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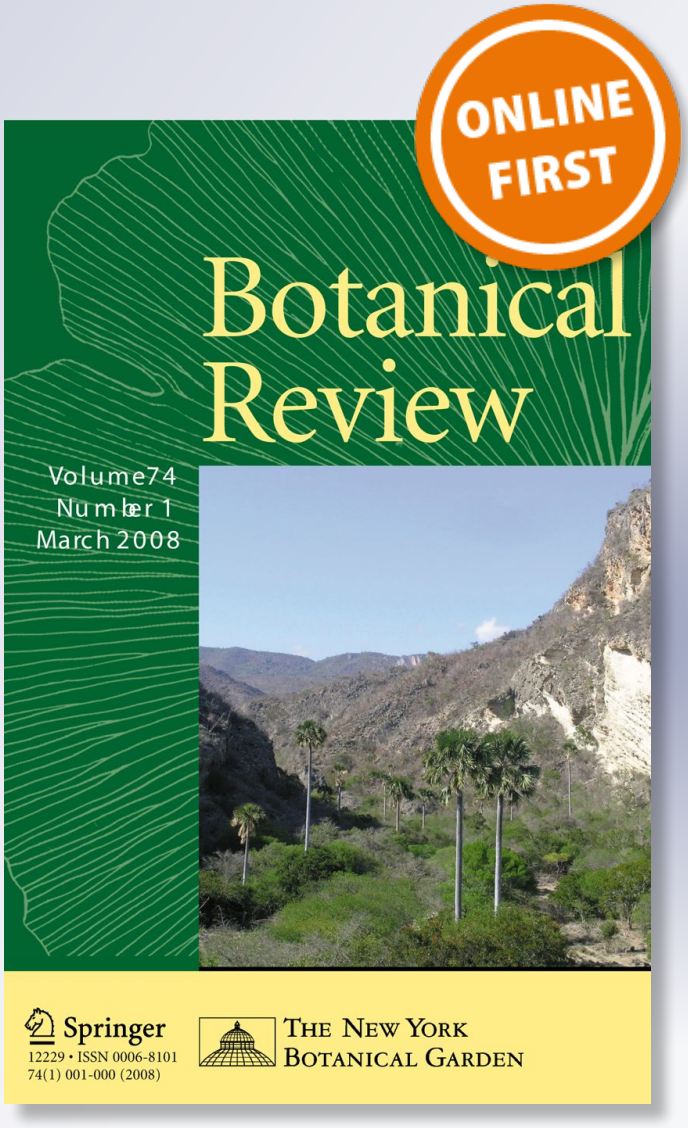

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Allotetraploids in Patagonia with Affinities to Western North American Diploids: Did Dispersal or Genome Doubling Occur First?

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Abstract Amphitropical disjunct distributions between western North America and western South America have intrigued botanists for over a century. Here, specific examples of migration and speciation are investigated using herbaceous species from the phlox family (Polemoniaceae) as a model for considering the timing of dispersal relative to speciation. Comparative DNA sequencing reveals that, in *Collomia* and *Navarretia*, the South American species are allopolyploids, suggesting either two dispersals prior to the allopolyploidization event for each species with subsequent extirpation of the diploid progenitors from South America, or allopolyploid formation prior to dispersal with extirpation of these polyploids from North America. Divergence time estimates support a Pliestocene dispersal hypothesis and sequence data indicate that, at least in *Collomia*, hybridization of the diploid progenitors occurred in South America.

Resumen Las distribuciones anfitropicales disyuntas entre el oeste de América del Norte y el oeste de América del Sur han intrigado a los botánicos durante más de un siglo. Aquí se investigan ejemplos específicos de migración y especiación usando especies de la familia del Flox (Polemoniaceae) como un modelo para considerar el efecto del orden temporal entre dispersión y especiación. La comparación de secuencias de ADN revela que las especies sudamericanas de *Navarretia* y *Collomia* son alopoliploides y sugiere para cada una de ellas, o bien dos eventos de dispersión anteriores a la formación del alopoliploide, seguido por la desaparición de los progenitores diploides en América del Sur, o bien la formación del alopoliploide antes de la dispersión, y la extinción de los progenitores diploides en América del Norte. La estimación de los tiempos de divergencia apoya la hipótesis de una dispersión durante el Pleistoceno, y los datos de las secuencias de ADN indican que, al menos en el caso de *Collomia*, la hibridación de los progenitores diploides ocurrió en América del Sur.

Keywords Allopolyploidy · Amphitropical disjunction · *Collomia biflora* · Long distance dispersal · *Navarretia involucrata* · Polemoniaceae

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Introduction

Pattern

Of many trans-oceanic biogeographic patterns, the amphitropical disjunct distribution of plant taxa between western North America and western South America has intrigued botanists for over a century (e.g., Gray & Hooker, 1880; Bray, 1898, 1900; Grant, 1959; Raven, 1963; Carlquist, 1966, 1983). The floras between the temperate regions of these two continents are far from homogenous, yet the cumulative result of chance events over thousands of millennia has resulted in a discernable pattern of disjunct relationships. Raven (1963) compiled over 130 instances of species or species pairs with established distributions in temperate regions on both continents that are separated by some 7000 km. Long distance dispersal is the favored hypothesis for diaspore movement in most instances (see also Thorne, 1972; Carlquist, 1983), and in the majority of cases, North America has served as the source and South America the sink. Many of these disjunct species groups share common characteristics (Raven, 1963) that function as preadaptations favoring establishment. For example, temperate disjuncts tend to be annuals from open habitats, primarily self-pollinating, and have seed, fruit, or other diaspore characteristics that aid in their attachment to migratory birds (Cruden, 1966; Carlquist, 1983). In a recent review, Wen and Ickert-Bond (2009) characterized the amphitropic disjunct pattern of the Americas as common and well-known, with a non-comprehensive list of 24 studies that have addressed this pattern to some measure within particular plant groups.

Process

Apart from pattern, processes play an essential role in the establishment and ultimate fate of dispersed lineages. Established disjunct species evidence diaspore movement and provide a baseline for assessing the frequency of dispersal—but this baseline recognizably fails to reflect dispersal with precision. Undoubtedly, many dispersal events failed to establish or have since been extirpated. Others, such as in the *Gilia laciniata* complex, have given rise to new species following dispersal (Morrell et al., 2000). Multiple dispersals are evident in yet other genera, such as *Sanicula* (Vargas et al., 1998), and multiple dispersals as well as speciation in *Tiquila* (Moore et al., 2006). Phylogenetic and biogeographic studies aided by DNA sequence data are illuminating the processes of speciation associated with long distance dispersal, a combination that, as noted by Mummenhoff and Franzke (2007), provides spectacular examples of evolutionary biology.

Allopolyploidy is recognizably an important mode of speciation in plants (Otto & Whitton, 2000; Soltis & Soltis, 2000, 2009). Stebbins (1942, 1947) early championed the value of allopolyploids in understanding plant biogeography. Raven (1963:157) reviewed some instances of ploidal levels that vary within particular species groups between North and South America and provided chromosome data for many other disjunct species pairs and groups. An important question when allopolyploidy is encountered in conjunction with intercontinental disjunct distributions is the timing of allopolyploidization relative to dispersal (Fig. 1). In a recent discussion, examples of both pre- and post-dispersal allopolyploidization were presented (Mummenhoff &

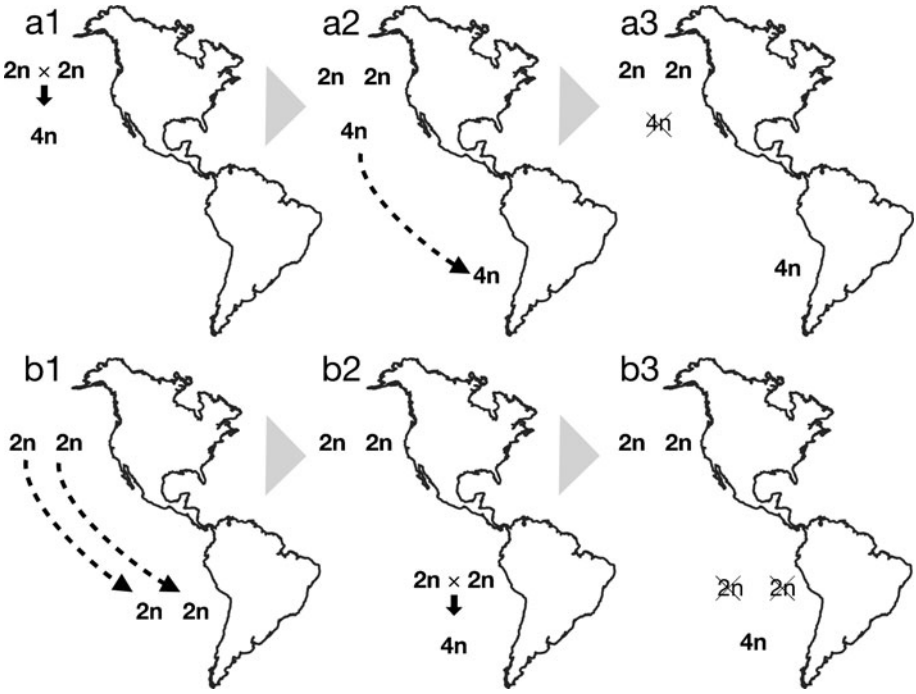

Fig. 1 Alternative hypotheses for the relative timing of dispersal vs allopolyploid formation for disjunct species groups where the allotetraploid species occurs in isolation from its apparent diploid progenitors. a1 = allopolyploid formation. a2 = dispersal. a3 = extirpation. b1 = dispersal; b2 = allopolyploid formation. b3 = extirpation. The pattern shown in a1–a3 requires three events (two if extirpation, represented by the crossed out entity, is not required); the pattern shown in b1–b3 requires five events (three if extirpation is not required)

Franzke, 2007). In *Microseris*, allopolyploidization is hypothesized to have occurred prior to dispersal from North America, followed by extirpation of the allotetraploid and one parent in North America and subsequent diversification of the allotetraploid in Australia and New Zealand (Vijverberg et al., 2000). Australian *Nicotiana* are similarly thought to have experienced allopolyploidization prior to their dispersal from South America (Goodspeed, 1954; Aoki & Ito, 2000). In contrast, the dispersal of diploids to Australia, subsequent allopolyploidization, and extirpation of the dispersed diploids from Australia is hypothesized for *Lepidium* (Mummenhoff et al., 2004). In *Lepidium*, the hypothesized timing of allopolyploidization relative to dispersal is supported by evidence that the diploid progenitors are presently established on different continents. For *Microseris* and *Nicotiana*, in contrast, the favored hypothesis is equivocal with respect to available data.

Polemoniaceae

The phlox family exhibits several instances of temperate amphitropical disjunct distributions unambiguously attributable to multiple dispersal events, both with and without subsequent speciation. Polemoniaceae contains some 400 species among 26 genera (Porter & Johnson, 2000). The temperate subfamily, Polemonioideae (22 genera/340 species) is predominantly western North American in distribution, with

some 70 % of this distribution occurring within California, USA. Fifteen Polemonioideae species from nine genera occur in South America (discounting putatively recently naturalized species such as *Collomia grandiflora*; Puntieri & Brion, 2005), primarily along the west coast, which shares many climatic and ecological similarities to western North America. Two species, *Microsteris gracilis* and *Polemonium micranthum*, are conspecific with their North American counterparts, while the rest are unique to South America. This uniqueness implies either stasis in South America following dispersal with concomitant extirpation (or lack of discovery) in North America, or sufficient change in South America following dispersal to diagnose these lineages now as distinct species. The origins of *Gilia valvidensis* and *Gilia laciniata* have been explored by Morrell et al. (2000), and relationships of *Leptosiphon* (*Linanthus*) *pusillis* by Bell and Patterson (2000).

Here, we investigate the origins of *Collomia biflora* and *Navarretia involucrata*, South American species from sister genera. In both instances, a single species is thought to exist in South America that is unique relative to any of the described North American diversity. This diversity is sufficiently rich (14 and 34 species, respectively) to support a North American source and South American sink dispersal hypotheses for these two species. This hypothesis is further supported by early phylogenetic analyses (Spencer & Porter, 1997; Johnson & Johnson, 2006). Whereas *Collomia biflora* has long been recognized to be polyploid, *Navarretia involucrata* has been assumed to be diploid. We examine the pattern of diversification and timing of diversification relative to dispersal in both species.

Materials and Methods

Phylogenetic relationships in *Collomia* have been recently reconstructed using a variety of chloroplast and nuclear markers (Green, 2010; Green et al., in prep.), but the pattern and processes detailed here were only tangentially discussed. Based on these analyses, we confined the sampling here to the annual, linear leaved *Collomia* species, with *C. tracy*i and *C. tinctoria* designated as the outgroup. This sampling excludes the perennial species (*Collomia* section *Collomiastrum*) and the annual species with more than one ovule per locule (*Collomia* section *Courtoisia*), neither of which are important to understanding the amphitropical disjunct distribution of this genus. The bulk of our data for *Collomia* comes from Green (2010), with additional populations of *Collomia biflora*, *Collomia grandiflora*, and *Collomia linearis* included here. In total, our sampling includes 34 populations representing nine species (Appendix I). Based on analyses of ITS sequences and morphology (Spencer & Porter, 1997) and ongoing work in our lab, we confined our sampling of *Navarretia* to the monophyletic *Navarretia* section *Navarretia*, with *Navarretia tagetina*, the sister to the remaining species in this section, specified as the outgroup. We sampled 25 populations representing all 11 named species in this section plus three additional species we have recently discovered that will be formally named elsewhere (Johnson et al., in prep).

For all sampled populations of *Collomia* and *Navarretia*, we sequenced four chloroplast regions (5′*trnK* intron and adjacent 5′ two-thirds of the *matK* gene; *trnL–trnL* intron and *trnL–trnF* intergenic spacer; *trnS–trnG* intergenic spacer; and

the *psbM–trnD* intergenic spacer) and the nuclear ITS-1, 5.8S, and ITS-2 region using primers as specified in Johnson et al. (2008). We used a somewhat reduced sampling (27 populations of *Collomia* for all 9 species, and 19 populations of *Navarretia* for 10 of the 11 named species and all three unnamed species) for low copy nuclear genes, primarily due to low quality DNA from herbarium specimens in the excluded samples; these low copy nuclear genes include portions of *PISTILLATA (PI)* and *g3pdh* in *Collomia*, and *PI* alone in *Navarretia*. Primers for *g3pdh* follow Strand et al. (1997), while primers developed in the Johnson lab by Weese (2004) for Polemoniaceae were used to access a 5′ portion of the *PI* gene, with internal primers developed as needed. These primers include PI-7F (5′-AGAGGAAAGATTGAGATAAAGAGG-3′) and PI-450R (5′-TTCTCTTCCTCCARCATCATT-3′) used for amplification and sequencing, with additional internal primers PI-900R (5′-ATCATTCTCTTTCTTGATCC-3′), PI-880F (5′-ATCCATGGACAGATCTGGTAA-3′), PiV1F (5′-CATAGGTTGGTTGAGAT CTTGG-3′), and PiV1R (5′-CTATCATTCTCTTTCTTGATCCTG-3′) used for sequencing if needed. We followed the methods of Johnson and Johnson (2006) for DNA extraction, PCR amplification, sequencing, and cloning (for the low copy nuclear genes and some ITS).

We compiled individual matrices for *Collomia* and *Navarretia* for the combined cpDNA regions, the ITS region, and each low copy nuclear gene. We manually aligned sequences within these matrices using Se-Al 2.0 (Rambaut, 2002), trimmed a few poly-N repeats with hypervariability (e.g., two introns in the *PI* gene contain poly CT strings of hypervariable length), and applied simple indel coding using SeqState (Müller, 2005). We made no attempt to analyze the matrices combined because visual comparison of results among the different matrices provides a stronger interpretive base for addressing dispersal hypotheses than would a single combined analysis of the chloroplast and putatively unlinked nuclear genes.

We performed parsimony analyses on each matrix using PAUP*4.0b10 (Swofford, 2002) with equal weighting of all characters, heuristic searches employing 1000 replications of random addition, TBR branch swapping, and collapsing branches with minimum lengths of zero (amb-). We assessed branch support via 100,000 replications of fast bootstrapping, and reconstructed base substitutions using DELTRAN optimization. DELTRAN optimization favored the reconstruction of many substitution between the outgroup and ingroup rather than along the two branches attached to the root, which better matched patterns of variation observed in these genera when matrices included broader (i.e., more phylogenetically distant) taxon sampling (unpubl. data) using either ACCTRAN or DELTRAN optimization.

We assessed the presence of a molecular clock in the cpDNA and ITS sequences using the likelihood ratio test and then calculated a rough approximation for the timing of dispersal in *Collomia* by multiplying the divergence between *C. biflora* and *C. grandiflora* (cpDNA), *C. biflora* and *C. linearis* (ITS), and *C. grandiflora* from Bariloche, Argentina, and *C. grandiflora* from the USA (cpDNA and ITS) by a range of published substitution rates for noncoding cpDNA (0.26–0.92 % Myr-1; Alsos et al., 2005; Mummenhoff et al., 2004) and ITS (0.172–0.834 % Myr-1; Kay et al., 2006) sequences. We estimated the average divergence between these taxa from branch lengths obtained from maximum likelihood analyses performed in PAUP* after first estimating the best model and parameters (using AIC) with Modeltest 3.7 (Posada & Crandall, 1998).

Results

Collomia

Parsimony analyses recovered three minimum length trees from the cpDNA matrix, two from the ITS matrix, four from the *PI* matrix, and one from the *g3pdh* matrix. Focusing solely on the inferred affinities of the South American species, *Collomia biflora* appears with strong bootstrap support (100 %) in a clade with *C. grandiflora* in the cpDNA trees. A single ITS copy was recovered for *C. biflora* via direct sequencing and also via sampling of cloned PCR fragments; *C. biflora* appears with marginal bootstrap support (68 %) in a clade with *C. linearis* in the ITS trees. In the *PI* and *g3pdh* trees, the two homeologs recovered from cloning PCR fragments of *C. biflora* appear separately in clades with *C. linearis* and *C. grandiflora*. With *PI*, both clades are supported with high bootstrap values (100 %), whereas with *g3pdh*, the *C. biflora* - *C. grandiflora* clade has moderate support (72 %) and the *C. biflora* - *C. linearis* clade has only weak support (<50 %). A population of *C. grandiflora* putatively naturalized in Argentina appears more closely related to the homeolog of *C. biflora* derived from a *C. grandiflora* like ancestor than to *C. grandiflora* itself in the *PI* and *g3pdh* trees.

Dispersal time estimates range from 137,000–485,000 yr based on the average cpDNA divergence between *C. biflora* and the sister clade of *C. grandiflora*; 92,000–328,000 yr based on the average cpDNA divergence between *C. grandiflora* from Bariloche, Argentina and the other *C. grandiflora* accessions within its same clade; 233,000–1,130,000 yr based on average ITS divergence between *C. biflora* and *C. linearis*; and 388,000–1,881,000 yr based on the average ITS divergence between *C. grandiflora* from Bariloche, Argentina and the other accessions of *C. grandiflora*.

Navarretia

Parsiomony analyses recovered two minimum length trees from the cpDNA matrix, five from the ITS matrix, and three from the *PI* matrix. The South American species *Navarretia involucrata* is placed isolated as sister to the remaining species exclusive of *N. tagetina* in the cpDNA trees (this result holds true when species outside section *Navarretia* are used to root the tree as well; results not shown). Similar to *C. biflora*, a single ITS copy was recovered from both direct sequencing and sequencing of cloned PCR fragments in *N. involucrata*; this sequence places *N. involucrata* with strong bootstrap support (98 %) within a largely unresolved clade also containing *N. prostrata*, *N. myersii*, *N. leucocephela*, and *N. willamettensis*. For the *PI* gene, two homeologs were recovered from cloning PCR fragments not only in *N. involucrata*, but also in *N. propinqua*, *N. saximontana*, *N. willametensis*, and *N. leucocephela*. Focusing again on the South American species, one homeolog from *N. involucrata* appears within a weakly supported clade including *N. prostrata*, *N. fossalis*, *N. leucocephela*, and *N. willamettensis*; within this clade, the placement of *N. involucrata* as sister to *N, prostrata* is strongly supported (99 % bootstrap). The other *N. involucrata* homeolog is placed weakly (<50 % bootstrap) in a clade that includes two recently discerned but as yet unnamed species from North America.

Discussion

Origins - *Collomia biflora*

Collomia biflora is a common annual foothills species along the Andean corridor in Argentina, Chile, and Bolivia. It is more or less upright in habit, bears flowers in head-like inflorescences, and is notable for having bright red adaxial corolla lobes. *Collomia biflora* is morphologically plastic and can occur in unbranched forms terminating in a single head-like inflorescence, or in much branched forms, as wide as tall, with many flowering heads. This plasticity has resulted in some nomenclatural confusion, but is not unexpected given that its closest extant relatives identified here, *C. linearis* and *C. grandiflora*, are notably morphologically plastic as well (e.g., Wilken, 1978, 1982). In North America, *C. linearis* is the most widespread *Collomia* species, ranging from the west to the east coast of the United States and Canada, and absent from only the "southern states" (USDA Plants database, 2010). As with the majority of annual Polemoniaceae, it is particularly abundant in western North America and can be found in virtually any mountain range within its distribution. It has pink (seldom white) corolla lobes and occupies an ecological zone parallel to that inhabited by *C. biflora* in South America. *Collomia grandiflora* is also widely distributed in Western North America and possesses, next to *C. linearis*, the broadest distribution of any *Collomia* species. It co-occurs with *C. linearis* in many locations, and is unique in the genus for producing salmon to yellow chasmogamous flowers as well as minute whitish cleistogamous flowers. Underscoring the morphological plasticity in these North American species, Wherry (1944) lists five subspecific taxa for *C. linearis* and five for *C. grandiflora*, none of which are in current use.

Morphological plasticity in *Collomia biflora*, compounded by the rule of priority in the Botanical Code, has led to some nomenclatural confusion involving the identity of *Collomia* diversity in South America. Eight names have been applied to what we here consider a single species. *Collomia linearis* (Cav.) Nutt, *C. bilfora* (Ruiz & Pav.) Brand, and *C. cavanillesii* Hook. & Arn. are the most commonly applied names in botanical literature, while *C. coccinia* Lehm. ex Lindl. remains popular in the horticultural trade. Wherry (1944), in opposition to Philippi (1895), argued that the epithat '*linearis*' belongs to the North American species so named when *Collomia* was erected by Nuttall (i.e., *Collomia linearis* Nutt.), rather than to the species from South America originally named in the genus *Phlox* (i.e., *Phlox linears* Cav. ≡ *Collomia linearis* (Cav.) Nutt.). This conclusion is supported by the current code (ICBN Article 11.4; McNeill et al. 2006). Wherry also cast doubt as to the true identity of *C. biflora* given that the protologue describes a plant with paired blue flowers and considered *C. cavanillesii* the correct name at the species level for South American *Collomia*. Having not yet viewed the type for *C. biflora* (as neither did Wherry), the lead author followed this line of reasoning in referring South American material to *C. cavanillesii* in an earlier publication (Johnson & Johnson, 2006). Other authors have also suggested South American *Collomia* likely represents but a single species (Reiche, 1910; Wilken et al., 1982). Soriano (1947) disagreed with Wherry regarding the validity of the combination *C. linearis* (Cav.) Nutt, but followed Wherry in restricting *C. biflora* to a species with non-red, solitary or paired flowers, noting that most of the material referred to *C. biflora* actually corresponds to *C.*

linearis (Cav.) Nutt (with *C. cavanillesii* considered by him a synonym of this species). Grant (1959) took a different approach, and recognized both *C. biflora* and *C. cavanillesii* in South America, with suggested affinities to *C. grandiflora* and *C. linearis*, respectively (with corolla size an apparent distinguishing feature based on specimen annotations at CONC). A recent worker, E. Servat, reviewing specimens at SI recognized *C. cavanillesii*, *C. biflora*, and *C. coccinea*, distinguishing the three species by branching, bract and leaf similarity, and leaf lobing. This confused nomenclature deserves attention and is being addressed in conjunction with a thorough examination of types (Johnson et al. in prep.). In approaching this study, the authors' working hypothesis is that just two named *Collomia* species are presently in South America: *Collomia biflora* circumscribing tetraploid material typically with red corolla lobes regardless of corolla size and plant habit, and *Collomia grandiflora* circumscribing diploid material with salmon flowers reported as naturalized in Argentina (Puntieri & Brion, 2005). We were unable to include material of the "yellow collomia" from Chile referred to *C. cavanillesii* (Hoffmann et al., 1998) but discuss some possible relationships below.

Sugiura (1936) and Flory (1937) report *Collomia coccinea* as tetraploid (synonymized to *C. cavanillesii* by Grant, 1959 and here treated as *C. biflora;* a direct tetraploid count for *C. biflora* also exists; Wilken, 1986). Raven (1963) records a diploid count for *C. cavanillesii*, but it is uncertain where this count originated or if it is a typographical error given that he acknowledged Grant (1959 and pers. comm.) as his source of information for Polemoniaceae. Following Grant (1959), Raven recognized both *C. cavanillesii* and *C. biflora* in South America, but reversed their putative sister species to *C. grandiflora* and *C. linearis*, respectively. Following this taxonomy, two sister pair relationships between single North American species and single South American species requires two dispersal events to South America within *Collomia*. Also, given Grant's knowledge of the polyploid condition in material he referred to *C. cavanillesii*, an affinity between this material and only *C. linearis* would imply autopolyploidy as the mechanism of genome doubling in the South American species. Johnson and Johnson (2006) first demonstrated that *Collomia biflora* was an allotetraploid using evidence from nuclear *idhA* and *idhB* genes.

An allopolyploid origin for *Collomia biflora* opens the door for additional hypotheses of dispersal relative to diversification. Allopolyploidization could have occurred in North America prior to dispersal, with a single dispersal event to South America followed by extirpation of the allopolyploid in North America (three events: allopolyploidization, dispersal, extirpation; Fig. 1a). Alternatively, two diploid species phylogenetically near *C. grandiflora* and *C. linearis* could have dispersed to South America with the allopolyploidization event occurring in South America followed by the extirpation of the two diploid species (five events: dispersal twice, allopolyploidization, extirpation twice; Fig. 1b). Eliminating extirpation as necessary components of either hypothesis still favors allopolyploidization prior to dispersal from a simple parsimony perspective (Fig. 1a; two steps, versus three in Fig. 1b).

Though less parsimonious, the second hypothesis of allopolyploidization following dispersal is supported by the data we present here. We sampled the naturalized population of *Collomia grandiflora* reported from Bariloche, Argentina (Figs. 2 and 3). This population occurs along the roadside in disturbed ground. The population is

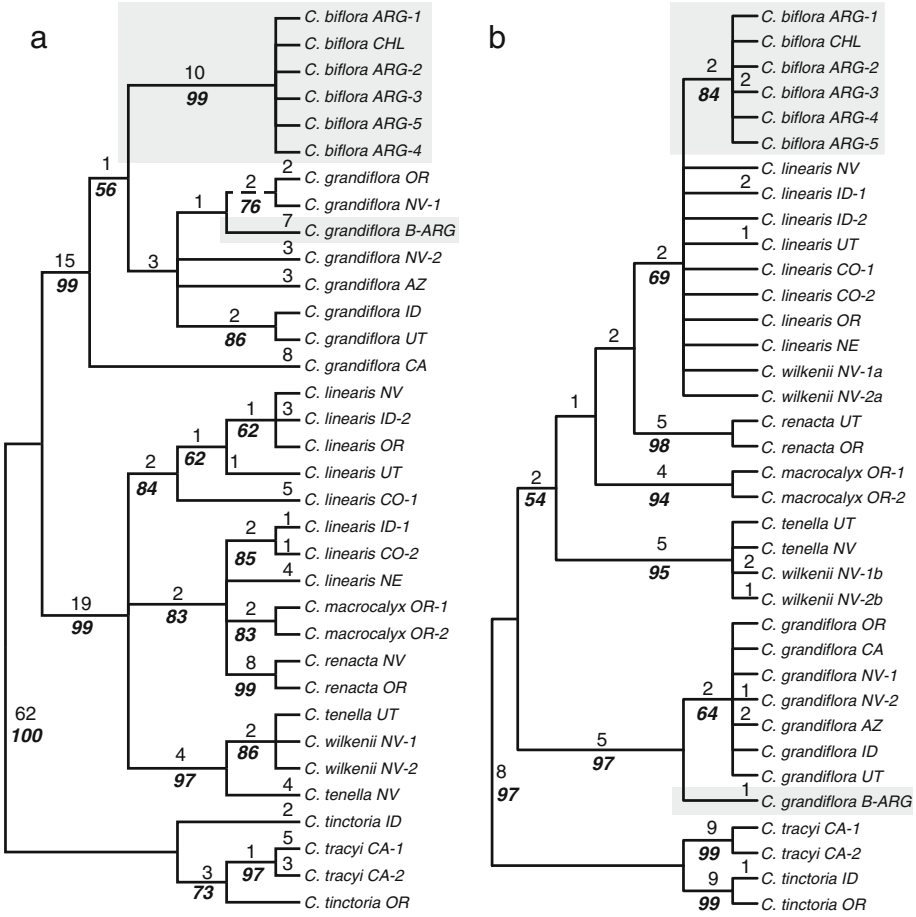

Fig. 2 Shortest trees recovered from parsimony analyses of *Collomia* DNA sequence matrices. Numbers above branches represent combined base substitutions and unique indel events reconstructed via Deltran optimization; numbers below branches represent bootstrap support values. Branches not found in all shortest trees are represented by dashed lines. Geographic distribution of samples is represented by three letter codes for Argentina (ARG), Chile (CHL) or, within the USA, using the standard postal abbreviation code for each state, and correspond to designations included in Appendix I. "B-ARG" designates the population of *Collomia grandiflora* sampled from Bariloche, Argentina. a = One of three trees of 195 steps recovered from the combined cpDNA matrix (CI=0.94; RI=0.99). b = One of two trees of 70 steps recovered from the ITS matrix (CI=0.81, RI=0.94)

small, was relatively recently discovered, and is the only reported instance of this species in Argentina (Puntieri & Brion, 2005). The physical description of the plants, including their flowers, match *C. grandiflora* as it is occurs in the United States. Furthermore, this species has been reported as naturalized in Europe (Pysek et al., 2002) and Australia (Hussey et al., 1997). The conclusion by Puntieri and Brion (2005) that the Bariloche population represents a naturalized introduction is thus logical; nevertheless, DNA sequence data indicate this population more likely represents a remnant of a much earlier dispersal event that established in South America prior to the allopolyploid formation of *C. biflora*.

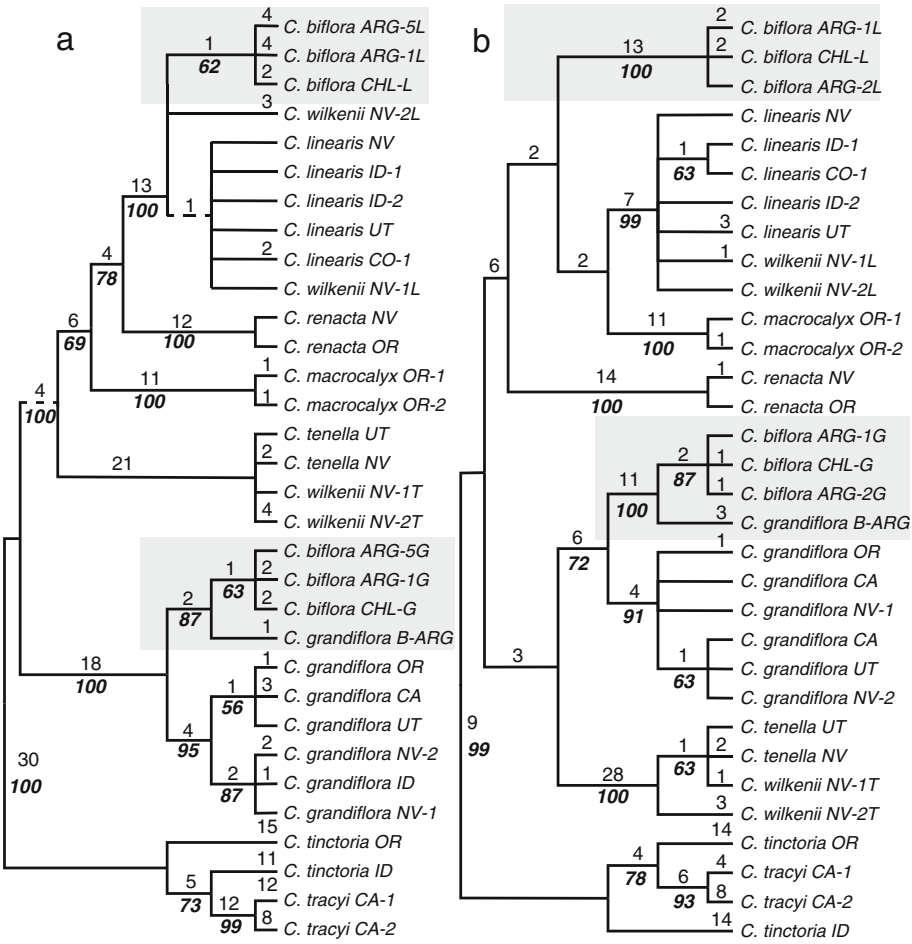

Fig. 3 Shortest trees recovered from parsimony analyses of *Collomia* DNA sequence matrices. Interpretation of details follows Fig. 2. A = One of four trees of 239 steps recovered from the *PI* matrix (CI=0.90; RI=0.96). b = Single tree of 193 steps recovered from the *g3pdh* matrix (CI=0.82, RI=0.93)

Specifically, *PI* and *g3pdh* sequences recover the Bariloche *C. grandiflora* as sister to *C. biflora's* maternal homeolog rather than among North American *C. grandiflora* (Fig. 3). Both low copy nuclear genes reveal synapomorphies between the Bariloche accession and *C. biflora*, as well as the lack of synapormophies in the Bariloche accession that unite the remaining accessions of *C. grandiflora* into a well supported clade. The ITS region in *C. biflora* has been homogenized through gene conversion or loss of loci to the single repeat typical of its paternal parent, as has been documented in some allotetraploids (e.g., Dierschke et al., 2009). Chloroplast DNA places the Bariloche accession, with some divergence, in a clade of *C. grandiflora* accessions from disparate locations in the United States that also includes *C. biflora*. Though cpDNA does not unite the Bariloche accession with *C. biflora* exclusive of *C. grandiflora*, the pattern of variation with both the Bariloche accession and *C. biflora* nesting within *C. grandiflora* indicates that the species that dispersed to South

America giving rise to *C. biflora's* maternal line was *C. grandiflora* itself. The yellow collomia from Metropoliana Chile (Hoffmann et al., 1998) may represent additional populations of this relic or derivatives from it, a hypothesis we are actively pursuing with recently obtained material. Relictual populations of *C. linearis* are unknown; specimens from CONC with smaller flowers and description of interior corollas 'deep pink' are late season plants. The lead author revisited one population earlier in the season and found only red, average-sized flowers (with 'red' itself being subjective). Persistent or not, our data indicate that the allopolyploidization event giving rise to *C. biflora* occurred in South America, and thus at least two dispersals of diploid species from North America also occurred.

Origins - *Navarretia involucrata*

Navarretia involucrata inhabits vernal pools and seasonally moist depressions along the Andean corridor in Chile and Argentina. Plants are small, spreading, with small corollas and an autogamous breeding system. This habit and predisposition to seasonally moist habitats are shared with its North American relatives, particularly *N. leucocephela*, *N. prostrata*, *N. myersii*, and *N. fossalis*—species that form the 'core vernal pool clade' of Spencer and Porter (1997). Remaining members of section *Navarretia*, such as *N. tagetina*, *N. subuligera*, and *N. interexta* are considered facultative or marginal vernal pool species; they are often associated with moist depressions and other seasonally wet habitats, but are not obligately tied to vernal depressions as are the core clade members. Grant (1959) and Spencer and Porter (1997) suggested *N. leucocephela* ssp. *minima* was the likely progenitor of *N. involucrata*.

Prior to work in our lab, reticulation and allopolyploidization have been scarcely discussed in the context of *Navarretia*. Instead, diversification has been thought to be divergent and possibly, in the case of the core vernal pool species, driven by isolation following fragmentation of a mid to late Pliestocene vernal pool that once covered the central plain of California, or by dispersal from pool to pool accentuated by genetic bottlenecks and drift. Our data are consistent, however, with an allopolyploid origin of *N. involucrata* as well as the North American species *N. propinqua* (long considered a subspecies of the diploid *N. interetexta*), and possibly *N. willametensis* and at least some populations of *N. leucocephela*. Although our sampling is less dense in *Navarretia* compared to *Collomia*, these results indicate that allopolyploidy has occurred multiple times within the vernal pool clade, which makes discerning the timing of alloplolyploidization in *N. involucrata* relative to dispersal a matter of conjecture without a relictual diploid persisting in South America.

The paternal parent of *N. involucrata* belongs to the core vernal pool clade as evidenced by ITS sequences (Fig. 4), with particularly close affinities to *N. prostrata* as evidenced by *PI* sequences (Fig. 5). The maternal parent is ambiguous. ITS sequences again show complete homogenization to the paternal homeolog. Chloroplast DNA places *N. involucrata* clearly within section *Navarretia*, but as sister to all species except *N. tagetina* rather than as sister to a single species. *PI* places, but without substantial bootstrap support, the maternal homeolog of *N. involucrata* as sister to two unnamed North American species, but the divergence along branches from their hypothetical ancestor is long relative to the branch uniting these species,

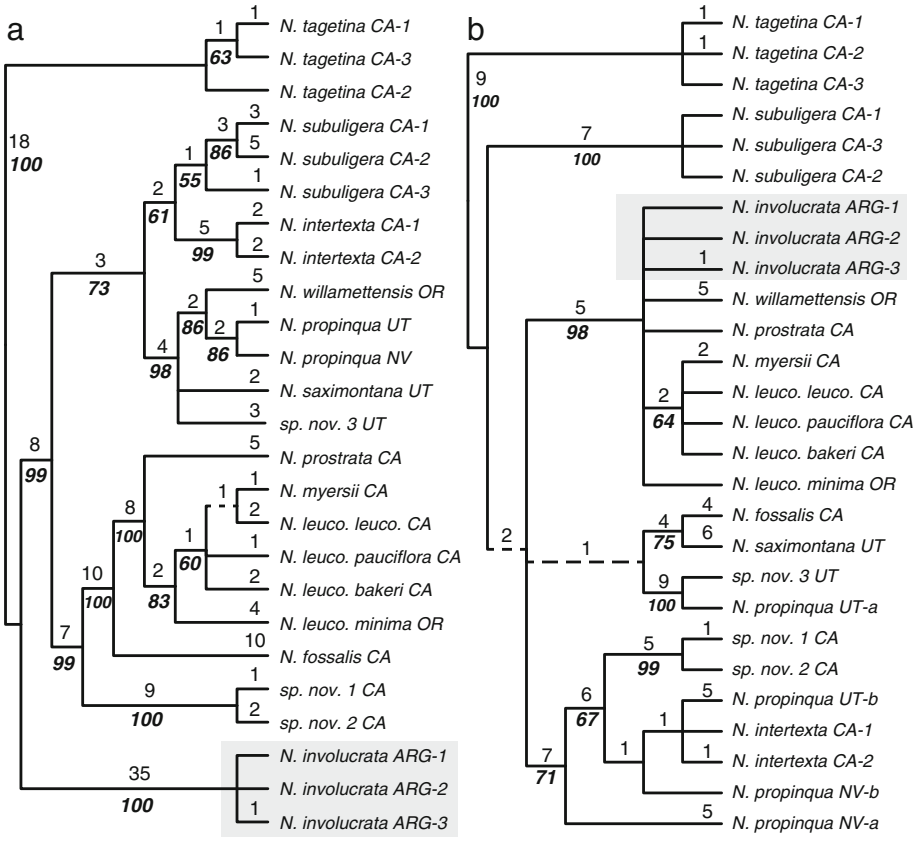

Fig. 4 Shortest trees recovered from parsimony analyses of *Navarretia* DNA sequence matrices. Interpretation of details follows Fig. 2. a = One of two trees of 176 steps recovered from the combined cpDNA matrix (CI=0.93; RI=0.97). b = One of five trees of 97 steps recovered from the ITS matrix (CI= 0.79, RI=0.90)

and trees three steps longer fail to resolve this relationship. Although we have initiated parallel studies with other low copy genes such as *g3pdh* and *idh*, our sampling is not yet sufficient to make inferences from those genes beyond confirming an allopolyploid pattern of variation in *Navarretia involucrata*.

We included the three as yet unnamed *Navarretia* discovered through our efforts, all native to North America, to see if sequence data implicated these taxa in the origins of *N. involucrata*. Though this does not appear to be the case, their discovery, combined with the discovery and naming of *N. saximontana*, *N. willametensis*, and a subspecies of *N. leucocephela* in the past decade (Spencer & Spencer, 2003; Björk, 2002) along with *N. myresii* (with two subspecies; Day, 1993, 1995) in the decade before that, indicates that our knowledge of species diversity in this portion of Polemoniaceae may yet be imperfect. Our sampling of *N. involucrata* from South America is recognizably limited and completely lacking in Chilean diversity. A diploid maternal parent or even closer paternal parent than *N. prostrata* may yet show up with additional sampling and closer attention to morphological detail on both continents.

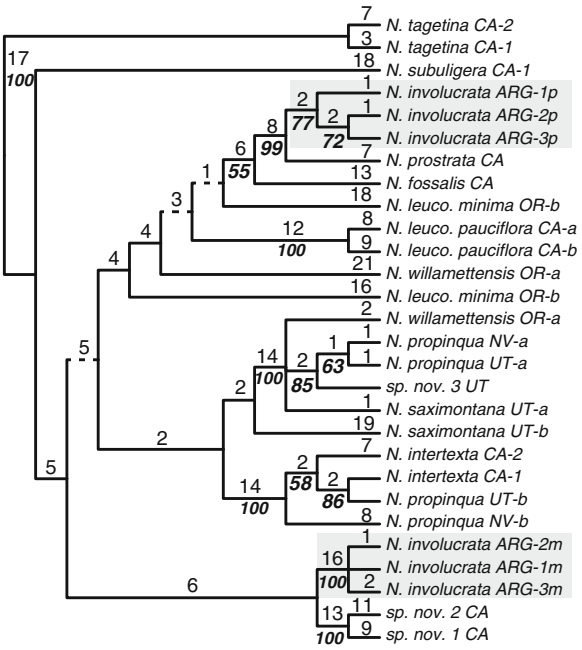

Fig. 5 One of three shortest trees recovered from parsimony analysis of *Navarretia PI* DNA sequences. Interpretation of details follows Fig. 2. Length = 327 steps (CI=0.75; RI=0.81)

Timing of Dispersal

Periods during which amphitropical regions shared the greatest ecological similarity, as well as fluctuating climatic regimes, are likely the times during which the greatest opportunity for dispersal and establishment existed (Axelrod, 1950; Raven, 1963). For the western North American-western South American disjunctions explored here, dispersal likely occurred in the Pliestocene (Raven and Axelrod 1978). Though rough and assumption-laden, our estimates of divergence times in *Collomia* derived from a range of nucleotide substation rates for both chloroplast and ITS sequences place the time of dispersal within the Pliestocene, with average values in the mid Pleistocene (Ionian period). Elsewhere in Polemoniaceae, Morrell et al. (2000) estimated a divergence time of 1–3 Myr in the South American *Gilia laciniata* complex, while *Leptosiphon pusillis* was estimated to have diverged 1–2 Myr from its North American relatives (Bell & Patterson, 2000). Thus, the divergences estimated here are within the range of divergence times estimated for relatives with similar disjunct distributions. We did not attempt to estimate a divergence time for *Navarretia* given extreme differences between the available maternal and paternal nodes as well as a paucity of change in the paternal line. However, vernal pools were common on both continents during the Pliestocene and a late Pliestocene/early Holocene dispersal hypothesis is compatible with available evidence.

In the absence of persistent diploid progenitors in the same region as an allopolyploid species, distinguishing the timing of allopolyploidization relative to dispersal is largely left to conjecture if the putative parents themselves

co-occur in a common region. From an events perspective, allopolyploidization prior to dispersal is more parsimonious (Fig. 1b), but factors attributed to the success of allopolyploids conceptually suggest the less parsimonious hypothesis should not be discounted. We list several such intrinsic factors here without exposition and refer readers to recent reviews for greater detail (Mummenhoff & Franzke, 2007; Soltis & Burleigh, 2009): 1) buffering effect of fixed heterozygosity; 2) allievement of inbreeding depression; 3) increased biochemical diversity via higher levels of heterozygosity leading to broader ecological tolerances; 4) genetic novelties via genome rearrangements enhancing the possibility for niche separation; and 5) the heightend contribution of all of these factors when the contributing genomes are themselves divergent.

Though successful long distance dispersal between North and South America is rare relative to extant plant diversity, the replicated pattern of modern plant distributions evidence the cumulative effects of such chance events. Fluctuating climatic conditions and reoccurring glacial cycles during the Pleistocene may well have favored the persistence of genetically rich allotetraploids over their diploid progenitors still recovering from post-dispersal genetic bottlenecks, just as polyploidy may have contributed to the success of genome-doubled lineages over diploids during the K-T mass extinction (Fawcett et al., 2009). Given the propensity of allotetraploids to be more successful than their diploid progenitors, their present distribution is more apt to reflect their site of origin. Otherwise, one must account for their formation, likely range expansion prior to dispersal (i.e., all else being equal, a wide-ranging species is more likely to participate in chance dispersal events than a geographically restricted species; Raven, 1963), and then extirpation in the same range where their diploid progenitors often still persist. The discovery in our data of diploid material plausibly relictual and persistent related to at least one of the diploid progenitors of *C. biflora* evidences both a post-dispersal allopolyploidization hypothesis in this species, as well as the greater success (based on current established range) of the allopolyploid over its diploid progenitors. Although no diploid "smoking gun" has yet been identified for *Navarretia involucrata*, we favor the hypothesis that it also was formed in South America following dispersal of its diploid progenitors.

While our study is not the first to distinguish the timing of allopolyploidization relative to dispersal, it contributes to a growing body of evidence documenting the post dispersal formation of allopolyploids in diverse lineages, including well studied examples such as *Tragapogon* (e.g., Ownbey, 1950; Lim et al., 2008; dispersal in the past century) and *Lepidium* (Mummenhoff et al., 2004; Dierschke et al., 2009; dispersal also during the Pliestocene). Post dispersal allopolyploidization may well be a general pattern in groups where multiple dispersals among close diploid relatives is followed by the establishment of contact zones, hybridization, and in many cases, an eventual decline in, or extirpation of, the diploid progenitors.

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Appendix I

GenBank accession numbers and voucher information. Acronyms following species names correspond to those used in Figs. 2 through 5. For *Collomia*, genbank numbers are given in the following order: *trnk*, *matK*, *trnL*, *trnS*, *psbM*, ITS, *PI*, *g3pdh*. For *Navarretia*, the same order was followed except for *g3pdh*, which was not included in this study. Semicolons are used to separate genes and commas are used to separate homeologs within genes for the polyploid species. All vouchers are housed at BRY unless indicated by an alternative herbarium within brackets.

Collomia biflora (Ruiz & Pav.) Brand—ARG1 = *Sersic s.n.* (DQ196463; DQ196886; DQ196955; DQ196934; HQ117098; DQ196896; HQ116874, HQ116875; HQ117235, HQ117236). ARG2 = [BAA 25056] (HQ911803; HQ911925; HQ911968; HQ911789; HQ911892; HQ911836; –-, –-; HQ911783, HQ911784). ARG3 = *Belgrano et al. 462* (HQ911804; HQ911926; HQ911969; HQ911790; HQ911893; HQ911837; –-, –-; –-, –-). ARG4 = *Belgrano et al. 519* (HQ911806; HQ911928; HQ911971; HQ911792; HQ911895; HQ911838; –-, –-; –-, –-). ARG5 = *Belgrano et al. 532* (HQ911805; HQ911927; HQ911970; HQ911791; HQ911894; HQ911839; HQ911962, HQ911963; –-,—). CHL = *Thibauat et al. 156* (HQ116975; HQ116935; HQ117058; HQ117020; HQ117099; HQ116834; HQ116876, HQ116877; HQ117237, HQ117238). ***Collomia grandiflora*** Douglas ex Lindl.—AZ = *Christy 449* [RSA] (HQ911808; HQ911930; HQ911973; HQ911794; HQ911897; HQ911841; –-; –-). B-ARG = *Belgrano 550* (HQ911811; HQ911933; HQ911976; HQ911797; HQ911900; HQ911844; HQ911965; HQ911785). CA = *Johnson 94-038* (HQ116983; HQ116942; HQ117065; HQ117027; HQ117107; HQ116840; HQ116886; HQ117247). ID = *Johnson & Johnson 95-034* (HQ911809; HQ911931; HQ911974; HQ911795; HQ911898; HQ911842; HQ911966; HQ911786). OR = *Johnson 93-086* (DQ196461; DQ196884; DQ196953; DQ196932; HQ117106; DQ196906; HQ116885; HQ117246). NV-1 = *Johnson 04-151* (DQ196462; DQ196885; DQ196954; DQ196932; HQ117109; DQ196907; HQ116887; HQ117248). NV-2 = *Howell s.n.* (HQ911807; HQ911929; HQ911972; HQ911793; HQ911896; HQ911840; HQ911967; HQ911787). UT =

Johnson & Matheson 03-073 (HQ911810; HQ911932; HQ911975; HQ911796; HQ911899; HQ911843; HQ911964; HQ911788). ***Collomia linearis*** Nutt.—CO-1 = *Johnson 99-010* (HQ116990; HQ116947; HQ117071; HQ117032; HQ117116; HQ116845; HQ116897; HQ117258). CO-2 = *Atwood & Higgins 5752* (HQ911812; HQ911934; HQ911977; HQ911798; HQ911901; HQ911845; –-; –-). ID-1 = *Johnson 92-045* [WS] (HQ116988; L34188; AF208170; EU628236; EU628359; AF208200; HQ116894; HQ117255). ID-2 = *Johnson 04-168* (DQ196451; DQ196874; DQ196943; DQ196922; HQ117114; DQ196895; HQ116895; HQ117256). NE = *Stephens & Brooks 24521* [KANU] (HQ911814; HQ911936; HQ911979; HQ911800; –-; HQ911847; –-; –-). NV = *Johnson & Johnson 04-104* (DQ196449; DQ196872; DQ196941; DQ196920; HQ117113; DQ196893; HQ116893; HQ117254). OR = *Johnson 97-134* (HQ911813; HQ911935; HQ911978; HQ911799; HQ911902; HQ911846; –-; –-). UT = *Thorne et al. 4171* (DQ196450; DQ196873; DQ196942; DQ196921; HQ117115; DQ196894; HQ116896; HQ117257). ***Collomia macrocalyx*** Leiberg ex Brand.—OR-1 = *Johnson & Johnson 05-071* (HQ116991; HQ116948; HQ117072; HQ117033; HQ117117; HQ116846; HQ116898; HQ117259). OR-2 = *Johnson & Johnson 05-079* (HQ116992; HQ116949; HQ117073; HQ117034; HQ117118; HQ116847; HQ116899; HQ117260). ***Collomia renacta*** Joyal—NV = *Johnson & Johnson 04-107* (DQ196455; DQ196878; DQ196947; DQ196926; HQ117119; DQ196900; HQ116900; HQ117261). OR = *Johnson & Johnson 05-103* (HQ116993; HQ116950; HQ117074; HQ117035; HQ117120; HQ116848; HQ116901; HQ117262). ***Collomia tenella*** A. Gray—NV = *Johnson 06-120* (HQ116998; HQ116954; HQ117078; HQ117039; HQ117126; HQ116852; HQ116908; HQ117269). UT = Johns*on 01-025* (DQ196457; DQ196880; DQ196949; DQ196928; HQ117124; DQ196902; HQ116906; HQ117267). ***Collomia tinctoria*** Kellogg—ID = *Porter 13769* (DQ196445; DQ196868; DQ196937; DQ196916; HQ117130; DQ196889; HQ116912; HQ117273). OR = *Johnson 95-048* (HQ117000; HQ116956; HQ117080; HQ117041; HQ117128; HQ116854; HQ116910; HQ117271). ***Collomia tracyi*** H. Mason—CA-1 = *Johnson 94-075* (DQ196447; DQ196870; DQ196939; DQ196918; HQ117131; DQ196891; HQ116913; HQ117274). CA-2 = *Johnson 94-078* (HQ117001; HQ116957; HQ117081; HQ911802; HQ117132; HQ116855; HQ116914; HQ117275). ***Collomia wilkenii*** L.A. Johnson & R.L. Johnson—NV1 = *Johnson & Johnson 04-105* (DQ196452; DQ196875; DQ196944; DQ196923; HQ117133; DQ196908, DQ196909; HQ116915, HQ116916; HQ117276, HQ117277). NV2 = *Johnson & Zhang 05-166* (HQ117002; HQ116958; HQ117082; HQ117043; HQ117134; HQ116856, HQ116857; HQ116917, HQ116918; HQ117278, HQ117279).

Navarretia fossalis Moran—CA = Spencer 4416-21 [RSA] (HQ911911; HQ912008; HQ911878; HQ911987; HQ911822; HQ911855; HQ911937). ***Navarretia intertexta*** (Benth.) Hook.—CA-1 = Johnson 93-088 (HQ911918; HQ912015; HQ911885; HQ911994; HQ911829; HQ911864; HQ911939). CA-2 = Johnson 04-038 (HQ911919; HQ912016; HQ911886; HQ911995; HQ911830; HQ911865; HQ911938). ***Navarretia involucrata*** Ruiz & Pav.—ARG-1 = *Belgrano et al. 470* (HQ911907; EU628547; EU628507; EU628244; EU628367; EU628293; HQ911940, HQ911944). ARG-2 = *Belgrano et al. 499* (HQ911908; HQ912005; HQ911875; HQ911984; HQ911819; HQ911852; HQ911941, HQ911943). ARG-3 =

Belgrano et al. 551 (HQ911909; HQ912006; HQ911876; HQ911985; HQ911820; HQ911853; HQ911942, HQ911945). ***Navarretia leucocephala*** Benth. ssp. ***bakeri*** (H. Mason) A.G. Day—CA = *Gowen 697*(HQ911924; HQ912021; HQ911891; HQ912000; HQ911835; HQ911870; –-, –-). ***Navarretia leucocephala*** Benth ssp. ***leucocephala***—CA = *Johnson 04-118* (HQ911921; HQ912018; HQ911888; HQ911997; HQ911832; HQ911867; –-, –-). ***Navarretia leucocephala*** Benth. ssp. ***minima*** (Nutt.) A.G. Day—OR = *Johnson 05-198* (HQ911922; HQ912019; HQ911889; HQ911998; HQ911833; HQ911868; HQ911946, HQ911947). ***Navarretia leucocephela*** ssp. ***pauciflora*** (H. Mason) A.G. Day—CA = *Johnson 04-036* (HQ911923; HQ912020; HQ911890; HQ911999; HQ911834; HQ911869; HQ911960, HQ911961). ***Navarretia myersii*** P.S. Allen & A.G. Day ssp. ***myersii***—CA = *Popp s.n.* [DAV] (HQ911920; HQ912017; HQ911887; HQ911996; HQ911831; HQ911866; –-, –-). ***Navarretia propinqua*** Suksd.—NV = *Howell s.n.* (HQ911917; HQ912014; HQ911884; HQ911993; HQ911828; HQ911862, HQ911863; HQ911948, HQ911949). UT = *Johnson 04-163*(HQ911916; HQ912013; HQ911883; HQ911992; HQ911827; HQ911860, HQ911861; HQ911950, HQ911951). ***Navarretia prostrata*** (A. Gray) Greene—CA = *Wilken s.n.* (HQ117012; HQ116967; HQ117091; HQ117052; HQ117143; HQ116866; HQ116928). ***Navarretia saximontana*** S.C. Spencer—UT = *Johnson 07-036* (HQ911912; HQ912009; HQ911879; HQ911988; HQ911823; HQ911856; HQ911952, HQ911953). ***Navarretia*** sp. nov. 1—CA = *Johnson et al. 09-021* (HQ911914; HQ912011; HQ911881; HQ911990; HQ911825; HQ911858; HQ911954). ***Navarretia*** sp. nov. 2—CA = *Johnson et al. 09-032* (HQ911915; HQ912012; HQ911882; HQ911991; HQ911826; HQ911859; HQ911955). ***Navarretia*** sp. nov. 3—UT = *Johnson & Johnson 05-197* (HQ911913; HQ912010; HQ911880; HQ911989; HQ911824; HQ911857; HQ911956). ***Navarretia subuligera*** Greene—CA-1 = *Johnson & Zhang 04-135* (HQ117016; HQ116970; HQ117094; HQ117055; HQ117146; HQ116869; HQ116932). CA-2 = *Johnson & Gowen 09-052* (HQ911906; HQ912004; HQ911874; HQ911983; HQ911818; HQ911851; –-). CA-3 = *Spencer 568-82* [RSA] (HQ911905; HQ912003; HQ911873; HQ911982; HQ911817; HQ911850; –-). ***Navarretia tagetina*** Greene—CA-1 = *Johnson 04-024* (HQ911903; HQ912001; HQ911871; HQ911980; HQ911815; HQ911848; HQ911957). CA-2 = *Johnson 04-046* (HQ117017; HQ116971; HQ117095; HQ117056; HQ117147; HQ116870; HQ116933). CA-3 = *Johnson & Zhang 05-164* (HQ911904; HQ912002; HQ911872; HQ911981; HQ911816; HQ911849; –-). ***Navarretia willamettensis*** S.C. Spencer—OR = *Johnson & Halse 05-208* (HQ911910; HQ912007; HQ911877; HQ911986; HQ911821; HQ911854; HQ911958, HQ911959).