



Transfer of four species of *Cryptantha* to the genus *Johnstonella* (Boraginaceae)

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Abstract

Based on a previous molecular phylogenetic analysis, *Cryptantha*, an herbaceous plant genus of the family Boraginaceae, subtribe Amsinckiinae, was split into five genera: *Eremocarya*, *Greeneocharis*, *Johnstonella*, *Oreocarya*, and a reduced *Cryptantha*, the last in two separate clades. As a result of this study, *Johnstonella* was expanded to 13 species and 15 minimum-rank taxa, these formerly classified in *Cryptantha* s.l. More recent analyses of this complex, with an increased sample size and high-throughput sequence data, indicate that four additional *Cryptantha* species not previously sampled—*C. albida*, *C. mexicana*, *C. texana*—plus what was originally identified as *C. hispida* nest within *Johnstonella* with strong support. However, the identity of *C. hispida* used in this analysis is now in doubt. The material used likely represents a new species, in the process of being investigated. Two additional species not sequenced to date—*C. geohintonii* and *C. gypsites*—are clearly close relatives of *C. albida* and *C. mexicana*, based on morphological similarity. In order to maintain monophyly of genera, we here make new combinations in transferring four of these species from *Cryptantha* to *Johnstonella*, with the new combinations *Johnstonella albida*, *J. geohintonii*, *J. gypsites*, and *J. mexicana*. We delay the transfer of *Cryptantha texana* to *Johnstonella* because of its morphological similarity to other species that clearly nest within *Cryptantha* s.s. These same molecular phylogenetic studies may also support the transfer of two previously recognized *Johnstonella* species—*J. echinosepala* and *J. micromeres*—to *Cryptantha*, one to each of two separate clades. Additional phylogenetic studies focusing on some of these taxa are needed to confirm the position of these latter three species and the possible recognition of a new genus in the complex.

Keywords: nomenclature, taxonomy, systematics

Introduction

The family Boraginaceae s.l. (e.g., as in APG IV 2016) has been split into several, separate families (see Luebert *et al.* 2016), including a reduced Boraginaceae s.s., this classification accepted here—henceforth cited as simply Boraginaceae. Chacón *et al.* (2016) proposed an infrafamilial classification of the Boraginaceae, dividing it into three subfamilies, ten tribes, and six subtribes. One of these subtribes, Amsinckiinae Brand (1931: 20) includes the genus *Cryptantha* Lehmann ex G. Don (1837: 373), with approximately 102 species and 114 minimum-rank taxa (Simpson 2007 onwards), one of the most diverse of the family (Chacón *et al.* 2016).

The first molecular phylogenetic study focusing on *Cryptantha* was that of Hasenstab-Lehman & Simpson (2012), who utilized ITS and chloroplast *trnLUAA* intron sequence data for 60 ingroup minimum-ranked taxa, using maximum parsimony and maximum likelihood analyses. These authors showed that *Cryptantha*, as previously circumscribed, is polyphyletic. Based on their analyses, *Cryptantha* was split into five previously named genera—*Eremocarya* Greene (1887a: 58), *Greeneocharis* Gürke & Harms (1899: 460), *Johnstonella* Brand (1925: 249), *Oreocarya* Greene (1887a: 57), and a reduced *Cryptantha*—the last occurring as two separate monophyletic groups in one analysis: a large *Cryptantha* s.s.1 clade and a smaller *Cryptantha* s.s.2 clade.

One of the segregates from this study, the genus *Johnstonella* Brand (1925: 249), was originally named and described with two species: *Johnstonella racemosa* (A.Gray) Brand (1925: 250), the designated type for the genus (see Simpson *et al.* 2014), and *J. inaequata* (I.M.Johnston) Brand (1925: 250). Among the diagnostic features described for the genus were the suffruticose habit and perennial plant duration (although note that *P. inaequata* is known to

be an annual) and the “sharp,” acutely margined nutlets that are heteromorphic, the larger nutlet persistent and the three smaller nutlets deciduous (Figure 1J,M; see Table 1, citing genus protologue description; see also Simpson & Hasenstab 2009 and Simpson *et al.* 2014). Hasenstab-Lehman & Simpson (2012) inferred a well-supported clade consisting of these two *Johnstonella* species plus six additional sequenced species (Figure 2A). Based on these results and on comparative, morphological studies with other *Cryptantha* species, the authors transferred 11 species and 13 minimally ranked taxa from *Cryptantha* to *Johnstonella* (Table 2), for a total of 13 species and 15 minimum-rank taxa in the genus. However, many of the *Johnstonella* taxa do not conform to Brand’s original diagnosis. Almost all are annual, not suffruticose and perennial (Table 2), and several have nutlets that are homomorphic (nutlets roughly the same in size, shape, and sculpturing; Figure 1A–E), as opposed to heteromorphic (Figure 1F–M) and/or with round margins (Figure 1C,G,H,K), as opposed to acute margins (Figure 1A,B,D–F,I,J,L,M; see Table 2 for a synopsis). The nutlets of *Johnstonella* taxa are generally similar, however, in being lance-ovate to ovate with white to whitish tubercles (Figure 1; see Simpson *et al.* 2014). Only *Johnstonella grayi* (Vasey & Rose) Hasenstab & M.G.Simpson var. *nesiotica* (I.M.Johnston) Hasenstab & M.G.Simpson (2012: 754; Figure 1C, right) and the large (odd) nutlet of the heteromorphic *J. micromeres* (A.Gray) Hasenstab & M.G.Simpson (2012: 754; Figure 1K, left) have nutlets that are smooth to somewhat smooth (occasionally being sparsely tuberculate). Two *Johnstonella* species—*J. diplotricha* (Phil.) Hasenstab & M.G.Simpson (2012: 754) and *J. parviflora* (Phil.) Hasenstab & M.G.Simpson (2012: 754)—are South American with an American amphitropic disjunction (see Guilliams *et al.* 2017; Simpson *et al.* 2017a); the remainder are North American (Table 2).

TABLE 1. Description of *Johnstonella* from protologue of Brand (1925).

Calyx usque ad basin partitus. Corolla campanulata fornicibus linearibus. Stamina superiori tubi parti affixa, inclusa. Gynobasis elevationibus cariniformibus ornata. Nuculae trigonae, acute marginatae, heteromorphae, una maior persistens, tres minores deciduae, facie interiore sulcatae. – Herbae, perennes, suffruticosae, follis linearibus, cincinnis axillaribus et terminalibus. Calyces fructiferi persistentes.

English translation: Calyx parted all the way to the base. Corolla campanulate, fornices linear. Stamens fused to the upper part of the tube, included. Gynobase elevated, ornately keel-shaped. Nutlets trigonous, margins acute, heteromorphic, the larger persistent, the three smaller deciduous, adaxial face sulcate. – Herbs, perennial, suffruticose, leaves linear, cymules axillary and terminal. Calyces in fruit persistent.

TABLE 2. Previously accepted species and varieties of *Johnstonella*, in chronological order of publication. 1=sequenced in Hasenstab-Lehman & Simpson (2012); 2=inferred from comparative morphology by Hasenstab-Lehman & Simpson (2012); 3=sequenced by Simpson *et al.* (2017b); 4=sequenced by Mabry & Simpson (2018). *=type species for genus, after Simpson *et al.* 2014. NA=North America; SA=South America.

Taxon	Distribution	Plant Duration	Nutlet Heteromorphism	Nutlet Margin
<i>J. inaequata</i> ^{1,3}	NA	annual	heteromorphic	acute
* <i>J. racemosa</i> ^{1,3,4}	NA	perennial to long-lived annual	heteromorphic	acute
<i>J. angelica</i> ^{2,3}	NA	annual	heteromorphic	acute
<i>J. angustifolia</i> ^{1,3,4}	NA	annual	heteromorphic	rounded
<i>J. costata</i> ^{1,3}	NA	annual	homomorphic	acute
<i>J. diplotricha</i> ^{1,3}	SA	annual	homomorphic to slightly heteromorphic	acute
<i>J. echinosepala</i> ^{2,3}	NA	annual	heteromorphic	rounded
<i>J. fastigiata</i> ^{2,3}	NA	annual	heteromorphic	acute
<i>J. grayi</i> ²				
var. <i>grayi</i> ^{2,3}	NA	annual	homomorphic	rounded
var. <i>cryptochaeta</i> ²	NA	annual	homomorphic	rounded
var. <i>nesiotica</i> ²	NA	annual	homomorphic	rounded
<i>J. holoptera</i> ^{1,3}	NA	annual to short-lived perennial	homomorphic	acute, narrowly winged
<i>J. micromeres</i> ^{1,3}	NA	annual	heteromorphic	rounded
<i>J. parviflora</i> ^{1,3}	SA	annual	heteromorphic	acute
<i>J. pusilla</i> ²	NA	annual	homomorphic	acute

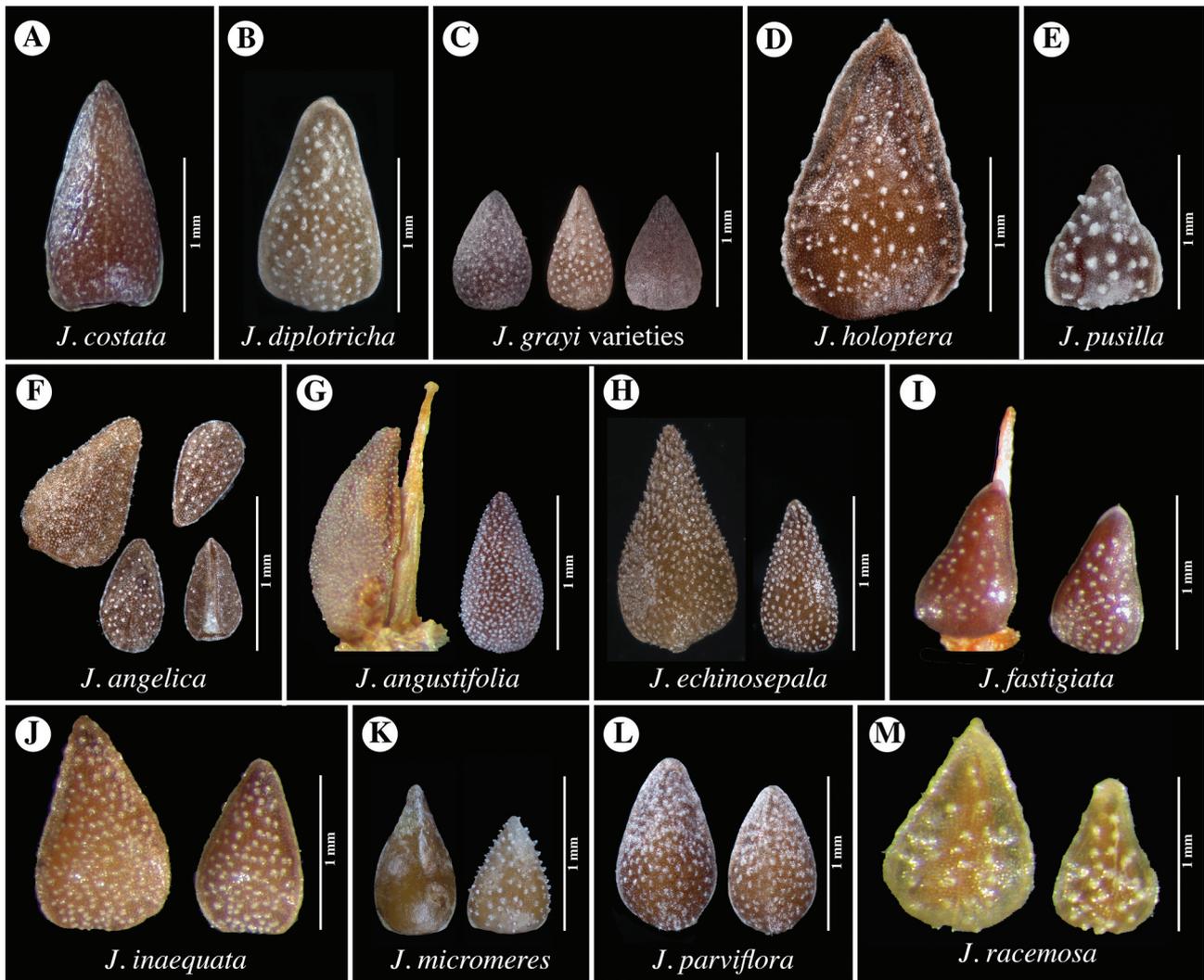


FIGURE 1. A–M. Nutlets of *Johnstonella* taxa, imaged at the same scale; source herbarium voucher accession numbers cited below (acronyms after Thiers, continuously updated). A–D. Taxa with homomorphic nutlets. A. *Johnstonella costata* (SD113307). B. *Johnstonella diplotricha* (SI47823). C. *Johnstonella grayi*; left to right: *J. g.* var. *cryptochaeta* (SD92565), *J. g.* var. *grayi* (SD94535), *J. g.* var. *nesiotica* (SD50143). D. *Johnstonella holoptera* (SD120959). E. *Johnstonella pusilla* (SD47400). F–M. Taxa with heteromorphic nutlets. F. *Johnstonella angelica* (SBBG46949). G. *Johnstonella angustifolia* (SDSU5676). H. *Johnstonella echinosepala* (SD153719). I. *Johnstonella fastigiata* (SD62789). J. *Johnstonella inaequata* (RSA732141). K. *Johnstonella micromeres* (SDSU14565). L. *Johnstonella parviflora* (MO4317600). M. *Johnstonella racemosa* (SDSU42216). Taxa at C, G, H, and K have nutlets with rounded margins, all others with acute (sharp) margins.

More recent molecular analyses of this complex of plants (Simpson *et al.* 2017b, Mabry & Simpson 2018) utilized high-throughput sequencing and genome skimming data. In these analyses sequence data of virtually the entire plastome (cpDNA), cistron (nrDNA), and of several mitochondrial (mtDNA) markers were obtained and analyzed. These two analyses incorporated both individual gene regions or concatenation of all three gene regions, and implemented maximum likelihood, Bayesian, and/or coalescent methods. Both studies consistently retrieved the bulk of *Cryptantha* as two separate clades (each strongly supported), termed *Cryptantha* s.s. (containing the type species, *C. glomerata* (Lehmann) ex G. Don (1837: 373), and congruent with *Cryptantha* s.s.1 of Hasenstab-Lehman & Simpson 2012) and what was termed the *Maritimae* clade (topologically congruent with *Cryptantha* s.s.2 of Hasenstab-Lehman & Simpson 2012). The examined species of *Johnstonella* were consistently retrieved as part of a well-supported clade, termed the *Johnstonella/Albidae* clade (see below), placed separate from both *Cryptantha* s.s. and the *Maritimae* clades in all analyses. However, although these clades were individually strongly supported, their interrelationships—and those of other clades of the Amsinckiinae, along the “backbone” of the group—varied in different analyses, with often low support.

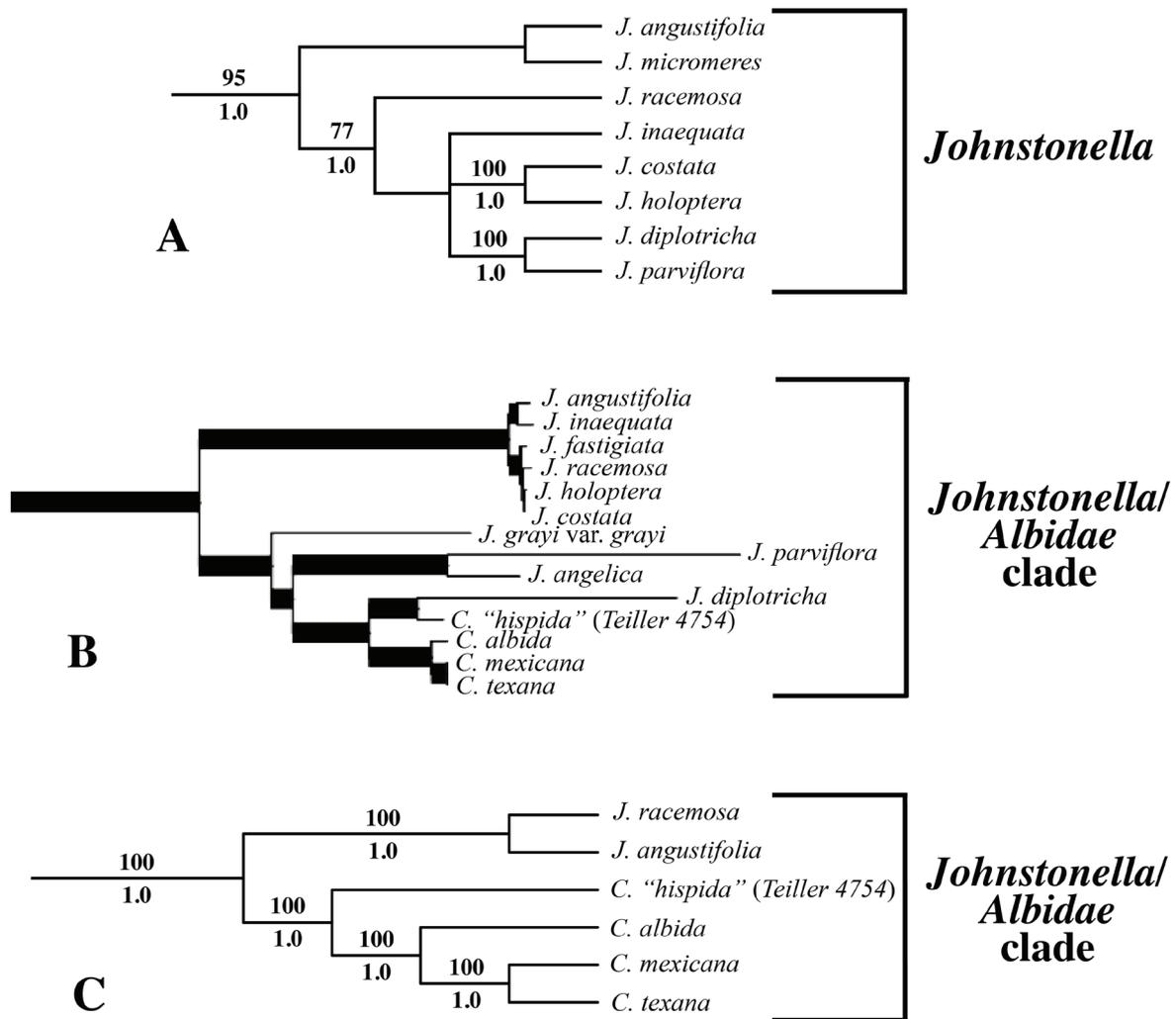


FIGURE 2. A. Maximum likelihood cladogram of *Johnstonea*, after Hasenstab-Lehman & Simpson (2012). Support values above lineages are bootstrap percentages (if strongly supported, i.e. $\geq 70\%$), those below are Bayesian posterior probabilities (if strongly supported, i.e., ≥ 0.95). If no number is present above or below, the clade is not strongly supported for that metric. B. Maximum likelihood cpDNA phylogram of *Johnstonea/Albidae* clade, after Simpson *et al.* (2017b), with three additional species of *Johnstonea* sampled. This clade differs in topology and relative branch lengths from that of their nrDNA and mtDNA trees, but all have the same species membership and strong support value for the clade itself. Note that *Cryptantha albida*, *C. "hispidia"*, *C. mexicana*, and *C. texana* are part of a clade with *J. diplotricha*. Thick lines=strong support: M.L. bootstrap $>70\%$ and Bayesian PP > 0.95 . C. Maximum likelihood concatenated cladogram of *Johnstonea/Albidae* clade, after Mabry & Simpson 2018, this is identical to that in their cpDNA, nrDNA, and coalescent trees. Support values as in "A." Taxon abbreviations of all studies: C.=*Cryptantha*; J.=*Johnstonea*.

The *Johnstonea/Albidae* clade

One novel result of both Simpson *et al.* (2017b) and of Mabry & Simpson (2018) was that four species of *Cryptantha*—*C. albida* (Kunth) I.M.Johnston (1923: 53), *C. hispidia* (Phil.) Reiche (1907: 831), *C. mexicana* I.M.Johnston (1961: 161), and *C. texana* (D.C.) Greene (1887b: 112)—were nested within a clade including all sampled taxa of *Johnstonea* with strong support, the *Johnstonea/Albidae* clade (after the genus *Johnstonea* plus *Cryptantha* series *Albidae* of Johnston 1925). In Simpson *et al.* (2017b), the focus of which was the entire subtribe, the *Johnstonea/Albidae* clade consisted of these four *Cryptantha* species and ten *Johnstonea* species—*J. angelica* (I.M.Johnston) Hasenstab & M.G.Simpson (2012: 754), *J. angustifolia* (Torr.) Hasenstab & M.G.Simpson (2012: 754), *J. costata* (Brandege) Hasenstab & M.G.Simpson (2012: 754), *J. diplotricha*, *J. fastigiata* (I.M. Johnston) Hasenstab & M.G.Simpson (2012: 754), *J. grayi* (Vasey & Rose) Hasenstab & M.G.Simpson var. *grayi* (2012: 754), *J. holoptera* (A.Gray) Hasenstab & M.G.Simpson (2012: 754), *J. inaequata*, *J. parviflora*, and *J. racemosa* (concatenated maximum likelihood seen in Figure 2B). In Mabry & Simpson (2018), the focus of which was *Cryptantha* itself, the *Johnstonea/Albidae* clade

contained the aforementioned four *Cryptantha* species along with *Johnstonella angustifolia* and *J. racemosa*, the only two members of the latter genus included in their analysis (concatenated maximum likelihood seen in Figure 2C). All trees of the *Johnstonella/Albidae* clade from these two analyses show no topological conflict (Figure 2B,C).

As alluded to earlier, placement of the *Johnstonella/Albidae* clade relative to the two *Cryptantha* s.s. clades and others of the subtribe varied in different analyses of these studies, but both clades were consistently monophyletic with strong support. Given that these four *Cryptantha* species—*C. albida*, *C. hispida*, *C. mexicana*, and *C. texana*—nested with strong support with members of the genus *Johnstonella* (Figure 1B,C), a review of their taxonomic history gives insight into this novel placement and the rationale for nomenclatural adjustments.

Series *Albidae*

In his treatment of North American members of *Cryptantha*, Johnston (1925) classified *C. albida* as the sole member of *Cryptantha* series *Albidae*. Johnston characterized the series as “Nutlets 4, coarsely tuberculate, dark, triangular-ovate, thickish, very broadly obtuse or convex on the sides, homomorphous but with the abaxial nutlet subpersistent; style much surpassing the nutlets” (p. 42; see Figure 3A). Johnston (1925) further stated that “*Cryptantha albida* is very closely related to *C. argentinica* Brand of northern Argentina, if indeed it is not the same species” (p. 44). In fact, Johnston (1927), in his treatment of South American *Cryptantha*, treated *C. argentinica* Brand (1924: 318) as a synonym of *C. albida*, which we accept here. Thus, *Cryptantha albida* is another example of an American amphitropic disjunct species (Guilliams *et al.* 2017; Simpson *et al.* 2017a). Johnston (1927) further stated that the South American *Cryptantha diplotricha* (Phil.) Reiche (1907: 821) [= *Johnstonella diplotricha*] is “most closely related to *C. albida*” (p. 39), agreeing with the close phylogenetic relationships of these species in our analyses.

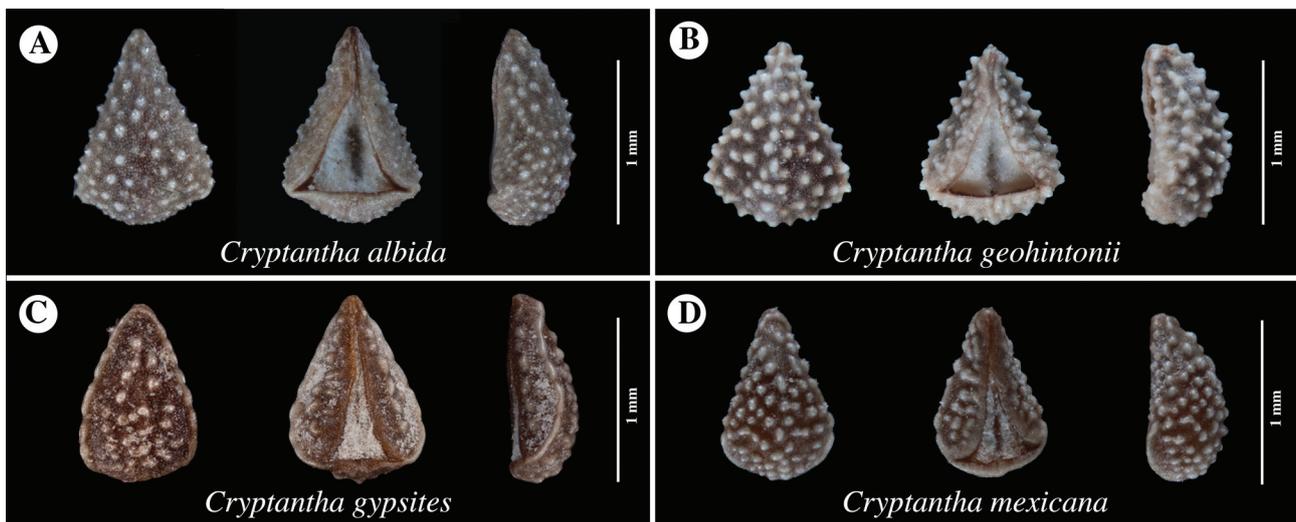


FIGURE 3. Nutlets of *Cryptantha* taxa, series *Albidae*, all homomorphous, imaged at the same scale; source herbarium voucher accession numbers cited below (acronyms after Thiers, continuously updated). A. *Cryptantha albida* (SD99139). B. *Cryptantha geohintonii* (TEX00208442). C. *Cryptantha gypsites* (LL00372644). D. *Cryptantha mexicana* (MO4011595). Nutlets, from left to right, in dorsal, ventral, and lateral views.

Cryptantha mexicana, also sequenced in our studies and also nesting within the *Johnstonella/Albidae* clade with strong support, was not validly published until considerably later by Johnston (1961). However, the species was cited by him in that publication as a close relative of *C. albida*, in fact one that “has been confused with *Cryptantha albida*” (p. 161; see Figure 3D). An additional species not included in our molecular analyses—*Cryptantha gypsites* I.M. Johnston (1959: 17)—was cited in the protologue to be a close relative of *Cryptantha albida*, resembling it in nutlet morphology (see Figure 3C). Another species not included in our analyses—*Cryptantha geohintonii* B.L. Turner (2008: 406)—was cited in the protologue of that species to be a close relative of both *Cryptantha gypsites* and *C. mexicana* (see Figure 3B). All four of these species—*Cryptantha albida*, *C. geohintonii*, *C. gypsites*, and *C. mexicana*—are similar to one another in fruit morphology and fit the concept of series *Albidae*, having nutlets that are four per fruit, homomorphous, small (1–ca. 1.2 mm long), and ovate to triangular in shape, all with prominent, white tubercles and a characteristic enlarged attachment scar, especially in the former two species (Figure 3A–D). Nutlets of *Cryptantha albida*, *C. geohintonii*, and *C. mexicana* resemble the nutlets of *Johnstonella angustifolia* and *J. grayi* in being radially thickened and in having rounded margins, whereas *C. gypsites* somewhat resembles the nutlets of other *Johnstonella* species in that the nutlets

are more radially compressed and have sharp, acute margins, these characteristic in *C. gypsites* in having a “beaded” appearance, with alternating tubercle-like thickenings. In general, nutlet morphology agrees well with the placement of *Cryptantha geohintonii* and *C. gypsites* in the *Johnstonella/Albidae* clade, these two not sequenced in the cited studies, but quite morphologically similar to *C. albida* and *C. mexicana* of series *Albidae*.

***Cryptantha* “hispidia”: A mis-identification and probable new species**

Aside from members of series *Albidae*, another *Cryptantha* taxon that nested with strong support within the *Johnstonella/Albidae* clade in our recent molecular analyses is a specimen (*S. Teillier* 4754, 17 Jun 2000, CONC150914) of what was identified as the South American *Cryptantha hispidia*. This species of *Cryptantha* had been placed in series *Phaceloides* by Johnston (1927), along with two other South American species: *Cryptantha dichita* (Phil.) I.M. Johnston (1927: 35) and *C. phaceloides* (Clos) Reiche (1907: 813). Johnston (1927) believed these three species to be quite similar, stating in his description of *C. hispidia*, “After *C. phaceloides*, from which species it [*C. hispidia*] is scarcely separable, the closest relative of this species is *C. dichita*. ... I should not be surprised if future collecting gave reason for treating *C. dichita* and *C. hispidia* as subordinate to *C. phaceloides*” (p. 36).

Simpson *et al.* (2017b) and Mabry & Simpson (2018) sequenced one sample of *Cryptantha phaceloides* (*Ackerman* 211, SGO146206), which nested with strong support in all analyses within the “core” *Cryptantha* s.s. clade, close to three other South American species: *Cryptantha diffusa* (Phil.) I.M. Johnston, *C. globulifera* (Clos) Reiche, and *C. peruviana* I.M. Johnston; all of these are distantly related to the *Johnstonella/Albidae* clade. (Material of *Cryptantha dichita* was unavailable for sequencing.) The fact that *C. phaceloides* and *C. hispidia* were placed in clades distant to one another was a puzzle given their close (possibly conspecific) similarity as noted by Johnston (1927). However, following our molecular studies, we realized that the *S. Teillier* 4754 specimen used in our studies, identified as *Cryptantha hispidia*, was evidently not that species. *Cryptantha hispidia* was described by Johnston (1927) as having “nutlets 1–2 [per fruit], smooth and shiny, pale, oblong-lanceolate, 3–3.3 mm. long, ca. 1.4 mm. broad, apex acute, base rounded, edges angled, back flat or broadly obtuse with a weak but definite medial ridge, ventrally right angled, groove closed to base and unforked” (p. 36). Measurement by us of the nutlets of *C. hispidia* from an herbarium specimen image of the holotype [*R. A. Philippi s.n.*, CHILE: Alto de Varas (SGO000004094=SGO54489)] and nutlet images of the isotype [*Philippi s.n.*, 1854, CHILE: Alto de Veras (GH00096396); see Figure 4A] indicate that they are smooth, lanceolate, and ca. 3.5 mm and 3.3 mm long, respectively, confirming Johnston’s description. The closely related *C. phaceloides* has nutlets similar in morphology to the former, cited by Johnston (1927) as being 3.5–3.8 mm long (p. 36). A visible nutlet from the image of the holotype of *C. phaceloides* (*Gay* 1621, Oct 1836 (P00606769) measures 3.7 mm long.

In contrast, the material of *C. “hispidia”* (*S. Teillier* 4754) that we sequenced has nutlets that are ovate, sharp-margined, smooth to rugulose, and ca. 1.2 mm long (Figure 4B). In addition, the calyx dimensions of the the *S. Teillier* 4754 specimen, which we measured at 2.8–3.2 mm long, do not fit those cited by Johnston (1927, p. 36) of either *C. phaceloides* (“ca. 4 mm long”) or *C. hispidia* (“4–5 mm long”). In Simpson (2017b) the *S. Teillier* 4754 specimen was placed with strong support in the *Johnstonella/Albidae* clade, sister to *Johnstonella diplotricha* (Figure 2B). In fact, the nutlet morphology of the *S. Teillier* 4754 specimen is somewhat similar to *J. diplotricha* (Figure 4C) and other species of *Johnstonella* (Figure 1) in outline and margin shape, although different in surface sculpturing from most. It is quite different from the type material of *C. hispidia* (Figure 4A) and from *C. phaceloides*, as based on both literature descriptions and personal observations.

Thus, the placement of our *Cryptantha “hispidia”* (*S. Teillier* 4754) sample within the *Johnstonella/Albidae* clade in our molecular analyses is corroborated by nutlet morphology, but we are now confident that it is not *Cryptantha hispidia*. We have to date been unable to find a previously described South American species of *Cryptantha* that fits this *S. Teillier* 4754 specimen. At present we think that this specimen is representative of a new species of *Johnstonella*, one that we are in the process of describing and naming.

Cryptantha texana* and series *Texanae

A final *Cryptantha* species that nests within the *Johnstonella/Albidae* clade in the molecular analyses cited is *Cryptantha texana*. In both Simpson *et al.* (2017b) and Mabry & Simpson (2018) this species is sister to *Cryptantha mexicana* in all analyses (maximum likelihood, Bayesian, and coalescence) using cpDNA, mtDNA, rDNA, or these data concatenated, almost always with strong support. However, Johnston (1925) placed *Cryptantha texana* not in series *Albidae*, but in his series *Texanae*, along with *Cryptantha pattersonii* Greene (1887b: 120) [now considered synonymous with *C. fendleri* (A.Gray) Greene (1887b: 120); not illustrated], *Cryptantha crassisejala* (Torrey & A.Gray) Greene (1887b: 112; Figure 5B), *Cryptantha kelseyana* Greene (1892: 232; Figure 5C), and *Cryptantha*

minima Rydberg (1901: 31; Figure 5D). The last three of these species are heteromorphic, with one large (odd) nutlet firmly attached to the gynobase and three (consimilar) nutlets that readily separate from the gynobase at maturity. These three species, which were included in both Simpson *et al.* (2017b) and Mabry & Simpson (2018), form a well-supported clade with *Cryptantha fendleri* (A.Gray) Greene and *C. recurvata* Coville (1893: 165), with all five species nesting with strong support within *Cryptantha* s.s., well apart from the *Johnstonella/Albidae* clade. Thus, based on our molecular analyses, series *Texanae* is diphyletic. [Curiously, as pointed out by Johnston (1925), the small, consimilar nutlets of *C. crassisepala* (Figure 5B, right) and of *C. minima* (Figure 5D, right) are strikingly similar to the homomorphic nutlets of series *Albidae* (Figure 3).]

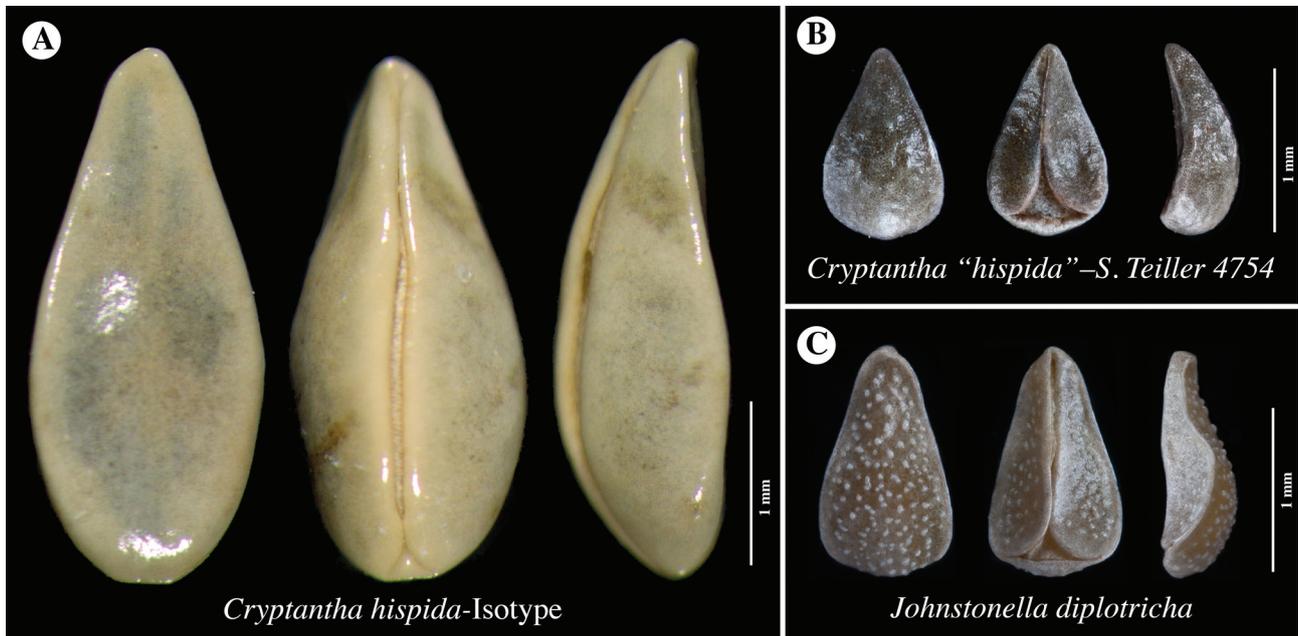


FIGURE 4. Nutlet morphology comparisons (see text for discussion); source herbarium voucher accession numbers cited below (acronyms after Thiers, continuously updated). A. *Cryptantha hispida*, series *Phaceloides* (*Philippi s.n.*, 1854; GH00096396-Isotype). B. *Cryptantha "hispida"* (*S. Teiller 4754*; CONC150914). C. *Johnstonella diplotricha* (SI47823). Note dissimilarity of "A" and "B" and greater similarity of "B" and "C". Nutlets, from left to right, in dorsal, ventral, and lateral views.

Cryptantha texana is rather unique in having solitary (one per fruit), relatively large (>2mm long) nutlets with a very densely papillate sculpturing (Figure 5A). This species resembles other members of series *Texanae* in having thick, indurate calyx midribs and in having the odd (solitary in *C. texana*) nutlet very firmly attached to the gynobase. The other members of series *Texanae*, however, have four nutlets per fruit that are heteromorphic (Figure 5B–D). Interestingly, the large (odd) nutlet of *Cryptantha minima* (Figure 5D, left) is quite similar in sculpturing (being very densely papillate) and general shape (ovate) to that of *Cryptantha texana* (Figure 5A).

Although nesting within the *Johnstonella/Albidae* clade, *Cryptantha texana* (Figure 5A) has a very different nutlet morphology from either *Johnstonella* (Figure 1) or series *Albidae* species (Figure 3). Several *Johnstonella* species have heteromorphic nutlets, and the reduction to a single nutlet can be considered a type of heteromorphism (relative to the three abortive ovules). However, *Cryptantha texana* must really be considered rather unique in the whole complex in nutlet morphology and its relationship within the Amsinckiinae subject to future analyses. Because of its morphological similarity to other members of series *Texanae* and its morphological dissimilarity to *Johnstonella* or series *Albidae* species, the phylogenetic placement of *Cryptantha texana* is in doubt.

New Combinations (see Table 3 for synopsis of characteristics)

Hasenstab-Lehman & Simpson (2012) had not examined any of these *Cryptantha* species that fall firmly within the *Johnstonella/Albidae* clade in the studies of Simpson *et al.* (2017b) and Mabry & Simpson (2018). Thus, their close relationship to *Johnstonella* was unknown at that time. Based on these more recent molecular analyses, and on comparative resemblances in nutlet morphology, we believe there is strong evidence warranting the transfer of the four species currently recognized in *Cryptantha* series *Albidae* to the genus *Johnstonella*. With these nomenclatural changes, the members of the *Johnstonella/Albidae* clade may simply be referred to as the genus *Johnstonella* in the future. As discussed earlier, the nomenclatural status of *Cryptantha texana* awaits additional, corroborative studies.

Specimens representative of the sequenced material of *Cryptantha* “*hispid*” are in the process of being investigated, and will be described as yet another species of *Johnstonella*.

We provide the new combinations below. Names of the two species occurring in the USA (*J. albida* and *J. mexicana*) will be reflected in the upcoming *Flora of North America* treatment (Kelley & Simpson, in prep.).

TABLE 3. New combinations of *Johnstonella*, this paper. NA=North America; SA=South America. 1=sequenced in Simpson *et al.* (2017b) and Mabry & Simpson (2018); 2=deduced from comparative morphology, this paper.

Taxon	Distribution	Plant Duration	Nutlet Heteromorphism	Nutlet Margin
<i>J. albida</i> ¹	NA/SA	annual	homomorphic	rounded
<i>J. geohintonii</i> ²	NA	annual	homomorphic	rounded
<i>J. gypsites</i> ²	NA	annual	homomorphic	acute
<i>J. mexicana</i> ¹	NA	annual	homomorphic	rounded

Johnstonella albida (Kunth) M.E.Mabry & M.G.Simpson, *comb. nov.* Basionym: *Myosotis albida* Kunth (in Humboldt *et al.* 1818: 91)—TYPE: MEXICO: S. Juan del Rio [Nov. gen. sp.: Crescit in alta planitie Mexicana, prope San Juan del Rio et Hacienda de Lira], *A.J.A. Bonpland, F.W.H.A. von Humboldt, s.n.* (holotype P00670727).

Johnstonella geohintonii (B.L.Turner) M.G.Simpson & R.B.Kelley, *comb. nov.* Basionym: *Cryptantha geohintonii* B.L.Turner (2008: 406)—TYPE: MEXICO: NUEVO LEON: Mpio. Mina, NE of Carricitos, 26.01386 N, 100.47018 W, 23 July 2007, *Hinton et al.* 28655 (holotype TEX00208442).

Johnstonella gypsites (I.M. Johnston) Hasenstab & M.E.Mabry, *comb. nov.* Basionym: *Cryptantha gypsites* I.M.Johnston (1959: 17–18)—TYPE: MEXICO: Nuevo Leon, 8 miles south of Galena. In gypsum open pinelands, 20 July 1958, *D. S. Correll 19872* (holotype LL00372644).

Johnstonella mexicana (Brandege) M.E.Mabry & M.G.Simpson, *comb. nov.* Basionym: *Krynitzkia mexicana* Brandege (1904: 182)—TYPE: MEXICO: Coahuila de Zaragoza, Viesca, 1903, *C. A. Purpus 126* (holotype UC126836, isotype US474666, barcode US00111058).

Taxonomic Ambiguities

Two species transferred from the genus *Cryptantha* to *Johnstonella* by Hasenstab-Lehman & Simpson (2012) show different phylogenetic affinities in the analyses of Simpson *et al.* 2017b. In the latter study *Johnstonella micromeres* nests within *Cryptantha* s.s. in all analyses with strong support. This was an unexpected result, as *J. micromeres* resembles other *Johnstonella* in having nutlets that are heteromorphic with mostly white tubercles (Figure 1K). In fact, *Johnstonella grayi* var. *cryptochaeta* (J.F.Macbr.) Hasenstab & M.G.Simpson (2012: 754) was originally described as a variety of *Cryptantha micromeres* (A.Gray) Greene (1887b: 113): *Cryptantha micromeres* var. *cryptochaeta* J.F.Macbride (1916: 46). *Johnstonella micromeres* is unique in the genus in having tubercles that are relatively elongate (muriculate) and in having uncinata (hooked) calyx trichomes. For the present, we are retaining *Johnstonella micromeres* in that genus, but note that the phylogenetic relationships of this taxon need confirmation. This species has shown considerable variation in phylogenetic position in other analyses (Hasenstab-Lehman, unpublished data), with possible reasons for this saltatory position being past hybridization or introgression events.

Second, what was named *Johnstonella echinosepala* (J.F.Macbr.) Hasenstab & M.G.Simpson (2012: 754) by Hasenstab-Lehman & Simpson (2012) based on morphology nests within the *Maritimae* clade with strong support in all analyses of Simpson *et al.* (2017b). This species, originally described as *Cryptantha echinosepala* J.F.Macbride (1918: 57), resembles other *Johnstonella* in having heteromorphic nutlets, with the smaller nutlets (Figure 1H, right) a close match to other *Johnstonella* species, such as that of *J. angustifolia* (Figure 1G, right) and all nutlets of *J. grayi* var. *grayi* (Figure 1C, middle). However, *Johnstonella echinosepala* is unique in the genus in that the large (odd), persistent nutlet is spinulose in sculpturing (Figure 1H, left) and in that the calyx in fruit is divergent to recurved with prominent, large, hispid trichomes on the upper (adaxial) side. The *Maritimae* clade [= *Cryptantha* s.s.2 of Hasenstab-Lehman & Simpson 2012], within which it is nesting, is a disparate assemblage of taxa but one that consistently is placed well separate from the *Johnstonella/Albidae* and *Cryptantha* s.s. clades of both Simpson *et al.* (2017b) and

Mabry & Simpson (2018). This complex needs further study, but may eventually be classified as a separate genus of the Amsinckiinae.

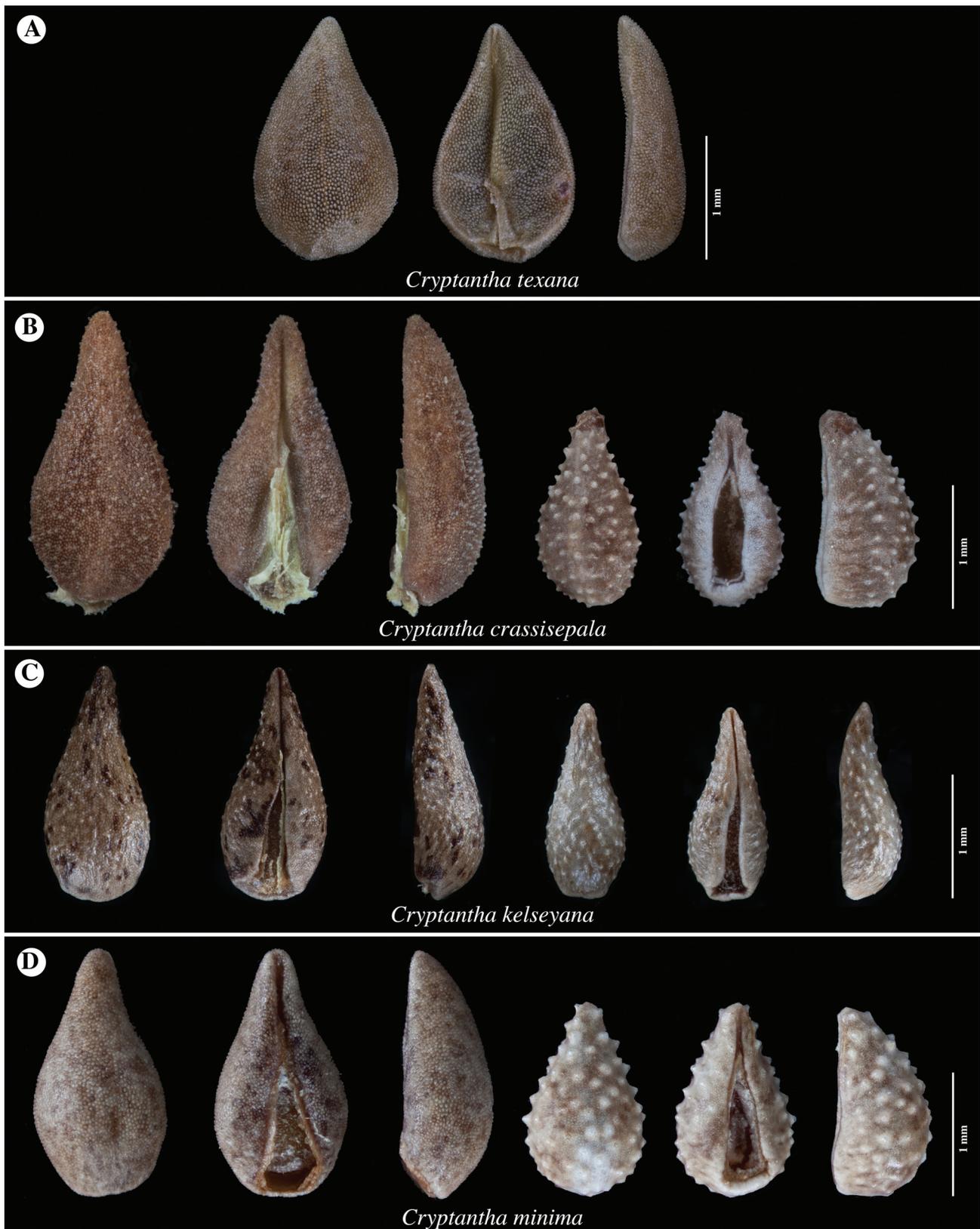


FIGURE 5. *Cryptantha* taxa of series *Texanae*; source herbarium voucher accession numbers cited below (acronyms after Thiers, continuously updated). A. *Cryptantha texana* (POM256905), nutlet one per fruit. B. *Cryptantha crassisepala* var. *elacantha* (SDSU20875), nutlets four per fruit, heteromorphic. C. *Cryptantha kelseyana* (Kelly 505, SDSU), nutlets four per fruit, heteromorphic. D. *Cryptantha minima* (POM369263), nutlets four per fruit, heteromorphic. In B–D, large (odd) nutlet to left in dorsal, ventral, and lateral views, one of the three small (consimilar) nutlets to right in dorsal, ventral, and lateral views.

Conclusions

In conclusion, we feel confident of four transfers of the *Cryptantha* species in series *Albidae* to the genus *Johnstonella*. We believe that an undescribed species in the subtribe also belongs in the genus *Johnstonella*, its placement awaiting valid publication of the name. The possible transfer of *Cryptantha texana* to *Johnstonella*, although supported by molecular data, is conflicted because of the morphological similarities of this species to members of *Cryptantha* series *Texanae*. Finally, we consider the possibility that two species previously placed in *Johnstonella* might be re-classified in *Cryptantha*, based on molecular phylogenetic analyses. Additional studies, ideally using different sequence data, such as multiple nuclear genes, are crucial to resolving these latter issues with confidence.

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