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CARDUUS CINEREUS (ASTERACEAE) – NEW TO NORTH AMERICA

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ABSTRACT

Carduus cinereus M.Bieb. populations have been discovered in the Hells Canyon Wilderness of northeastern Oregon and western Idaho. These plants were initially identified as *C. pycnocephalus* L. They are distinguished from that species by loosely clustered, usually pedunculate, heads and by scarious-margined phyllaries. These plants are genetically distant from other North American *Carduus* species (<94% similarity) based on DNA sequence comparisons of the internal transcribed spacers between the 18S and 26S nuclear rDNA regions. *Carduus cinereus* is new to North America. A revised key to the species of *Carduus* in North America is presented.

Key Words: Asteraceae, *Carduus cinereus*, taxonomy, non-native, invasive species.

Carduus L. comprises about 90 species from Eurasia and northern Africa, mainly in the Mediterranean region (Susanna and Garcia-Jacas 2007). Currently, five *Carduus* species are listed as introduced in North America (Keil 2006). A thistle collected in the Hells Canyon Wilderness in the northeastern corner of Oregon was initially identified by noxious weed managers as *Carduus pycnocephalus* L. (Italian thistle). Over several years, the same thistle was found at additional locations. Identification of the plants was challenged in 2014 by Eric Coombs (then State Biological Control Entomologist for the Oregon Department of Agriculture), who noted morphological differences between populations of *C. pycnocephalus* from southeastern Oregon and the plants that had been identified as *C. pycnocephalus* in northeastern Oregon. Based on morphology and the molecular phylogenetic analysis described below, we conclude that a species new to North America, *Carduus cinereus* M.Bieb., is present in northeastern Oregon and adjacent Idaho.

TAXONOMIC TREATMENT

Carduus cinereus M.Bieb., Fl. Taur.-Caucas. 2:270. 1808. *Carduus pycnocephalus* L. subsp. *cinereus* (M.Bieb.) P.H.Davis, Notes Roy. Bot. Gard.

Edinburgh 33(3):429. 1975; *Carduus arabicus* Jacq. subsp. *cinereus* (M.Bieb.) Kazmi, Mitt. Bot. Staatssamml. München 5:451. 1964.

Annuals, 15–120 cm tall. **Stems** simple to openly branched, loosely tomentose; winged, teeth of wings to 5 mm long, wing spines to 10 mm long. **Leaves** basal, tapering to winged petioles, blades to 10 cm long, margins pinnately 2–5-lobed, abaxial faces tomentose, adaxial faces loosely tomentose; cauline, sessile, shorter, distally greatly reduced. **Heads** borne singly or clustered in \pm loose groups of 2–5 at ends of branches and in distal axils, sessile or short-pedunculate. **Peduncles** 0–3 cm long, unwinged to sparsely winged, tomentose. **Involucres** cylindrical to ellipsoid, 12–20 mm long \times 7–20 mm in diameter. **Phyllaries** linear-lanceolate, the outer, with appressed, loosely tomentose bases 2–3 mm wide and ascending, linear appendages 0.5–1.0 mm wide, scarious-margined, distally scabrous on faces and margins, spine tips 1–2 mm long, inner, straight, erect, with armed or minutely armed tips. **Corollas** \pm purple, 12–15 mm long; lobes ca. 2.5 times longer than throat. **Cypselae** golden brown to brown, 4–6 mm long, finely-nerved; **pappus** bristles 10–20 mm long.

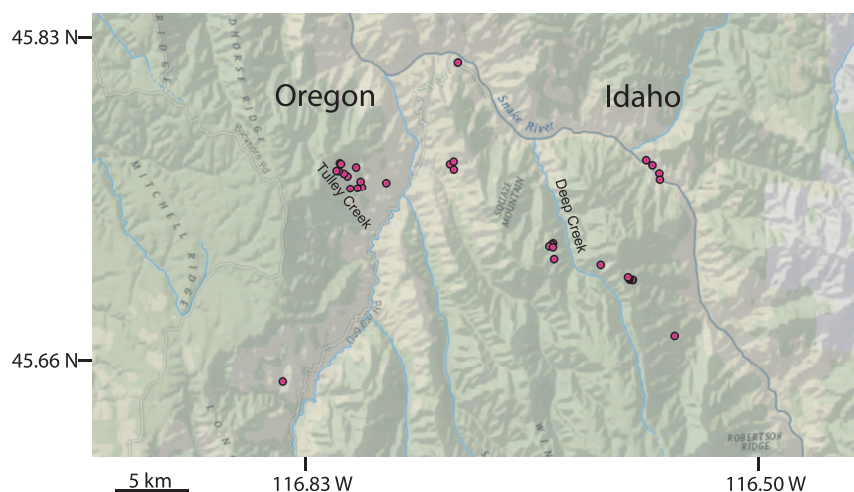


FIG. 1. Map of *Carduus cinereus* locations in northeastern Oregon and adjacent Idaho, USA.

Specimens examined: USA. OREGON. Wallowa Co.: Tyron Creek, Snake River Canyon, 45.6737°, -116.5555°, 1650 m, 26 May 2016, *Mark Porter s.n.* (UC); Tulley Creek, Imnaha Canyon, 45.7582°, -116.7895°, 1650 m, 4 May 2016, *Mark Porter s.n.* (CDA, UC); Idaho. Idaho Co.: Pittsburgh Landing, Snake River Canyon, 45.7653°, -116.5705°, 2500 m, 10 May 2016, *B. Smengut s.n.* (UC);

TAXONOMIC RELATIONSHIPS

Carduus cinereus has been considered to be part of a species complex including *C. arabicus* Jaqu. and *C. pycnocephalus* (Kazmi 1964, Davis 1975). Kazmi (1964) and Rechinger (1979) treated *C. cinereus* as a subspecies of *C. arabicus*. Davis (1975) subordinated both *C. arabicus* and *C. cinereus* within a broadly defined *C. pycnocephalus*. The three taxa involved are closely related. Typical *C. pycnocephalus* from the western Mediterranean and *C. cinereus* from the Eastern Mediterranean and the Irano-Turanian region differ morphologically. Identifying the numerous intermediate forms noted by Davis (1975) is problematic. We treat the three main taxa of the complex as species, as did Tamamschian (1963).

DISTRIBUTION AND ECOLOGY

To date, two relatively large sites are known for *C. cinereus* for North America: Tulley Creek and Deep Creek, Oregon, and satellite populations are known (Fig. 1). We presume that future inventories will reveal a wider distribution of *C. cinereus*. The infested area is a rugged canyon grassland with scattered shrubby and forested areas in a temperate continental climate (Fig. 2A). Local variation in topography and aspect result in a variety of plant community types over small areas. Largely basaltic soils are mixed with ash and Loess deposits and provide a wide variety of substrates that impact plant

community distribution. In northwestern North America, *C. cinereus* often occurs in plant communities dominated by native bunchgrasses, including bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve) and Idaho fescue (*Festuca idahoensis* Elmer) (for regional plant community descriptions, see Johnson and Simon, 1987). To date, *C. cinereus* has been found in sites ranging from dry rocky talus on southern aspects to deeper soil in more mesic sites. Reproduction is by seed. Spread is presumed to be by wind and animals.

ORIGIN

When and how *C. cinereus* arrived in northwestern North America is unknown. The area was settled sparsely during the early 19th century; human activities were initially small homesteads. Later agricultural activities centered around sheep grazing, followed in the 1940s by cattle ranching. *Crupina vulgaris* Cass. (common crupina) is also found in the area and it has been postulated that its seed came to the area in sheep fleeces brought by Basque herders (Roché et al., 2003). *Carduus cinereus* is not known from western Europe; a different pathway for its introduction is likely.

METHODS

DNA was extracted from ~10 to 20 mg samples of silica-dried branchlet material from 21 individual plants from Oregon and Idaho (specimen information in Table 1) using a modified CTAB method (Hillis et al. 1996). We sequenced the internal transcribed spacers (ITS 1 and ITS 2) between the 18S and 26S nuclear rDNA regions, including 5.8S. We PCR amplified and sequenced using the forward and reverse primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') from White et al. (1990). PCRs were conducted using the thermal

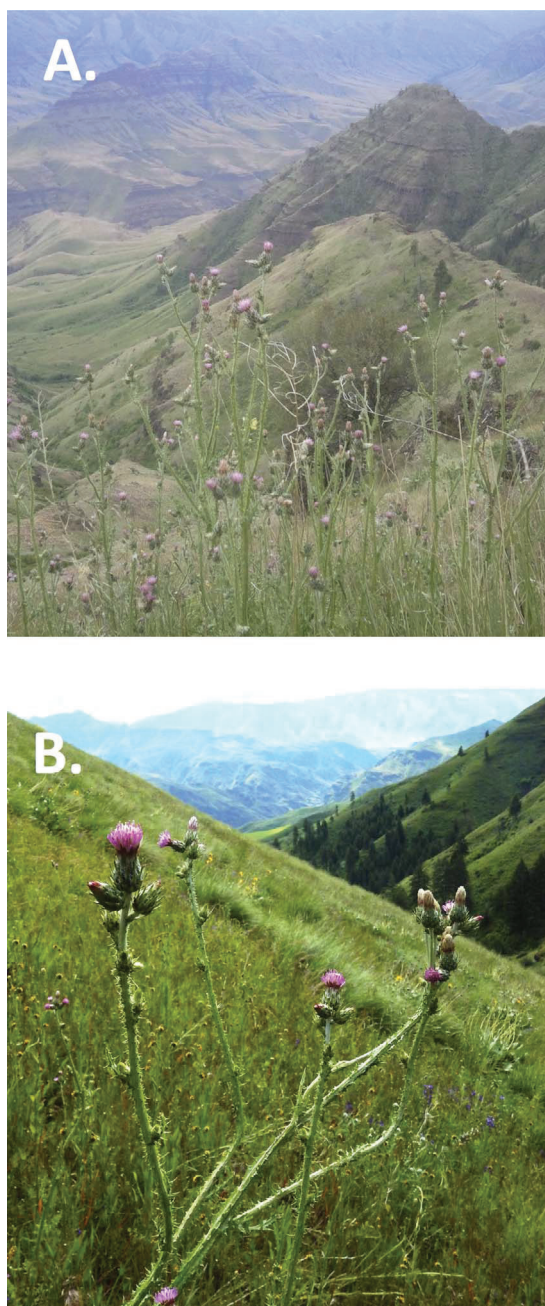


FIG. 2. *Carduus cinereus* found in northeast Oregon, USA. A. *Carduus cinereus* growing in the lower Imnaha region of Hells Canyon near the Idaho border in the rugged and variable topography. B. *Carduus cinereus* inflorescences.

cycling program: one cycle of 120 sec at 95°C, then 30 cycles of 60 sec at 95°C, 60 sec at 54°C, 120 sec at 72°C, and followed by one cycle of 300 sec at 32°C; each 30 μ L reaction contained 3 μ L of genomic DNA, 3 μ L 10X NH₄ PCR buffer (Bioline, Taunton, Massachusetts), 1.5 μ L 50 mM MgCl₂, 0.24 μ L 100 mM dNTP mix, 3 μ L 2 μ M of each primer, and 0.15 μ L (0.75

units) of Biolase DNA Polymerase (Bioline). PCR products were purified using QIAquick Gel Extraction kits (Qiagen, Germantown, Maryland). Our sequence reaction cycling program consisted of one cycle of 60 sec at 96°C, then 25 cycles of 10 sec at 96°C, 5 sec at 50°C, 240 sec at 60°C; each 10 μ L reaction contained 1.5–3 μ L of PCR template, 3 μ L 5X sequence buffer (Thermo Fisher Scientific, Waltham, Massachusetts), 0.85 μ L each primer, 1 μ L BigDye Terminator Reaction Ready Mix v 3.1 (Thermo Fisher Scientific). Sequencing was performed on an ABI 3130 Genetic Analyzer (Thermo Fisher Scientific). ITS sequences were downloaded from GenBank for other taxa included in the analyses. The analyzed taxa included all *Carduus* species known from North America (*C. acanthoides* Gren. & Godr., *C. crispus* Huds., *C. nutans* L., *C. pycnocephalus*, and *C. tenuiflorus* Curtis), selected *Carduus* species not found in North America (*C. defloratus* L., *C. personata* Jacq., *C. carlinoides* Steud., and *C. tmoleus* Boiss.), putative *C. cinereus* from Oregon and Idaho, USA, plants from Oregon morphologically identified as *C. pycnocephalus* and *C. tenuiflorus*, and outgroup species from *Cirsium*, *Onopordum*, and *Centaurea*. Sequences were aligned using ClustalW in MEGA 6.05 (Tamura et al. 2007). Genetic distance between each accession and a maximum likelihood tree with 1000 bootstraps (using a General Time Reversible model) were constructed in MEGA 6.05. Missing nucleotide sequence data from GenBank accessions were coded as N. Insertion/deletion events were included in the analysis. Within *Carduus*, there were two single base pair insertion/deletion events.

RESULTS

The ITS region provided 637 alignable base pairs. There were 22 nucleotide sites (22/637 = 3.4%) in which the USA *C. cinereus* contained alleles not found in other *Carduus* from North America. The maximum likelihood tree (Fig. 3) separated the USA *C. cinereus* from all other North American *Carduus* taxa with 99% bootstrap support. The pairwise genetic distance between the USA *C. cinereus* and all North American *Carduus* taxa ranged from 3.9–4.9%, as compared to a maximum distance of 2.9% between known *Carduus* in North America. The pairwise genetic distance between *C. pycnocephalus* (perhaps the closest North American match to the USA *C. cinereus* morphologically) and the USA *C. cinereus* was 4.9%. The genetic distance between *Carduus* taxa and the closest outgroup (*Cirsium vulgare*) was 4.3–5.8%; the genetic distance between the USA *C. cinereus* and the closest outgroup (*Cirsium vulgare*) was 5.9%. The GenBank accession number for our *C. cinereus* collections (all have identical ITS DNA sequences) is MK253665. There were no *C. cinereus* ITS sequences in GenBank; we could not make a genetic distance comparison with ITS sequences obtained from conspecific specimens. There were “*C. pycnocephalus* subsp. *arabicus*” and *C. pycnocephalus* subsp. *albidus* (M.Bieb) Kazmi

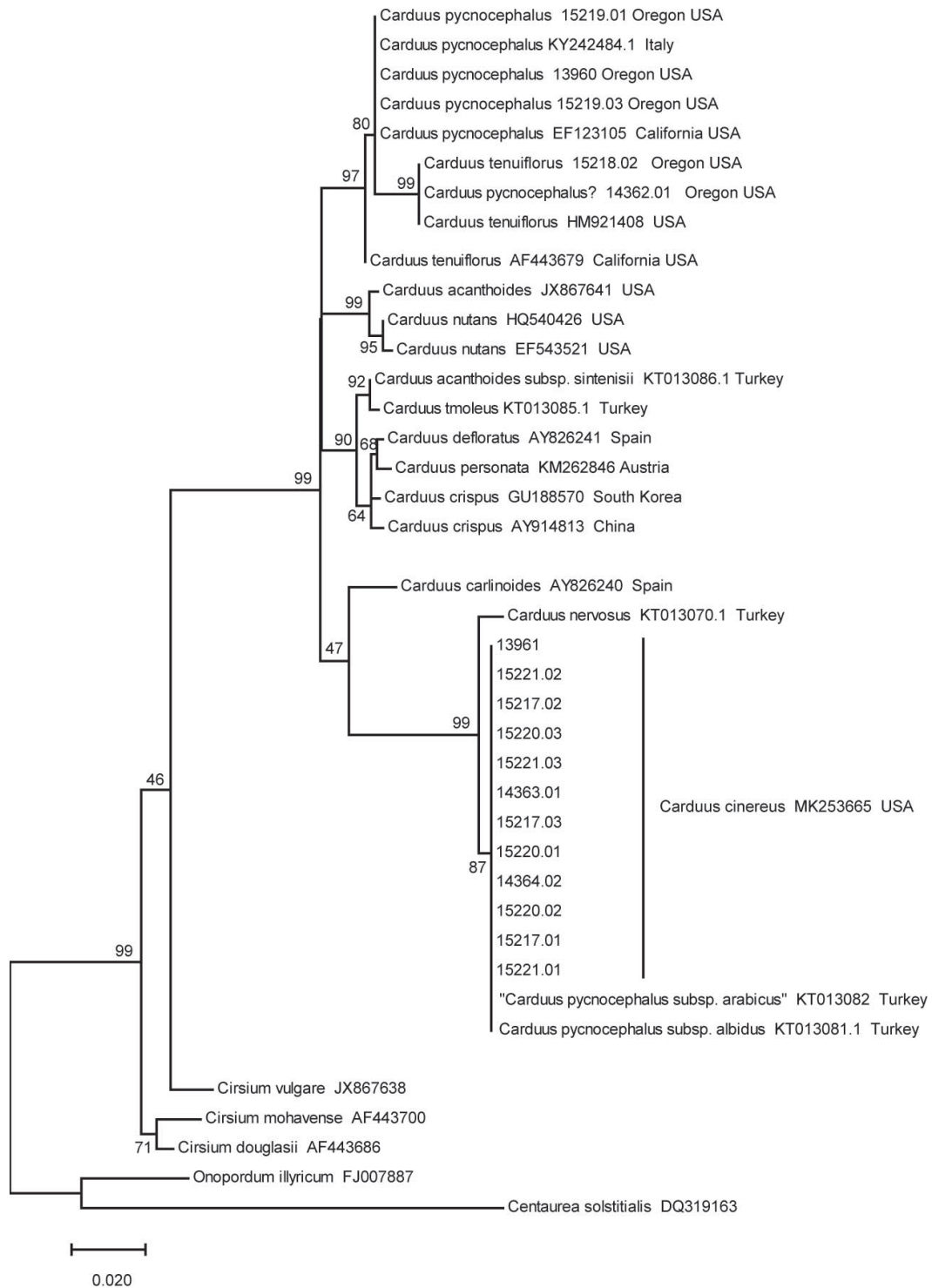


FIG. 3. Maximum likelihood tree of *Carduus* and outgroups for the internal transcribed spacer of 18S to 26S nuclear rDNA region. Accession information includes genus, species, GenBank accession number or our DNA number, and geographic origin, if known. Bootstrap values are posted next to branches.

TABLE 1. Accession Information for DNA Samples of *Carduus* Species from Oregon and Idaho, USA.

DNA sample #	Genus	Species	State	Location	Nearest town or river	Site code	Latitude	Longitude
13960	<i>Carduus</i>	<i>pycnocephalus</i>	Oregon	Douglas County	Winston		43.0965	-123.3889
13961	<i>Carduus</i>	?	Oregon	Lower Innaha Canyon	Innaha		45.7582	-116.7896
14362.01	<i>Carduus</i>	<i>pycnocephalus?</i>	Oregon	North Bank Rd.	Roseburg			
14363.00	<i>Carduus</i>	?	Oregon	Tulley Creek Site	Imnaha		45.7582	-116.7896
14364.00	<i>Carduus</i>	?	Oregon	Holmes Site	Imnaha		45.7685	-116.7186
15217.01	<i>Carduus</i>	?	Oregon	Tryon 1	Imnaha	Tryon 1	45.7046	-116.5878
15217.02	<i>Carduus</i>	?	Oregon	Tryon 2	Imnaha	Tryon 2	45.7046	-116.5878
15217.03	<i>Carduus</i>	?	Oregon	Tryon 3	Imnaha	Tryon 3	45.7046	-116.5878
15218.01	<i>Carduus</i>	<i>temuiflorus</i>	Oregon	Dixonville	Dixonville	CATE-1		
15218.02	<i>Carduus</i>	<i>temuiflorus</i>	Oregon	Dixonville	Dixonville	CATE-2		
15218.03	<i>Carduus</i>	<i>temuiflorus</i>	Oregon	Dixonville	Dixonville	CATE-3		
15219.01	<i>Carduus</i>	<i>pycnocephalus</i>	Oregon	Dixonville	Dixonville	CAPY-1		
15219.02	<i>Carduus</i>	<i>pycnocephalus</i>	Oregon	Dixonville	Dixonville	CAPY-2		
15219.03	<i>Carduus</i>	<i>pycnocephalus</i>	Oregon	Dixonville	Dixonville	CAPY-3		
15220.01	<i>Carduus</i>	?	Idaho	Cat Creek	Snake River	P. snake 1	45.7657	-116.5705
15220.02	<i>Carduus</i>	?	Idaho	Cat Creek	Snake River	P. snake 2	45.7657	-116.5705
15220.03	<i>Carduus</i>	?	Idaho	Cat Creek	Snake River	P. snake 3	45.7657	-116.5705
15221.01	<i>Carduus</i>	?	Oregon	Tulley Creek 1	Tulley Creek	Tulley Creek 1	45.7582	-116.7896
15221.02	<i>Carduus</i>	?	Oregon	Tulley Creek 2	Tulley Creek	Tulley Creek 1	45.7582	-116.7896
15221.03	<i>Carduus</i>	?	Oregon	Tulley Creek 3	Tulley Creek	Tulley Creek 1	45.7582	-116.7896
15837.00	<i>Carduus</i>	<i>crispus</i>	Oregon	Wallowa County	Enterprise		45.3948	-117.2994

GenBank accessions collected by S. Kostekci and T. Arabaci from Turkey; these had a 0.3% genetic distance from our USA *C. cinereus*. These taxa may prove to be conspecific with the USA *C. cinereus*.

DISCUSSION

If our USA *C. cinereus* with the distinct morphology were a hybrid of existing *Carduus* already found in North America, one would expect that the ITS sequence from such a recent hybridization event might not be subject to concerted evolution (Elder and Turner 1995) and would contain heterozygotic loci (or nucleotide additivity patterns) where there are sequence differences between the two parental taxa. We found only 2/637 loci (0.3%) in the USA *C. cinereus* that were heterozygotic, and only one of these loci showed multiple alleles within North American *Carduus*. In addition, the presence in the

USA *C. cinereus* of alleles unique to sampled North American *Carduus* renders its origin as a recent interspecific hybrid unlikely. The minimum genetic distance of USA *C. cinereus* from any *Carduus* listed as present in North America (3.9%), compared to the maximum genetic distance between all other North American *Carduus* species (2.9%) suggests that this is a distinct taxon. Our collections differ morphologically from *C. pycnocephalus* in North America by having heads usually pedunculate and loosely clustered (Fig. 2B) vs. usually sessile and tightly clustered and phyllaries scarious-margined vs. not scarious-margined. Given the differences in morphology and DNA sequence between *C. pycnocephalus* subsp. *pycnocephalus* and our collections from Oregon and Idaho, we suggest that we have found a species new to North America, and identify it as *C. cinereus* M.Bieb.

MODIFIED KEY TO *CARDUUS* SPECIES IN NORTH AMERICA

1. Phyllaries 2–7 mm wide, usually wider than the appressed bases; peduncles often elongate, distally wingless; heads often nodding, usually borne singly or in leafy corymbiform arrays; involucre 20–70 mm diam *Carduus nutans*
- 1' Phyllaries 0.5–2.0 mm wide, usually narrower than the appressed bases; peduncles short, if present, usually winged throughout, less often sparsely winged or unwinged; heads erect, 1–many, often clustered or loosely clustered at branch tips or upper axils; involucre 7–30 mm diam
 2. Involucre spheric or hemispheric
 3. Corollas 13–20 mm long; heads 18–25 mm long; involucre 14–20 mm long; abaxial leaf faces glabrate except for long, curled, septate hairs along veins *Carduus acanthoides*
 - 3' Corollas 11–16 mm long; heads 15–18 mm long; involucre 12–17 mm long; abaxial leaf faces sparsely to densely tomentose with fine, non-septate hairs and often with curled, septate hairs along veins as well *Carduus crispus*
 - 2' Involucre cylindrical or narrowly ellipsoid

- 4. Heads 5–20 at ends of branches; phyllaries glabrous or sparingly tomentose, distally ciliate or glabrous. *Carduus tenuiflorus*
- 4' Heads 1–5 at ends of branches; phyllaries ± persistently tomentose, distally scabrous on margins and faces
- 5. Heads usually pedunculate and loosely clustered; phyllaries scarious-margined. *Carduus cinereus*
- 5' Heads usually sessile and tightly clustered; phyllaries not scarious-margined. *Carduus pycnocephalus*

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