



Cryptic speciation: distinguishing serpentine affiliated sister species *Navarretia paradoxiclara* and *N. paradoxinota* from *N. intertexta* (Polemoniaceae)

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Abstract

Two species endemic to California in the western United States, *Navarretia paradoxiclara* and *N. paradoxinota*, are here described. Both species occur on serpentine influenced soils and have been previously collected as *N. intertexta*, with which they are sympatric. However, they vary from *N. intertexta* subtly, yet consistently, in floral features and remarkably in surveyed DNA regions. *Navarretia paradoxiclara* and *N. paradoxinota* are sister species phylogenetically well separated from *N. intertexta*. With respect to each other, these new species are allopatric and diagnosable by differences in their corollas.

Key words: cryptic species delimitation, DNA sequence data, morphometrics, taxonomy, unified species concept

Introduction

The *Navarretia intertexta* (Bentham 1833: 1622) Hooker (1838: 74) complex of *Navarretia* Ruiz & Pavón (1794: 20) section *Navarretia* has doubled in numbers during the past decade from two taxa of uncertain rank to four species: *N. intertexta*, *N. propinqua* Suksdorf (1906: 26; with the latter long treated as a variety or subspecies of the former), *N. saximontana* Spencer (in Spencer & Spencer 2003: 198) and *N. furnissii* L.A.Johnson & L.M.Chan (in Johnson *et al.* 2012a: 56). These species are associated with seasonally moist topography although they are not obligate to vernal pools as are several other species in *Navarretia* section *Navarretia*.

The systematics of *Navarretia saximontana* and *N. furnissii*, species from the intermountain region of the western United States long confused with *N. propinqua* or *N. leucocephala* Bentham (1849: 324 in Bentham 1839–1857) subsp. *minima* (Nuttall 1848: 13) Day (1993: 337), was examined recently (Johnson *et al.* 2012a). Here, we present evidence delimiting two species that are morphologically similar to *N. intertexta*, a large statured and widely distributed species in Western North America.

Navarretia intertexta was first described as *Aegochloa intertexta* Bentham (1833: 1622) from material collected by David Douglas from California and Northwest America. As noted by Bentham, the stamens of this species are exserted. From herbarium surveys and field work, we have observed that the anthers are not just exserted from the throat/tube of the sympetalous corolla, but that they are positioned beyond the tips of the corolla lobes when fully extended—a consistent feature despite some plasticity in gross structure in this species across its considerable distribution that ranges along the west coast of North America from Baja California to British Columbia and eastward into Idaho and Nevada. In Northern California's inner coast range and the north/central Sierra foothills, material heretofore referred to *N. intertexta* but with shorter stamens and equal or larger corollas exists. This material also varies ecologically and molecularly from *N. intertexta*. Following the unified species concept (de Queiroz 1998, 2007) and applying evidence of non-homogenizing gene flow of both morphological and molecular data as criteria for distinguishing the

evolutionary independence of metapopulation lineages, we here delimit two sister species: *N. paradoxiclارا* and *N. paradoxinota*. Despite great morphological similarity to *N. intertexta*, these species show remarkable molecular divergence from this species as well as other members of *Navarretia* section *Navarretia*.

Materials and methods

We analyzed DNA sequence data with an emphasis on sampling multiple populations (twelve, five, and five, respectively) of *Navarretia intertexta*, *N. paradoxiclارا*, and *N. paradoxinota* from across their range to assess the degree of divergence among these focal species. We included six additional species representing the diversity of *Navarretia* section *Navarretia* for a total of 31 populations sampled (Appendix 1). *Navarretia tagetina* Greene (1887: 137) was used to root the tree based on its position as sister to the remaining members of the section in earlier studies (e.g., Spencer & Porter 1997). We isolated DNA and PCR amplified selected regions using standard conditions as described in Johnson *et al.* (2012b).

We sequenced three chloroplast regions (*trnL-trnF-trnF* intergenic spacer and intron (*trnL*; Taberlet *et al.* 1991), *psbM-trnT* intergenic spacer (*psbM*; Shaw *et al.* 2005), and *trnS-trnG* intergenic spacer (*trnS*; Hamilton 1999)), the nuclear ribosomal ITS1, 5.8s, and ITS2 region (White *et al.* 1990, Porter 1996), and a portion from the 5' end of the nuclear *Pistillata* (*PI*) region as described in Johnson *et al.* (2012b). Four populations of *N. intertexta* were excluded from the *PI* analyses due to amplification problems likely associated with poor quality DNA from herbarium specimens. All sequences are available from GenBank, either through prior publication (Johnson *et al.* 2012a, 2012b) or new submissions (84 sequences, accession numbers KC145177–KC145260).

We aligned sequences by eye in Se-AL (Rambaut 1996) and coded indels as present or absent using simple indel coding (Simmons & Ochoterena 2000). We conducted unweighted parsimony analyses to infer species relationships using PAUP* 4.0b10 (Swofford 2003) with 10,000 random addition replications, collapsing branches with minimum length of zero, and assessing support with 100,000 bootstrap replications using fast stepwise addition.

We compared morphology under the framework of population aggregate analysis/specimen aggregate analysis (Davis & Nixon 1992, Snow *et al.* 2003) using specimens from field work and herbarium visits to, or loans from, the following herbaria: BRY, CAS, CPH, IDS, RSA, and JEPS. We examined two to five individuals per population from five populations per species. We measured several vegetative and reproductive features using digital calipers for larger features and, for smaller features, made measurements from digital images taken with an Olympus SZX-12 dissecting microscope using MicroSuite Five Basic Edition software (Olympus Soft Imaging Solutions Corp.). Seed color was assessed by comparison with published standards (Munsell Color Company 2000). We assessed variation in measured features using Aabel 3.0.3 (Gigawiz Ltd. Co.).

Results

Parsimony analyses recovered six trees of 92 steps from the concatenated cpDNA matrix (CI = 0.90; RI = 0.96), 32 trees of 83 steps from the ITS matrix (CI = 0.80; RI = 0.92), and six trees of 211 steps from the *PI* matrix (CI = 0.79; RI = 0.96; Fig. 1). In the cpDNA trees, *Navarretia paradoxiclارا* and *N. paradoxinota* form a well-supported clade sister to two species representing the core vernal pool clade. The *N. paradoxiclارا/N. paradoxinota* clade is well separated patristically from *N. intertexta* (Fig. 1A). In the ITS trees, *N. paradoxiclارا* and *N. paradoxinota* again form a well-supported clade patristically well differentiated from all *N. intertexta* sampled; however, in this instance, *N. intertexta* weakly forms a paraphyletic grade with respect to *N. paradoxiclارا* and *N. paradoxinota* (Fig. 1B). In the *PI* trees, *N. paradoxiclارا* and *N. paradoxinota* again form a well-supported clade with considerable patristic distance

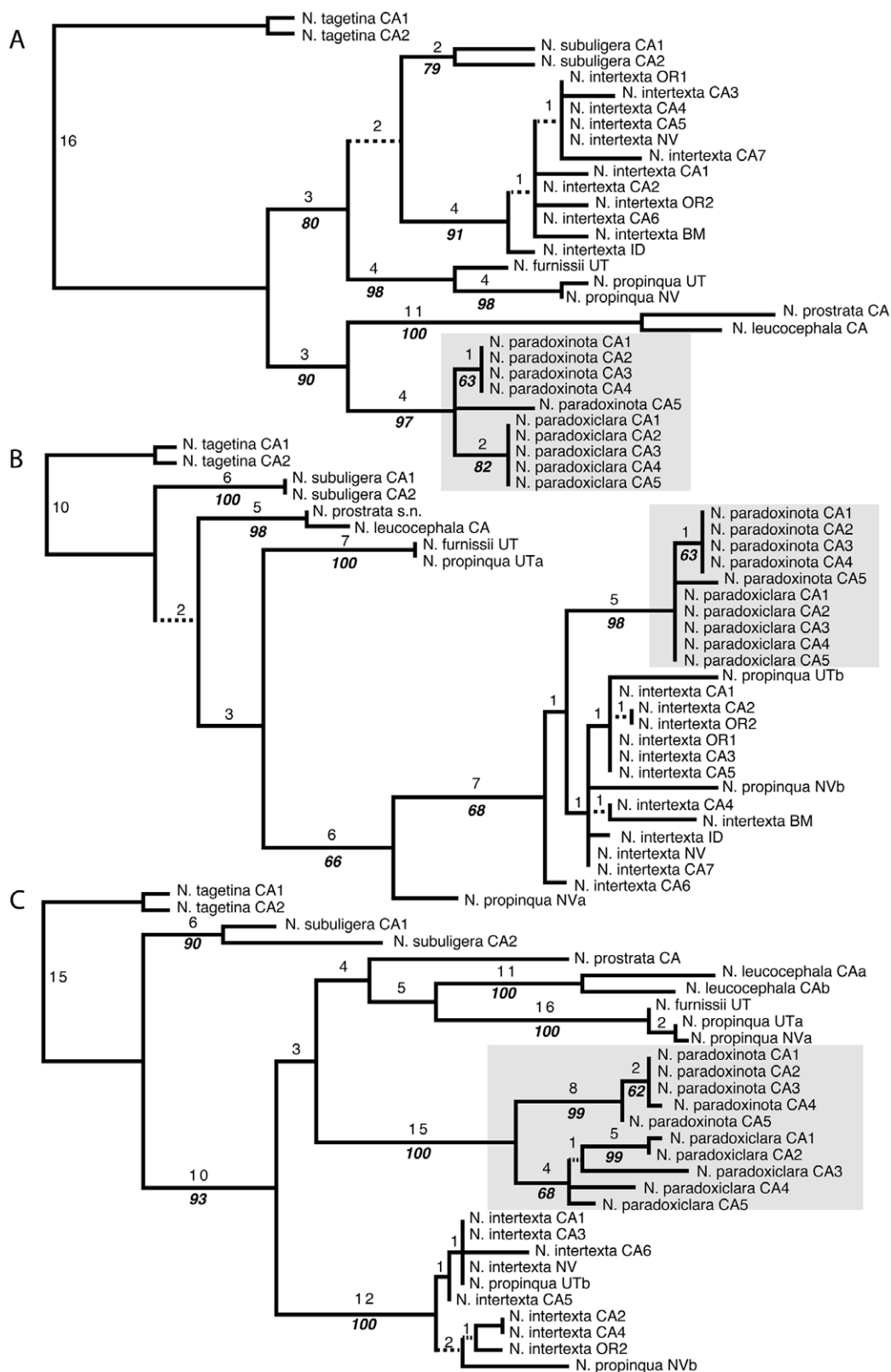


FIGURE 1. Representative most parsimonious phylograms inferred from analysis of DNA sequence data. Acronyms following species names are correlated to specimens in Appendix 1. Lower case letters ('a' and 'b') following acronyms in Figs. 1B, C indicate multiple copies indicative of either polyploidy (e.g. *N. propinqua*) or possibly gene duplication or intrapopulation variation among multiple individuals (e.g. *N. leucocephala*). Total character change (base substitutions and indels) are reconstructed above interior branches (terminal values can be inferred by branch length). Branches not found in all shortest trees are indicated by dotted lines. Bootstrap support values are shown in bold italics below branches. A. One of six trees inferred from concatenated cpDNA sequences. B. One of 32 trees inferred from nrDNA ITS sequences. C. One of six trees inferred from nuclear *PI* sequences.

separating these species from a monophyletic *N. intertexta* (*N. propinqua* is an allotetraploid; Fig. 1C). *PI* sequences also recover *N. paradoxi-clara* and *N. paradoxinota* as reciprocally monophyletic lineages. In comparison, chloroplast sequences resolve *N. paradoxi-clara* as monophyletic while the northernmost population of *N. paradoxinota* is unresolved with respect to the remaining populations of this species, and ITS sequences lack synapomorphies uniting *N. paradoxi-clara* and again exclude the northern most population of *N. paradoxinota* from a lineage supported by a synapomorphy uniting the remaining populations of this species.

Navarretia paradoxi-clara is larger in several measurable floral traits relative to both *N. paradoxinota* and *N. intertexta* (Fig. 2), with the latter two species similar to each other in several traits. Nevertheless, the combination of slightly longer corolla lobes with shorter stamen filaments in *N. paradoxinota* (Fig. 2) results in anthers positioned such that they do not exceed the corolla lobes on pressed flowers whereas anthers do exceed the corolla lobes in *N. intertexta* (Fig. 3).

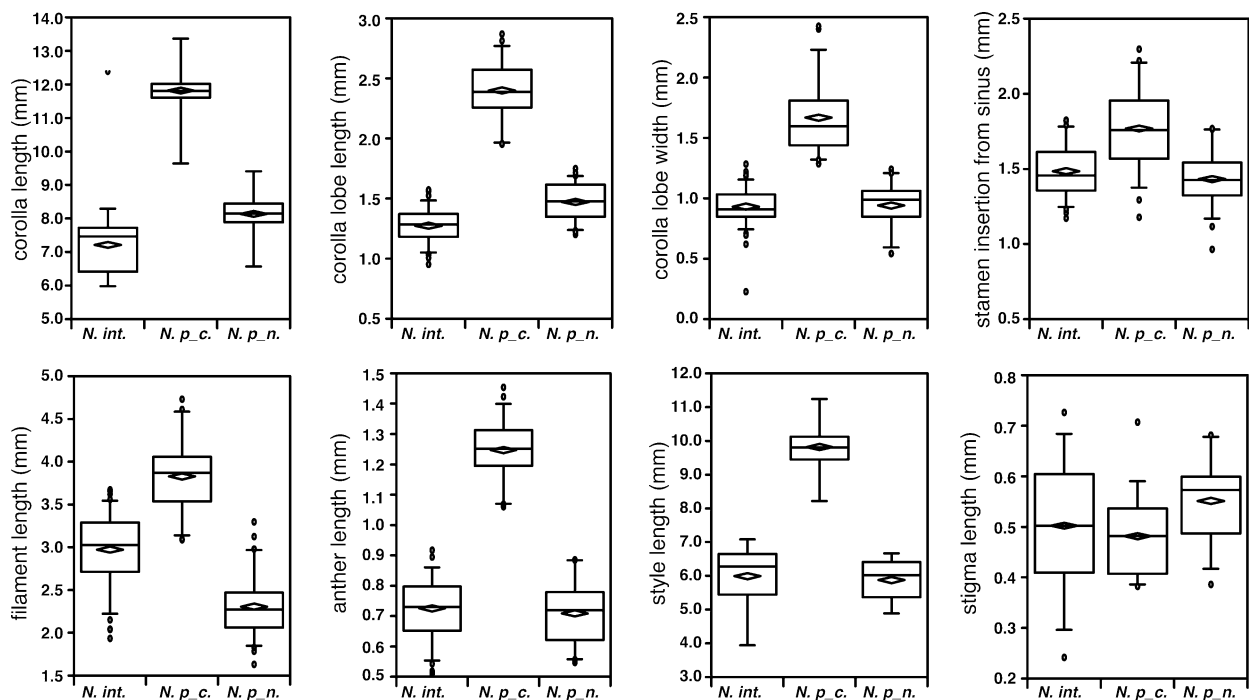


FIGURE 2. Box plots of variation in floral features among *N. intertexta* (*N. int.*), *N. paradoxi-clara* (*N. p.c.*), and *N. paradoxinota* (*N. p.n.*). Boxes bound the 25 and 75 percentiles; horizontal line marks the 50 percentile, whiskers extend to the 5 and 95 percentiles with outliers shown as dots. The diamond demarks the mean (horizontal vertices) and standard deviation (vertical vertices).

Discussion

Navarretia intertexta, *N. paradoxi-clara*, and *N. paradoxinota* are extremely similar in gross morphology, vegetative stature and structure, trichome morphology, and distribution. Chloroplast, nuclear ITS, and nuclear *PI* DNA sequences, however, reveal considerable anagenic change at the molecular level indicating an early divergence of *N. paradoxi-clara* and *N. paradoxinota* from their common ancestor with *N. intertexta*. Though agreeing in revealing substantial anagenic change, the three surveyed DNA regions recover somewhat different sister relationships, though in general these sister relationships are not strongly supported (Fig. 1). More thorough taxon sampling within this section of *Navarretia*, with *N. paradoxi-clara* and *N. paradoxinota* included as “sp. nov. 2 CA” and sp. nov. 1 CA”, respectively, recovers similar and more strongly supported relationships with cpDNA and ITS sequences, with *PI* placing the new species nearer to the base of the section, just inside *N. tagetina* and *N. subuligera* Greene (1887: 137) (Johnson *et al.* 2012b). These alternative

hypotheses may reflect a rapid diversification following divergence obscuring synapomorphy, early homoploid hybridization, or lineage sorting of ancestral polymorphisms. Both new species are inferred to be diploid based on experimental results, though a direct chromosome count has not yet been made.

The denser population sampling of *Navarretia paradoxiclارا*, *N. paradoxinota*, and *N. intertexta* here, compared to Johnson *et al.* 2012b, affirms consistency between morphological and molecular results in supporting not only differences between both new species and *N. intertexta*, but also the distinctiveness of the new species with respect to each other. *PI* sequences provide evidence for reciprocal monophyly between *N. paradoxiclارا* and *N. paradoxinota*. Weaker differentiation in the surveyed cpDNA and ITS regions does not recover reciprocally monophyletic lineages, but neither does it strongly reject this hypothesis for these two species.

Morphological differentiation among *Navarretia intertexta*, *N. paradoxiclارا*, and *N. paradoxinota* is minimal. However, observable differences do exist consistent with the inferences derived from DNA sequence data. Both *N. paradoxiclارا* and *N. paradoxinota* have anthers that, when flowers are pressed (dry or rehydrated), reach, but do not exceed, the corolla tips (Fig. 3E, F). This is more obvious in *N. paradoxinota* than *N. paradoxiclارا* when flowers are fresh (Fig. 3A, B); in the former species, corolla lobes are ascending and the anthers appear from the top to be just beyond the throat (Fig. 3B). In *N. paradoxiclارا*, the anthers appear more strongly exerted because the corollas are larger, the anthers themselves bigger, and the corolla lobes more often presented at right angles to the corolla tube or even slightly reflexed (Fig. 3A). *Navarretia intertexta* corollas are generally smaller with a narrower, shorter throat than either *N. paradoxiclارا* or *N. paradoxinota* (Fig. 3C). We did observe one collection of *N. intertexta* from Sutter County, California (*L. Ahart 3454*; CAS 884645), with unusually long corollas (Fig. 3G, flower on the right) relative to the many specimens we examined from throughout this species' range. In all cases, *N. intertexta* stamens are strongly exerted with anthers presented beyond the corolla lobe tips when pressed, and observable on fresh flowers as well (Fig. 3C, G). The large corollas of *N. paradoxiclارا* generally exceed all calyx lobes, whereas corollas may or may not exceed the calyx lobes of *N. paradoxinota* and *N. intertexta* (Fig. 3A–C). *Navarretia paradoxiclارا* corollas are pale blue (fading to white), similar to *N. intertexta*'s corollas that vary towards white suffused with blue. In contrast, *N. paradoxinota* corollas are plain white. In all three species, calyx lobes are unequal and generally entire, though one or two lobes may be two or three pronged. Pronged calyx lobes are infrequent in *N. paradoxinota* and *N. paradoxiclارا*—a quick survey suggests generally only one calyx per head—but are variable in number per head in *N. intertexta*. As with other species in *Navarretia* section *Navarretia*, there is no particular odor, differentiating or not, associated with these species.

Geographically, *Navarretia intertexta* is sympatric with the narrow distributions of *N. paradoxiclارا* and *N. paradoxinota*, whereas *N. paradoxiclارا* and *N. paradoxinota* are wholly allopatric (Fig. 4). We have yet to observe truly intermixed populations of *N. intertexta* with either of the new species, but have collected and observed collections of *N. intertexta* in close proximity to both species.

Agreeing with molecular and morphological data, the new species are also edaphically divergent from *N. intertexta*. Both *N. paradoxiclارا* and *N. paradoxinota* occur on serpentine influenced soils (Fig. 4). All three species share the propensity for seasonally moist (but not fully vernal/seasonal pool inundation) characteristic of several other members of *Navarretia* section *Navarretia* such as *N. tagetina*, *N. subuligera*, and *N. propinqua*. Undoubtedly *N. intertexta* can tolerate some degree of serpentine influence and we have grown *N. paradoxinota* in “normal” soil. However, natural populations of the new species show a strong association with serpentine whereas *N. intertexta* does not. Specialization towards vernal pool habitats appears to have evolved just once in *Navarretia*, but serpentine endemism has evolved multiple times (Spencer & Porter 1997). The new species described here mark yet another origin of serpentine endemism unique in that the species belong to the section that includes the vernal pool species and serpentine specialization was apparently followed by differentiation into two species, perhaps following a long distance dispersal or vicariance that separated their common ancestor into two geographically distinct groups. Other origins of serpentine endemism in *Navarretia* are restricted to single species (though some belong to the same local clades; Spencer & Porter 1997).

Given consistent differences in morphology between *N. paradoxiclara* and *N. paradoxinota* with no sign of intergradation, and molecular data consistent with the existence of separate metapopulation lineages, we treat these sister taxa as distinct at the species level.

Systematic treatment

Navarretia paradoxiclara L.A.Johnson & D.Gowen, *sp. nov.* (Fig. 3A, E, I, K, M–P).

A species similar to *Navarretia intertexta* in gross morphology, but distinguished by having larger flowers with stamens shorter relative to the corolla lobes and predominantly occurring on serpentine derived soils. Similar and genetically closer to *Navarretia paradoxinota*, but distinguished by having notably larger corollas that are light blue (fading to white) rather than pure white.

TYPE:—U.S.A. California: Calaveras County, In moist drainage (serpentine nearby) leading to New Hogan Reservoir, within the Oak Knoll Campground, ca. 2.5 miles SE of Hwy 12 on S. Petersburg Rd., 38.18049° N 120.79178° W, 208 m, 28 May 2009, *Johnson, Gowen, & Mort 09-032* (holotype BRY!; isotypes JEPS! MO!, NY!, RSA!).

Taprooted annuals, erect with primary axis exceeding in length secondary axes, infrequently much (± 6) branched from the base with subequal secondary axes; primary axis to 30 cm tall; secondary axes 0–10(–20) in number, 0.2–5(–15) cm long, all axes terminating in an inflorescence; tertiary axes rare, and only from very well developed secondary axes. Stem and branches mostly tan to reddish brown, pubescent with white, retrorse eglandular hairs generally less than 1 mm long, sparsely pubescent at lowermost nodes, \pm evenly pubescent distally. Cotyledons two, linear, entire. Lowermost nodes, ± 4 in number, bearing opposite leaves; leaves flexuous, entire to pinnate with filiform rachis and lobes, \pm glabrous with blunt tips. Higher nodes alternate, leaves rigid, once to twice pinnate, tips acerose. Proximal nodes often congested, forming a rosette, leaves to 5 cm long, withering with age; internodes above the base commonly 2–3 cm long, associated leaves (subtending secondary axes, if present), diminishing upward from 4 cm to 2 cm in length, typically with 1–2 pairs of short linear lobes proximally, 2–3 pair of unequally branched lateral lobes, and the rachis then extending to a well separated, unequally three-pronged apex (the central prong or rachis terminus longer than the two lateral prongs), glabrous to sparsely pubescent with uniseriate hairs along the abaxial rachis and margins. Inflorescence 1–10(–20) in number, head-like, terminating all axes, ca. 8–26 mm wide exclusive of bract tips, up to 40 mm inclusive of bract tips, bearing up to 30 flowers in 1–3 flowered cymose units. Bracts strongly 3-dimensional from lobes diverging from rachis in multiple planes; outer bracts pinnate with a non-green, flattened and linear proximal rachis ± 2 mm wide by 5 mm long, the lateral margin bearing 3–4 pairs of branched needle-like lobes; the proximal rachis transitions into a green, needle-like apex equal in texture/appearance to the lateral lobes, three pronged distally with the central prong (rachis terminus) longer than the two lateral prongs; the abaxial rachis base and margins pubescent. Inner bract proximal rachis non-green, obdeltoid, concave-clasping with entire lateral margins such that all lobes are \pm distal, flanking either side of the 3-pronged rachis apex; rachis base long pubescent abaxially and marginally. Flowers. Calyx (6.5–)8–11(–12.5) mm, accrescent, of 5 costae connected into a tube 4–5 mm long by an intercostal hyaline membrane, this membrane v-shaped distally; the costae broadest at the distal tube, non-green proximally and tapering to a narrow base, narrower than the intercostal membrane, green distally, the lobes unequal with two typically longer than the other three, sometimes one or both of the longer lobes 3-pronged; calyx tube glabrous internally, pubescent and minutely glandular externally with uniseriate chain-like hairs increasing in length from the base upwards, longest near mouth of calyx tube, the lobes pubescent just above this region both adaxially and abaxially; minute glands short stalked, ± 50 –60 μm in diameter. Corolla funnelform, light blue-lavender or white (fading to white), exserted from calyx, 9.5–13.5 mm long, lobes rounded, 2.0–2.9 mm long, 1.25–2.5 mm wide, with a single vein entering the base and branching pedately soon after. Stamen filaments 3.0–4.7 mm long and inserted 1.2–1.85 mm below sinuses; the anthers 1–1.45 mm long, exserted to tips of

corolla lobes or below. Pollen deep yellow; apertures pantoporate, acolpate; sexine semitectate, reticulate, heterobrochate. Ovary oblong, two-chambered; style 8.0–11.5 mm long, stigma lobes two, 0.38–0.71 mm, Capsule 2.3–3(–3.8) mm long, proximal $\frac{3}{4}$ membranous, distal $\frac{1}{4}$ corneous, short apiculate, indehiscent at maturity. Seeds (4–)8(–10), medium brown (hue 5YR, value 3, chroma 4 to hue 2.5YR, value 3 chroma 4), ovoid-angular, 1–2.1 mm long \times 0.8–1.1 mm wide, testa reticulate, mucilaginous when wet.

Habitat, Distribution, and Phenology:—*Navarretia paradoxiclara* occurs in serpentine influenced soils that are at least seasonally moist or best characterized as drainages, alone or among grasses. This taxon is presently known from at least 10 sites in Tuolumne and Calaveras counties, California and blooms primarily from mid May to late June (early July).

Etymology:—This specific epithet is derived from the Latin *paradoxus*—contrary to expectation, and *clarus*—bright, famous. *Clarus* is to honor Dr. Robert (Germanic for fame + bright) "Bob" Patterson, a faculty at San Francisco State University with a long interest in student training and in the systematics of Polemoniaceae. His selfless help has contributed meaningfully to the first author's research through the years. *Paradoxus* refers to the unexpected genetic similarity of this taxon to *N. paradoxinota*, and unexpected degree of molecular divergence from *N. intertexta*.

Additional specimens examined (paratypes):—U.S.A. California: Calaveras County, dry serpentine soil along a stream bank at the tip of New Hogan Reservoir in the N shore camp area, 2.5 mi S. E. of Hwy 12 near Valley Springs, 31 May 1972, *McNeal* 999 (BRY, CPH, UT); Burson, 500 ft, 30 May 1923, *Jepson* 9948 (JEPS); Salt Springs Valley, 1000 ft, *Tracey* 5650 (JEPS); Upper Littlejohns Creek Drainage about 2.7 airmiles SW of Copperopolis, 1200 ft, 18 June 1984, *Stone & Clifton* 630 (& 633, JEPS); 5.8 miles west Altaville (just west of Elkhorn Station, along Calif. Highway 4), 1420 ft, 6 June 1958, *Bacigalupi, Constance & Alava* 6375 (JEPS); Dry serpentine soil in small gullies beside the Hwy 49 bridge over the N Fork of the Calaveras River, 16 May 1972, *McNeal* 887a (BRY, CPH, UT); Serpentine drainage leading to New Hogan Reservoir, 38.17692°N, 120.78546°W, 206 m, 28 May 2009, *Johnson, Gowen, & Mort* 09-031 (BRY, JEPS, RSA); Off Hwy 49, in a small channel in steep slope leading to N. Fork of the Calaveras river near bridge, serpentine soils, 38.21907°N, 120.69914°W, 227 m, 28 May 2009, *Johnson, Gowen, & Mort* 09-033 (BRY, JEPS, RSA). Tuolumne County, 3.6 miles west of Yosemite Junction, Highway 120, 24 May 1953, *Cantelow s.n.* (CAS); Six-Big Gulch, upper portion, along Red Hills road, Red Hills, 1050 ft, 22 May 1984, *Taylor* 8516C (JEPS); Chinese Camp, 1300 ft, 30 May 1915, *Jepson* 6328 (JEPS); Dry rocky serpentine soil by a small stream crossing Sims Rd, 0.6 miles south of Hwy 120 between Yosemite Jct. and Chinese Camp, T1S R14E S5, 11 May 1972, *McNeal* 849 (CPH, IDS); Just north of jct of State Hwy 59 and Hwy 108/120, ca. 3.9 miles southwest of Yosemite Jct., seep area with serpentine soils, 37.84379°N, 120.5067°W, 324 m, 28 May 2009, *Johnson, Gowen, & Mort* 09-035 (BRY, JEPS, RSA); Northwest side of Red Hills Road en route to Chinese Camp, serpentine, 37.84887°N, 120.45863°W, 313 m, 28 May 2009, *Johnson, Gowen, & Mort* 09-039 (BRY, JEPS); North side of Red Hills Road ca. 0.7 miles southwest of jct. of Hwys 120 and 49, serpentine influenced soils, 37.86542°N, 120.43976°W, 381 m, 28 May 2009, *Johnson, Gowen, & Mort* 09-040 (BRY, JEPS, RSA).

Notes:—We first became aware of this species when reviewing herbarium sheets from BRY in 2008 and made our first collections in 2009. Though suspecting this material was not typical *N. intertexta*, we were surprised to see the close genetic relationship between this material and the smaller flowered, allopatrically distributed material under study at that time that we here designate *N. paradoxinota*. The ecological propensity for both species to occur on serpentine derived soils, however, is congruent with the genetic relationships. The larger flowers of *N. paradoxiclara* compared to *N. intertexta* and *N. paradoxinota* are striking, and make this species showier both in the field and on herbarium sheets. This species was included in *Johnson et al.* (2012b) as "*Navarretia* sp. nov. 2-CA."

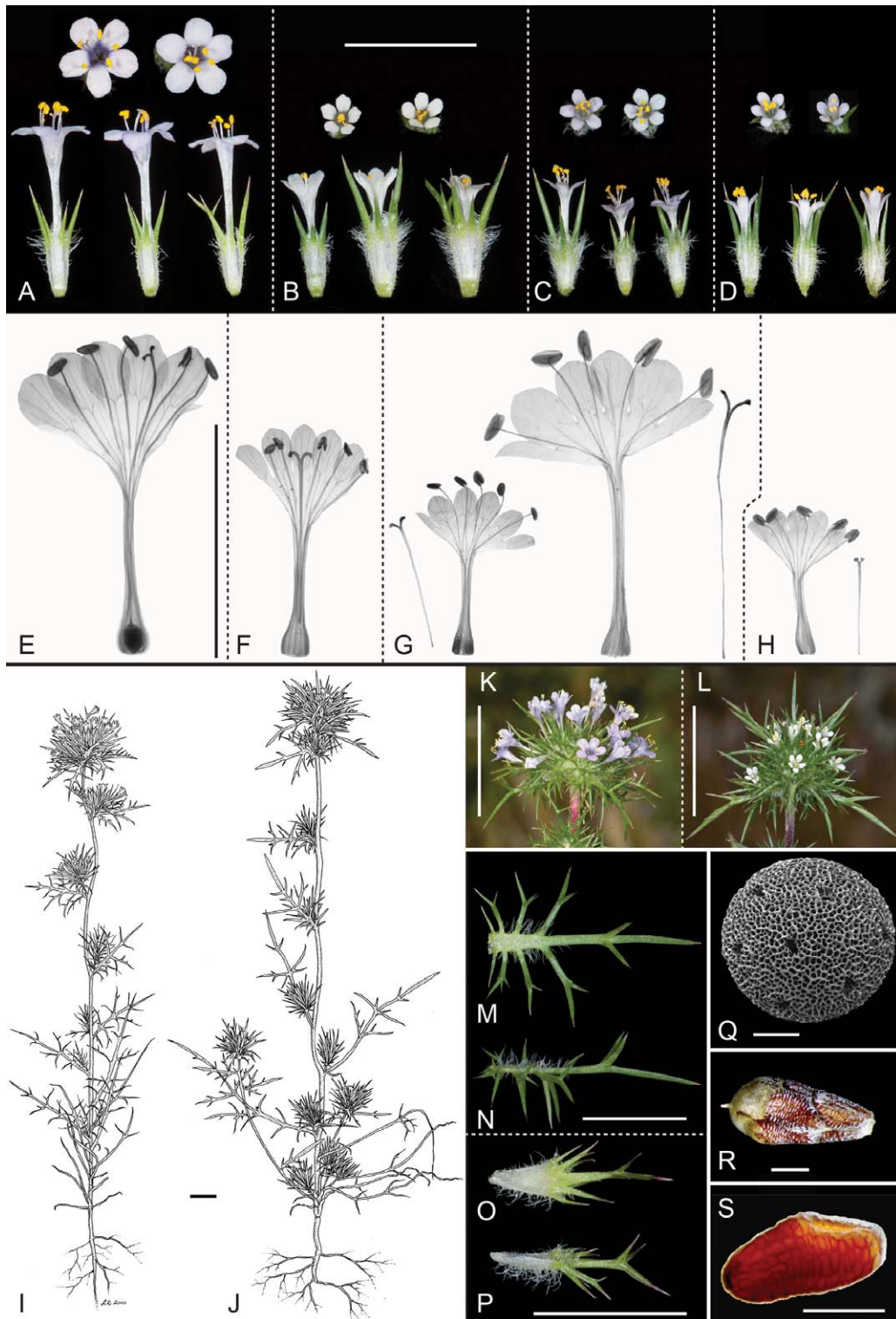


FIGURE 3. Features of *Navarretia paradoxiclara* (all Johnson, Gowen & Mort 09-032) and *N. paradoxinota* (all Johnson, Gowen & Mort 09-021), with some comparison to *N. intertexta* and *N. propinqua*. All vouchers deposited at BRY unless otherwise indicated. A–D. flowers, top and side views, scale bar = 1 cm. A. *N. paradoxiclara*. B. *N. paradoxinota*. C. *N. intertexta* Gowen 1133, 1134-B. D. *N. propinqua* Johnson & Johnson 11-076. E–H. Corolla dissections, scale bar = 1 cm. E. *N. paradoxiclara*. F. *N. paradoxinota*. G. *N. intertexta* (left = Gowen 1133; right = Ahart 3453 [CAS]). H. *N. propinqua* Johnson & Johnson 09-067. I–J. Plant habit (note, either species can have a single leader (I) or be variously branched (J)), scale bar = 1 cm. I. *N. paradoxiclara*. J. *N. paradoxinota*. K–L. Inflorescence, scale bars = 2 cm. K. *N. paradoxiclara*. L. *N. paradoxinota*. M–N. Outer inflorescence bract, *N. paradoxiclara*, scale bar = 1 cm. M. Adaxial view. N. Lateral view. O–P. Inner inflorescence bract, *N. paradoxiclara*, scale bar = 1 cm. O. Adaxial view. P. Lateral view. Q. Pollen grain, *N. paradoxinota*, scale bar = 10 μ m. R. mature capsule, *N. paradoxinota*, scale bar = 1 mm (distal end to the left). S. Partially hydrated seed with thin halo of mucilaginous spiracles, *N. paradoxinota*, scale bar = 1 mm.

Navarretia paradoxinota L.A.Johnson & D.Gowen, *sp. nov.* (Fig. 3B, F, J, L, Q–S).

A species similar to *Navarretia intertexta* in gross morphology, but distinguished by having stamens shorter relative to the corolla lobes and predominantly occurring on serpentine derived soils. Similar and genetically closer to *Navarretia paradoxiclara*, but distinguished by having smaller corollas that are pure white rather than light blue fading to white.

TYPE:—U.S.A. California: Napa County, In depressions along southeast side of Snell road within 50 yards of jct. with Butts Canyon Road. Serpentine influenced soils, 38.69642° N 122.44412° W, 180 m, 27 May 2009, *Johnson, Gowen, & Mort 09-021* (holotype BRY!; isotypes JEPS!, MO!, NY!, RSA!).

Taprooted annuals with architecture, stem, leaf, and inflorescence descriptions paralleling *N. paradoxiclara*, but varying in the following particulars: Flowers. Calyx 6.4–9.5 mm long. Corolla white, exserted or included in calyx, 6.5–9.5 mm long, lobes rounded, 1.2–1.75 mm long, 0.5–1.25 mm wide. Stamen filaments 1.6–2.8(–3.3) mm long and inserted 0.9–1.8 mm below sinuses; the anthers 0.55–0.9 mm long, exserted to tips of corolla lobes or below. Style 4.5–7 mm long.

Habitat, Distribution, and Phenology:—*Navarretia paradoxinota* occurs in serpentine influenced soils that are at least seasonally moist or best characterized as drainages, alone or among grasses. This taxon is presently known from fewer than 10 sites in Colusa, Lake, and Napa counties, California, and blooms primarily from mid May to late June (early July).

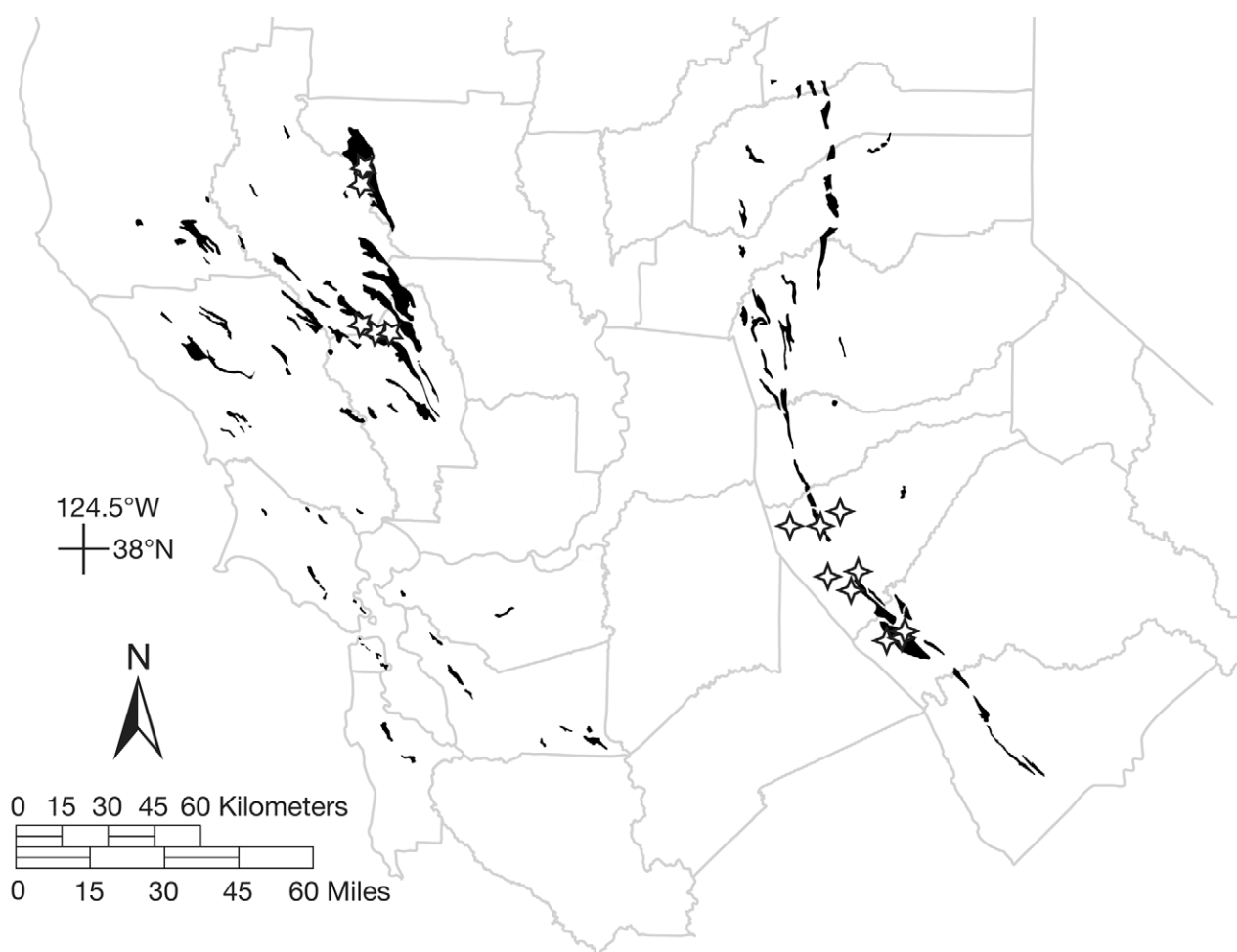


FIGURE 4. Map of California, U.S.A., with county borders (gray lines) showing the distribution of *Navarretia paradoxiclara* (stars with four points) and *Navarretia paradoxinota* (stars with five points). Serpentine areas are shaded black (derived from 2010 Geologic Map of California; <http://www.quake.ca.gov/gmaps/GMC/stategeologicmap.html>).

Etymology:—This specific epithet is derived from the Latin *paradoxus*—contrary to expectation, and *Nota*—mark. *Nota* is to honor Dr. J. Mark Porter, a faculty at the Claremont Graduate University, researcher at Rancho Santa Ana Botanic Garden, and friend with a career long interest in Polemoniaceae that has contributed substantially to our present understanding of relationships in this family. *Paradoxus* refers to the unexpected genetic similarity of this taxon to *N. paradoxiclara* and unexpected molecular divergence from *N. intertexta*.

Additional specimens examined (paratypes):—U.S.A. California: Colusa County, serpentine soil along a wet drainage, east edge of Buck Flat meadow adjacent to Walker Ridge Road, 39°7.798'N, 122°29.598'W, 2758 ft, 6 June 2012, *Gowen 1182* (BRY, JEPS); Lake County, Serpentine chaparral, Butts Canyon Road, 5–8 miles south of Middletown, 900 ft, 29 June 1980, *Breedlove 44829* (CAS); 3 miles east Middletown, 30 May 1946, *Mason 12798* (JEPS); West of Walker Ridge Road, in meadow near camping area north of Barrel Spring, serpentine, 792 m, 14 June 2010, *Gowen 1129* (BRY, JEPS, RSA); North of Butts Canyon Road, 7.9 miles east of Middletown, Serpentine, 38.71842°N, 122.49565°W, 311 m, 30 May 2009, *Johnson & Gowen 09-053* (BRY, JEPS); Butts Canyon Road, 0.6 miles North of Lake Co. line, along stream near pond, 15 June 2009, *Gowen 1068* (BRY, JEPS); Serpentine area, open flat near Barrel Spring, Walker Ridge, 39°08'48.2"N, 122°28'42.0"W, 691 m, 2 July 2005, *Ahart & Schlising 12131* (BRY, CHSC, JEPS); Napa County, Snell Wildflower Preserve, SW side of Snell Valley Road, east of Butts Canyon Road, 165 m, 15 June 2009, *Gowen 1062* (BRY, JEPS, RSA); Along Snell Valley Road within 50 yards of jet with Butts Canyon road, 26 May 1996, *Johnson 96-012* (BRY).

Notes:—The first author first collected this species in 1996 at the type locality where the label records “stamens equally exerted to middle of corolla lobes”, a diagnostic observation not appreciated at that time. Later lab work revealed the genetic differences between this material and *N. intertexta*, which led to subsequent field and lab work cumulating in this work. This species was included in Johnson *et al.* (2012b) as “*Navarretia* sp. nov. 1-CA.”

Key distinguishing *Navarretia paradoxiclara* and *N. paradoxinota* from *N. intertexta* and *N. propinqua*

This short key effectively replaces lead 20 in the recent treatment of *Navarretia* in California (Johnson 2012), and distinguishes the new species from their closest morphological allies.

1. Plants generally mounding with multiple heads in close proximity; corolla (4.5–)5–6.5 mm long; stamens exerted ± to corolla tips..... *Navarretia propinqua*
- Plants generally upright, or much branched with multiple heads well separated along stems; corolla 6–13.5 mm long 2
2. Corolla 6–8(–13) mm long; anthers exerted beyond corolla tips when flowers pressed..... *Navarretia intertexta*
- Corolla 6.5–13.5 mm long; anthers less than or equal to corolla lobe tips when pressed 3
3. Corolla 9.5–13.5 mm long, pale blue to white; corolla lobes 1.25–2.5 mm wide, broadly ovate; plants of Calaveras and Tuolumne Counties *Navarretia paradoxiclara*
- Corolla 6.5–9.5 mm long, white; corolla lobes 0.5–1.25 mm wide, narrowly ovate. Plants of Lake, Napa, and Colusa Counties *Navarretia paradoxinota*

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References

- Bentham, G. (1833) *Collomia coccinea*. *Edward's Botanical Register* 19: pl. 1622.
- Bentham, G. (1839–1857) *Plantas Hartwegianas*. W. Pamplin, London, 393 pp.
- Davis, J.I. & Nixon, K.C. (1992) Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Biology* 41: 421–435.
<http://dx.doi.org/10.1093/sysbio/41.4.421>
- Day, A.G. (1993) New taxa and nomenclatural changes in *Allophyllum*, *Gilia*, and *Navarretia* (Polemoniaceae). *Novon* 3: 331–340.
<http://dx.doi.org/10.2307/3391379>
- de Queiroz, K. (1998) The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: Howard, D.J. & Berlocher, S.H. (eds.) *Endless forms: species and speciation*. Oxford University Press, Oxford, pp. 57–75.
- de Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879–886.
<http://dx.doi.org/10.1080/10635150701701083>
- Greene, E.L. (1887). Some American Polemoniaceae I. *Pittonia* 1: 120–139.
- Hamilton, M.B. (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8: 521–523.
- Johnson, L.A. (2012) *Navarretia*. In: Baldwin, B.G., Goldman, D.H., Keil, D.J., Patterson, R., Rosatti, T.J. & Wilken, D.H. (eds.) *The Jepson manual: flowering plants of California* edition 2. University of California Press, Berkeley. pp. 1062–1068.
- Johnson, L.A., Chan, L.M., Burr, K. & Hendrickson, D. (2012a) *Navarretia furnissii* (Polemoniaceae), a new diploid species from the intermountain western United States distinguished from tetraploid *Navarretia saximontana*. *Phytotaxa* 42: 51–61.
- Johnson, L.A., Chan, L.M., Pozner, R. & Glazier, L.D. (2012b) Allotetraploids in Patagonia with affinities to western North American diploids: did dispersal or genome doubling occur first? *Botanical Review* 78: 288–306.
- Mason, H.L. (1946) Five new species of *Navarretia*. *Madroño* 8: 196–200.
- Munsell Color Company (2000) *Munsell soil color charts, year 2000 revised washable edition*. Munsell Color Company, Inc., Grand Rapids, MI.
- Nuttall, T. (1848) Descriptions of plants collected by Mr. William Gambel in the Rocky Mountains and Upper California. *Proceedings of the Academy of Natural Sciences of Philadelphia* 4: 7–26.
- Porter, J.M. (1996) Phylogeny of Polemoniaceae based on nuclear ribosomal internal transcribed spacer DNA sequences. *Aliso* 15: 57–77.
- Rambaut, A. (1996) *Se-Al: sequence alignment editor*. Published by the author. Available from <http://tree.bio.ed.ac.uk/software/> (accessed: 15 Feb 2013).
- Ruiz, L.H. & Pavón, J.A. (1794) *Flora Peruviana, et Chilensis Prodromus*. Impr. De Sancha, Madrid.
- Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166.
<http://dx.doi.org/10.3732/ajb.92.1.142>
- Simmons, M.P. & Ochoterena, H. (2000) Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.
<http://dx.doi.org/10.1093/sysbio/49.2.369>
- Snow, N., Guymier, G.P. & Sawvel, G. (2003) Systematics of *Austromyrtus*, *Lenwebbia*, and the Australian species of *Gossia* (Myrtaceae). *Systematic Botany Monographs* 65: 1–95.
<http://dx.doi.org/10.2307/25027907>
- Spencer, S.C. & Porter, J.M. (1997) Evolutionary diversification and adaptation to novel environments in *Navarretia* (Polemoniaceae). *Systematic Botany* 22: 649–668.
<http://dx.doi.org/10.2307/2419433>
- Spencer, S.C. & Spencer, A.E. (2003) *Navarretia willamettensis* and *Navarretia saximontana* (Polemoniaceae), new species from ephemeral wetlands of western North America. *Madroño* 50: 196–199.
- Suksdorf, W.N. (1906) Washingtonische Pflanzen. II. *Allgemeine Botanische Zeitschrift für Systematik, Floristik, Pflanzengeographie* 12: 26–27.
- Swofford, D.L. (2003) *PAUP* Phylogenetic analysis using parsimony (*and other methods) 4.0b10*. Sinauer Associates, Sunderland.
- Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1110.
- White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J. & White, T. (eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp. 315–322.

Appendix I. Voucher specimens used in DNA sequencing studies

Presented with acronym label used in Fig. 1, followed by U.S. state and county of origin, the collector, and collector number. All specimens are deposited at BRY, available online at <http://lib.byu.edu/sites/scholarsarchive/life-sciences/s-l-welsh-herbarium-bry/>.

Navarretia tagetina. CA1 = California, Lake County, *Johnson 04-024*. CA2 = California, Lake County, *Johnson 04-046*.

Navarretia subuligera. CA1 = California, Shasta County, *Johnson 04-135*. CA2 = California, Napa County, *Johnson & Gowen 09-052*.

Navarretia intertexta. BM = Mexico, Baja California Sur, *Brey 229*. CA1 = California, Lassen County, *Johnson 93-088*. CA2 = California, Lake County, *Johnson 04-038*. CA3 = California, Shasta County, *Johnson 04-129*. CA4 = California, Lake County, *Johnson & Zhang 05-146*. CA5 = California, Shasta County, *Johnson & Zhang, 05-163*. CA6 = California, Tulare County, *Gowen 1057*. CA7 = California, Mariposa County, *Clines 2337*. ID = Idaho, Owyhee County, *Atwood 20326*. NV = Nevada, Elko County, *Howell s.n.* OR1 = Oregon, Grant County, *Johnson 93-084*. OR2 = Oregon, Benton County, *Johnson & Halse, 05-206*.

Navarretia propinqua. UT = Utah, Utah County, *Johnson 04-163*. NV = Nevada, Elko County, *Howell s.n. (44405)*.

Navarretia furnissii. UT = Utah, Summit County, *Johnson & Johnson 05-197*.

Navarretia prostrata Greene (1887: 130). CA = California, Monterey County, *Wilken s.n.*

Navarretia leucocephala subsp. *pauciflora* (Mason 1946: 200) Day 1993: 337. CA = California, Lake County, *Johnson 04-036*

Navarretia paradoxiclara. CA1 = California, Napa County, *Johnson 96-012*. CA2 = California, Napa County, *Gowen 1062*. CA3 = California, Lake County, *Johnson & Gowen 09-053*. CA4 = California, Lake County, *Gowen 1068*. CA5 = California, Lake County, *Gowen 1129*.

Navarretia paradoxinota. CA1 = California, Calaveras County, *Johnson et al. 09-032*. CA2 = California, Calaveras County, *Johnson et al. 09-033*. CA3 = California, Tuolumne County, *Johnson et al. 09-035*. CA4 = California, Tuolumne County, *Johnson et al. 09-039*. CA5 = California, Tuolumne County, *Johnson et al. 09-040*.