with a Bayesian posterior probability (PP) of 1.0 (Fig. 3). Within this clade are all *Lycium* and *Grabowskia* species (PP = 0.87), which are divided into two monophyletic groups. One well-supported lineage (PP = 0.99) includes all *Grabowskia* accessions (PP = 1.0) sister to a strongly supported clade (PP = 1.0) of five *Lycium* species (*L. cooperi*, *L. macrodon*, *L. pallidum*, *L. puberulum*, *L. shockleyi*). The other well-supported lineage (PP = 1.0) includes all other sampled *Lycium* species. Within *Grabowskia* there is no resolution of relationships, with a notable absence of any species-specific signal that groups individuals within species.

DISCUSSION

Previous molecular phylogenetic studies (Levin & Miller, 2005; Levin & al., 2007; Olmstead & al., 2008) clearly established tribe Lycieae as a monophyletic group, with a paraphyletic genus *Lycium*. *Grabowskia* species are always nested within *Lycium*, and *Phrodus microphyllus* is either at the base of the tribe or nested within *Lycium*. Within *Grabowskia* itself, results from the present study suggest that the genus may be more appropriately considered as including a single widespread species, rather than three separate species. Phylogenetic relationships among *Grabowskia* accessions exhibit no species-specific phylogenetic signal (Figs. 2–3). In contrast, these same data have sufficient signal to group different accessions within *Lycium* as monophyletic species (e.g., *L. puberulum* in Fig. 2; *L. pallidum* in Fig. 3). Furthermore, comparison of pairwise distances among *Grabowskia* individuals (across three different species) with pairwise distances between individuals within four *Lycium* species does not support the definition of the three *Grabowskia* species examined. The average pairwise distance across all 15 *Grabowskia* individuals from different geographic locations in three different species was less (0.0013) than all other conspecific distances for four *Lycium* species (average pairwise distances ranged from 0.0024 to 0.0044). Thus, the level of nucleotide variation among formally named *Grabowskia* species is similar to, and even lower than, levels of variation within several *Lycium* species. These comparisons involve *Lycium* species that are relatively distantly related to *Grabowskia*. However, pairwise distances across eight individuals of five species (*Lycium cooperi*, *L. macrodon*, *L. pallidum*, *L. puberulum*, *L. shockleyi*) in the sister clade to *Grabowskia* were also greater (0.0032) than across the 15 accessions of *Grabowskia* species. These observations suggest strongly that there is little molecular differentiation among the named

Grabowskia species. From the morphological point of view, the few floral traits used by Hunziker (1997) to differentiate the species (corolla size and color, anther size, number of flowers and size of inflorescences, inflorescence location) are very variable and do not follow a geographic pattern. Coupled with the lack of molecular data supporting the three *Grabowskia* species, it is preferable to recognize only a single species of *Grabowskia*. As the binomial *Grabowskia boerhaviifolia* has priority (Schlechtendal, 1832), we synonymize *G. obtusa* and *G. duplicata* within it.

Regarding the Patagonian *Grabowskia megalosperma*, it was collected only once in 1899 with no specific locality data other than “Golfo de San Jorge” (Spegazzini, 1902). This area was extensively searched by numerous botanists in the last century, but the “species” was never found again. Hunziker (1997) questioned its validity and suggested that it could be a form of *G. duplicata*. Here we treat it as another synonym of *G. boerhaviifolia*.

Given that *Grabowskia* is strongly supported as sister to a group of *Lycium* species (*L. cooperi*, *L. macrodon*, *L. pallidum*, *L. puberulum*, *L. shockleyi*), and that the genus is nested within *Lycium* (Figs. 2–3) not only in this study, but also in previous studies using different taxon sampling and genomic regions (Miller, 2002; Levin & Miller, 2005; Levin & al., 2007; Olmstead & al., 2008), we propose to include *Grabowskia boerhaviifolia* within *Lycium*, using its basionym: *Lycium boerhaviifolium* L. f. Distinctions between *Grabowskia* and *Lycium* have historically been based on fruit structure, but in reality their fruit morphologies overlap to a great extent (Fig. 1). *Grabowskia* has drupaceous fruits with two apically septate pyrenes with 2–4 seeds each (Fig. 1F–H), whereas most *Lycium* produce fleshy multi-seeded berries (Fig. 1J, L, N). However, there are several South and North American species with fruits that range from berries with microscopic (Fig. 1K) or macroscopic (Fig. 1Q) sclerifications to drupaceous fruits with two non-septate one-seeded pyrenes (Fig. 1B–D) (Bernardello, 1986a, b). Indeed, it is apparent that fruit morphology is somewhat labile across the genus, given that two-seeded drupaceous fruits evolved three separate times in *Lycium* (once in North America and twice in South America; Levin & al., 2007). Furthermore, the group of five *Lycium* species that are more closely related to the *Grabowskia* clade than other *Lycium* species have variously hardened fruits, from fleshy red berries with small distal sclerifications in *L. pallidum* to yellow-green fruits with a reduced seed number (<8 seeds) and indurated endocarp in *L. cooperi*, *L. macrodon*, and *L. puberulum* (Chiang-Cabrera, 1981; Miller, 2002). In addition to fruit morphology, cytological data also support an affinity between *Lycium* and *Grabowskia* species. Karyotype data for 25 South American and Asian *Lycium* species (e.g., Stiefkens & Bernardello, 1996, 2000, 2002, 2006; Sheidai & al., 1999; Zhao & al., 2000) and the three *Grabowskia* species studied here (Bernardello & al., 2008) suggest a common formula composed of 11 metacentric + 1 submetacentric chromosome pairs. Thus, there is considerable evidence from molecular, morphological, and cytological data supporting the inclusion of *Grabowskia* within *Lycium*.

Phylogenetic results suggest that *Phrodus microphyllus* is at the base of tribe Lycieae (Fig. 3). Other studies have indicated conflicting placements, with plastid data suggesting that *P. microphyllus* is nested well within *Lycium*, and nuclear data often placing it at the base of tribe Lycieae (Levin & al., 2007, 2009a, b; Olmstead & al., 2008; Miller & al., 2009). Morphological characteristics of *Phrodus* are likewise equivocal in differentiating this taxon from the rest of the tribe (Fig. 1). Historically, only the presence of somewhat larger flowers, a plicate corolla in bud, and the fact that the corolla falls off completely without leaving a small ring of tissue were used to differentiate the genus (Bernardello & Hunziker, 1987; Hunziker, 2001). *Phrodus microphyllus* has also been distinguished using its apical sclerifications in fruit; however, it is clear that fruit sclerifications are not uncommon across the other species in the tribe (see above; also compare Fig. 1Q–R), and the presence of stone cells (i.e., sclerified areas) may well be symplesiomorphic (see below, also reviewed in Knapp, 2002). Cytological data indicate that *Phrodus* has a comparatively distinct, more asymmetrical karyotype than *Lycium* species (Bernardello & al., 2008). There are several examples in diverse plant groups showing that increased asymmetry is a less derived character (e.g., Moretti, 1990; Cota & Wallace, 1995; Mercado-Ruaro & Delgado-Salinas, 1998), which is concordant with a phylogenetic position of *Phrodus* at the base of the tribe.

If *Phrodus microphyllus* is indeed nested within *Lycium*, then this monotypic genus should be transferred to *Lycium*. Even if the “true” phylogenetic placement of this species is at the base of the tribe, there is no phylogenetic conflict in combining *P. microphyllus* with *Lycium*, eliminating the monotypic genus *Phrodus*. Thus, we propose the transfer of *Phrodus microphyllus* to the genus *Lycium*. As this epithet has been previously used (*Lycium microphyllum* Phil., synonymized in Bernardello, 1986b to *L. chilense* Miers ex Bertero), it cannot be applied. Instead, we use *Phrodus bridgesii* Miers. This species was described simultaneously (Miers, 1849) and is synonymous with *P. microphyllus* (Bernardello & Hunziker, 1987). Therefore, we propose a new combination with this basionym.

The transfer of these two taxa, one of which was originally included within *Lycium*, is a natural follow-up to recent molecular studies (e.g., Olmstead & al., 2008). Tribe Lycieae now includes the single genus *Lycium*, and, as such, tribal delimitation is now unnecessary. The genus likely evolved in South America approximately 5 Ma, with subsequent dispersal between North and South America (Levin & al., 2007; Miller & al., 2011). There was also a single dispersal of *Lycium* from South America to Africa approximately 3.5 Ma (Miller & al., 2011). Geographically, *Grabowskia* and *Phrodus* are almost entirely restricted to South America, the region of highest species richness for *Lycium*; thus, the proposed inclusion of these genera within *Lycium* has no effect on the overall geographic distribution of the genus *Lycium* as a whole.

As one of the largest genera in Solanaceae, *Lycium* encompasses much diversity, especially in terms of sexual strategies and fruit type (e.g., Miller & Venable, 2000; Miller, 2002; Levin & Miller, 2005; Yeung & al., 2005; Levin & al., 2007).

A more inclusive genus facilitates investigations of character evolution and biogeography. Within *Lycium*, fruit type varies from a fleshy multi-seeded berry to a hardened fruit with reduced seed number (Bernardello, 1983). Consequently, the pyrenes of *Grabowskia* and the somewhat sclerified berries of *Phrodus* are readily incorporated within the already diverse *Lycium*. A monophyletic *Lycium* now includes ca. 90 species, with the equally species-rich *Nolana* as its closest relative (Levin & al., 2007; Olmstead & al., 2008). In contrast to the cosmopolitan distribution of *Lycium*, *Nolana* is restricted to western South America and the Galapagos Islands. Most likely the fleshy, bird-dispersed fruits of *Lycium* species aided in this dispersal, compared to the “passive” dispersal (Knapp, 2002) of the sclerified *Nolana* mericarps. Fruit type is also likely important to mating system evolution in *Lycium*. Gametophytic self-incompatibility (GSI) is known to be ancestral in *Lycium*, and, quite remarkably, it has been maintained through a long-distance dispersal to the Old World (Miller & al., 2008). Its maintenance was undoubtedly aided by the dispersal of a fruit with multiple seeds, most likely a New World fleshy-fruited ancestor (as opposed to a taxon with hardened fruits) that dispersed to the Old World. Multi-seeded fruit likely contain a greater number of self-incompatibility alleles among progeny, thus increasing the potential for the maintenance of GSI following long-distance dispersal in colonizing lineages (Miller & al., 2008).

■ TAXONOMY AND NOMENCLATURE

Tribe **Lycieae** Hunz. in Kurtziana 10: 28. 1977.

Lycium L., Sp. Pl. 1: 191. 1753 – Lectotype: *Lycium afrum* L. (designated by Britton & Brown, 1913: 168).

= *Grabowskia* Schldtl. in Linnaea 7: 71. 1832 – Type: *Grabowskia boerhaviifolia* (L. f.) Schldtl.

= *Phrodus* Miers in Ann. Mag. Nat. Hist., ser. 2, 4: 33. 1849 – Lectotype: *Phrodus microphyllus* (Miers) Miers (designated by D’Arcy, 1986: 28).

Lycium boerhaviifolium L. f., Suppl. Pl., ed. 13: 150. 1782 = *Grabowskia boerhaviifolia* (L. f.) Schldtl. in Linnaea 7: 72. 1832 – Lectotype (here designated): *C. Linnaeus 259.12* (LINN).

= *Grabowskia duplicata* Arn. in Linnaea 11: 485. 1837 – Lectotype (here designated): Argentina, Buenos Aires, *Tweedie s.n.* (K 000640374).

= *Grabowskia obtusa* Arn. in Linnaea 11: 485. 1837 – Lectotype (here designated): Argentina, Mendoza, hedge near Sanjon, Albaricoquillo, 22 September 1827, *J. Gillies s.n.* (K 000640373).

= *Grabowskia megalosperma* Spig. in Anales Soc. Ci. Argent. 53: 166. 1902 – Holotype: Argentina, Golfo de San Jorge, 1899, *Ameghino 36* (BA).

Note that the species epithet “*boerhaviifolium*” is a change from the original Linnaean “*boerhaviaefolium*” following Art. 60.8 of the *Vienna Code* (McNeill & al., 2006).

Lycium bridgesii (Miers) Levin, Miller & Bernardello, **comb. nov.** ≡ *Phrodus bridgesii* Miers in Ann. Mag. Nat. Hist., ser. 2, 4: 34. 1849 – Lectotype (here designated): Chile, Coquimbo, *Bridges 1332* (K 000585988; isolectotypes: BM 000941672, P, E, FI).

= *Phrodus microphyllum* (Miers) Miers in Ann. Mag. Nat. Hist., ser. 2, 4: 33. 1849 ≡ *Alona microphylla* Miers in London J. Bot. 4: 501. 1845 – Lectotype (here designated): Chile, Coquimbo, *Bridges 1330* (BM 000994066).

The epithet “*microphyllum*” cannot be used for the new combination because of the existence of *Lycium microphyllum* Phil. (Anales Univ. Chile 36: 197. 1870), a synonym of *L. chilense* var. *minutifolium* (Miers) Barkley (cf. Bernardello, 1986b). It should be noted that the binomial *Phrodus bridgesii*, used here for the new combination, was published by Miers at the same time as *P. microphyllum*.

■ ACKNOWLEDGEMENTS

The authors thank C. Giaimo, N. Feliciano, and M. Dhond for laboratory contributions. This work was supported by U.S. National Science Foundation grants DEB-0343735 and DEB-0843364 to R.A.L. and J.S.M. Additional support was provided by the Howard Hughes Medical Institute Fellowship Program and Department of Biology at Amherst College. G.B. acknowledges support from the Universidad Nacional de Córdoba and CONICET.

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Appendix. Taxa, collection localities, voucher information, and GenBank accession numbers for all sequences included in this study. To differentiate the *Grabowskia* collections from Argentina, provincial localities are also given. Abbreviations in parentheses correspond to those used in Figs. 2–3: AZ, Arizona; CA, California; NV, Nevada; TX, Texas; BOL, Bolivia; GAL, Galapagos; MEX, Mexico; ARG, Argentina; CAT, Catamarca; CHA, Chaco; FOR, Formosa; LAR, La Rioja; SJU, San Juan; SFE, Santa Fe. GenBank accession numbers are listed in the following order: NIA, GBSSI, *trnH^{GUC}-psbA*, *rpl32-trnL^{UAG}*, *ndhF-rpl32*, *trnD^{GUC}-trnT^{GCU}*. Voucher specimens are deposited in the following herbaria: ARIZ, University of Arizona; BLFU, University of the Free State; CORD, Museo Botánico de Córdoba; F, Field Museum; NY, New York Botanical Garden; RSA, Rancho Santa Ana Botanic Garden; TAIC, Texas A&M University, Kingsville; US, U.S. National Herbarium; UT, University of Utah.

Grabowskia boerhaviifolia Schltdl.—Bolivia, *Nee 51864* (NY); JF284393, JF284368, JF284425, JF284441, JF284409, JF284380; (BOL). *G. boerhaviifolia* Schltdl.—Ecuador, Galapagos Islands, *Dillon & Tye 8502* (F); JF284394, EF137752, JF284423, JF284439, JF284407, JF284378; (GAL). *G. boerhaviifolia* Schltdl.—Mexico, *Chiang & al. F-2206* (ARIZ); JF284395, EF137750, JF284424, JF284440, JF284408, JF284379; (MEX). *G. boerhaviifolia* Schltdl.—Peru, *Plowman 5401* (US); JF284398–9, JF284371, JF284426, JF284442, JF284410, JF284381; (PERU). *G. boerhaviifolia* Schltdl.—Argentina, Prov. Catamarca, *Bernardello 894* (CORD); FJ444896, EF137751, HM195022, HM194982, HM195002, FJ444876; (ARG-CAT). *G. boerhaviifolia* Schltdl.—Argentina, Prov. Formosa, *Miller & al. 05-20* (NY); JF284397, JF284370, JF284422, JF284438, JF284406, JF284377; (ARG-FOR). *G. boerhaviifolia* Schltdl.—Argentina, Prov. La Rioja, *Barboza 594* (CORD); JF284396, JF284369, –, –, –, (ARG-LAR). *G. duplicata* Arnott—Argentina, Prov. Catamarca, *Miller & al. 04-55* (NY); JF284402–3, JF284373, JF284428, JF284444, JF284412, JF284383; (ARG-CAT). *G. duplicata* Arnott—Argentina, Prov. Chaco, *Barboza 526* (CORD); JF284400–1, JF284372, –, –, –, (ARG-CHA). *G. duplicata* Arnott—Argentina, Prov. Santa Fe, *Bernardello & Vesprini 898* (CORD); JF284391, JF284366, JF284427, JF284443, JF284411, JF284382; (ARG-SFE). *G. duplicata* Arnott—Argentina, Prov. Santa Fe, *Miller & al. 05-12* (NY); JF284392, JF284367, –, –, –, (ARG-SFE). *G. obtusa* Arnott—Argentina, Prov. Catamarca, *Bernardello 891* (CORD); FJ444897, JF284364, JF284429, JF284445, JF284413, FJ444877; (ARG-CAT). *G. obtusa* Arnott—Argentina, Prov. Catamarca, *Miller & al. 04-58* (NY); JF284389, JF284363, –, –, –, (ARG-CAT). *G. obtusa* Arnott—Argentina, Prov. La Rioja, *Barboza 543* (CORD); JF284404–5, JF284374, JF284430, JF284446, JF284414, JF284384; (ARG-LAR). *G. obtusa* Arnott—Argentina, Prov. San Juan, *Miller & al. 04-113* (NY); JF284390, JF284365, –, –, –, (ARG-SJU). *Lycium americanum* Jacq.—Argentina, *Barboza 525* (CORD); FJ444898, DQ124502, FJ189606, FJ189735, FJ189668, FJ189630; *L. arenicola* Miers—South Africa, *Venter 647* (BLFU); FJ444899, JF284375, JF284431, JF284447, JF284415, FJ444878; *L. berlandieri* Dunal—Arizona, U.S.A., *Miller 01-1* (ARIZ); FJ444901, DQ124506, JF284432, JF284448, JF284416, FJ444880; *L. carolinianum* Walt.—Texas, U.S.A., *Hempel 843* (TAIC); FJ444904, JF284376, GQ301192, GQ301193, GQ301194, FJ444883; *L. cooperi* A. Gray—Arizona, U.S.A., *Miller 97-1* (ARIZ); FJ444906, JF284361, JF284433, JF284449, JF284417, FJ444884; (AZ). *L. cooperi* A. Gray—California, U.S.A., *Miller & Levin 08-27* (RSA); JF284388, JF284362, –, –, –, (CA). *L. elongatum* Miers—Argentina, *Bohs 2940* (UT); FJ444908, DQ124520, JF284434, JF284450, JF284418, FJ444885; *L. macradon* A. Gray—Arizona, U.S.A., *Miller 97-21* (ARIZ); FJ444914, DQ124530, HM195020, HM194980, HM195000, FJ444889; (AZ). *L. nodosum* Miers—Argentina, *Barboza 515* (CORD); FJ444915, EF137783, FJ189617, FJ189746, FJ189679, FJ189641; *L. pallidum* Miers—Arizona, U.S.A., *Miller 97-20* (ARIZ); FJ444916, DQ124534, JF284435, JF284451, JF284419, FJ444890; (AZ). *L. pallidum* Miers—California, U.S.A., *Miller & Levin 08-31* (RSA); JF284387, JF284360, JF284436, JF284452, JF284420, JF284385; (CA). *L. parishii* A. Gray—Arizona, U.S.A., *Miller 97-22* (ARIZ); FJ444917, DQ124535, FJ189619, FJ189748, FJ189681, FJ189643; *L. puberulum* A. Gray—Texas, U.S.A., *Levin 97-6* (ARIZ); FJ444918, JF284359, FJ189620, FJ189749, FJ189682, FJ189644; (TX). *L. puberulum* A. Gray—Texas, U.S.A., same location as *Levin 97-6* (ARIZ); JF284386, JF284358, –, –, –, (TX). *L. shockleyi* A. Gray—Nevada, U.S.A., *Miller 98-1* (ARIZ); FJ444920, JF284357, FJ189624, FJ189753, FJ189686, FJ189648; (NV). *L. villosum* Schinz—South Africa, *Venter 652* (BLFU); FJ444924, DQ124547, FJ189626, FJ189755, FJ189688, FJ189650; *L. vimineum* Miers—Argentina, *Bernardello & Vesprini 896* (CORD); FJ444925, EF137796, JF284437, JF284453, JF284421, FJ444894; *Phrodus microphyllus* (Miers) Miers—Chile, *Miller & al. 04-92* (NY); FJ444928, EF137801, FJ189627, FJ189756, FJ189689, FJ189651. — OUTGROUP: *Nolana werdermannii* I.M. Johnst.—Chile, *Miller & al. 04-77* (NY); FJ444927, EF137799, FJ189604, FJ189733, FJ189666, FJ189628.