Research Article

Phylogeny and subgeneric classification of *Bouteloua* with a new species, *B. herrera-arrietae* (Poaceae: Chloridoideae: Cynodonteae: Boutelouinae)

Paul M. Peterson[1]*, Konstantin Romaschenko[1,2], and Yolanda Herrera Arrieta[3]

[1]Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington DC 20013-7012, USA
[2]M.G. Kholodny Institute of Botany, National Academy of Sciences, Kiev 01601, Ukraine
[3]Instituto Politécnico Nacional, CIIDIR Unidad-Durango-COFAA, Durango C.P. 34220, Mexico
*Author for correspondence. E-mail: peterson@si.edu. Tel.: 1-202-633-0975. Fax: 1-202-786-2653.
ORCID (http://orcid.org): PMP, 0000-0001-9405-5528; KR, 0000-0002-7248-4193.

Abstract *Bouteloua* (Poaceae: Chloridoideae: Cynodonteae; Boutelouiane) is an important genus of forage grasses containing 60 species found primarily in the Americas with a center of diversity in northern Mexico. A modern subgeneric classification is lacking. The goals of our study were to reconstruct the evolutionary history among the species of *Bouteloua* using molecular data with increased species sampling compared to previous studies. A phylogenetic analysis was conducted on 209 samples, of which 59 species (206 individuals) were in *Bouteloua*, using two plastid (*rpl32-trnL* spacer and *rps16-trnK* spacer) and nuclear ITS 1&2 (ribosomal internal transcribed spacer) sequences to infer evolutionary relationships and produce a subgeneric classification. Overall, ITS and plastid phylogenies rendered similar patterns. However, the ITS phylogeny lacked backbone structure, recovering only four internal clades out of nine found in the plastid phylogeny. The ITS network shows a radiative evolutionary pattern and indicates a number of incompatible splits, suggesting past hybridization between species of different sections. The maximum-likelihood tree from the combined plastid and ITS regions is well resolved and depicts a strongly supported monophyletic *Bouteloua* that includes ten strongly supported clades and one moderately supported clade. The molecular results support the recognition of 10 sections and two subsections within *Bouteloua* s.l.; three sections are new: *Barbata*, *Hirsuta*, and *Trifida*; four sections are new combinations: *Buchloe*, *Cyclostachya*, *Opizia*, and *Triplathera*; and two subsections are new: *Eriopoda* and *Hirsuta*. Based on our molecular results and the possession of unique morphological characters we describe a new species from Nuevo León, *Bouteloua herrera-arrietae*.

Key words: *Bouteloua*, classification, DNA, ITS, phylogeny, plastid DNA sequences.

Subtribe Boutelouinae Stapf (Poaceae: Chloridoideae: Cynodonteae) consists of the single genus, *Bouteloua*, with 60 species (Columbus et al., 1998, 2000; Columbus, 1999a; Clayton et al., 2006; Kinney et al., 2007; Simon, 2014; Soreng et al., 2015) centered in the southwestern USA and Mexico, including the dominant range grasses, *B. gracilis* (Kunth) Lag. ex Griffiths (blue grama) and *B. dactyloides* (Nutt.) Columbus (buffalograss). Together or separately, these two grasses are widely recognized as key species in shortgrass communities and both are highly drought and grazing tolerant (Hyder et al., 1975; Hook et al., 1991; Burke et al., 1995; Coffin et al., 1996). The genus is characterized by an inflorescence of 1–many spicate primary branches distributed along the main axis, branch axes persistent or falling entire, spikelets bisexual, unisexual (plants monoecious or dioecious), or sterile, solitary, paired, or in triplets, sometimes secund, biseriate, glumes awned or awnless, lemmas 3-nerved, awned or unawned, a base chromosome number of $x = 10$, and a preference for xeric habitats (Watson & Dallwitz, 1992;

Clayton et al., 2006). The inflorescences show significant structural variation, and this was used initially to subdivide *Bouteloua* into two subgenera, *Bouteloua* and *Chondrosum* (Desv.) A. Gray (Gray, 1956; Gould, 1980; Columbus et al., 1998; Pilatti & Vegetti, 2014).

The New World genus *Bouteloua* was established by Lagasca (1805) who listed five species with no indication of the type. Griffiths (1912), Hitchcock (1920), and Swallen (1939) all accepted the first species listed, *B. racemosa* Lag. [=*B. curtipendula* (Michx.) Torr.], as the type. Other genera, such as, *Buchloe* Engelm., *Buchlomimus* Reeder, C. Reeder & Rzed., *Cathestecum* J. Presl, *Cyclostachya* Reeder & C. Reeder, *Opizia* J. Presl, *Pentarrhaphis* Kunth, *Pringleochloa* Scribn., and *Soderstromia* C.V. Morton were known to be morphologically similar. The two monotypic genera, *Buchlomimus* and *Cyclostachya*, described by John Reeder and others, each included a species that was transferred from *Bouteloua* based primarily on the possession of sexual dimorphism (Reeder & Reeder, 1963a; Reeder et al., 1965). Phylogenetic relationships among

these eight genera and *Bouteloua* were unknown until DNA molecular studies revealed these satellite genera belonged in *Bouteloua*, now a large putative monophyletic assemblage (Columbus et al., 1998, 2000; Columbus, 1999a). Based on these earlier molecular studies no subgeneric or sectional names have been provided for the species in the much expanded, *Bouteloua*. Only *Neobouteloua* Gould (Gould, 1968), a recent segregate of *Bouteloua* (type = *Bouteloua lophostachya* Griseb.), has remained distinct and since has been found to be sister to the *Dactyloctenium* Willd.–*Brachychloa* S.M. Phillips clade and not immediately related to the Boutelouinae (Peterson et al., 2010a, 2015, unpublished).

The main goals of this study were to estimate the phylogeny of *Bouteloua* species with increased sampling and provide a subgeneric classification that reflects evolutionary units. We present here a new phylogenetic hypothesis based on ITS and two plastid regions (*rpl32-trnL* and *rps16-trnK*) for 59 of the 60 species (98.3%) that occur in *Bouteloua*. The number of samples for many of these species is expanded compared to earlier studies, including for the first time specimens of *B. megapotamica* (Spreng.) Kuntze, *B. swallenii* Columbus, and a new species. We discuss morphological and anatomical characters supporting relationships and propose a completely new subgeneric classification. We also include description of the new species, *Bouteloua herrera-arrietae*.

Material and Methods

Taxon sampling

Our sampling consists of 206 samples, representing 59 species of *Bouteloua* (59/60 or 98%), and three outgroups species: *Muhlenbergia schreberi* J.F. Gmel. (subtribe Muhlenbergiinae), *Scleropogon brevifolius* Phil. (subtribe Scleropogoninae), and *Distichlis littoralis* (Engelm.) H.L. Bell & Columbus (subtribe Monanthochloinae) (Peterson et al., 2010a, 2010b). In previous studies (Peterson et al., 2010a; Soreng et al., 2015) the closest sister to the Boutelouinae was the Monanthochloinae, followed by, in order of divergence, the Scleropogoninae and the Muhlenbergiinae. We selected the type species of each of these subtribes to include as outgroups in our study. A complete list of taxa, voucher information, and GenBank numbers can be found in Appendix S1. All collections gathered by PMP after 1998 were collected in silica but the majority of samples used in this study were taken from herbarium specimens.

DNA extraction, amplification, and sequencing

All procedures were performed in the Laboratory of Analytical Biology (LAB) at the Smithsonian Institution. DNA isolation, amplification, and sequencing of *rpl32-trnL* spacer, *rps16-trnK* spacer, and ITS followed procedures outlined in Peterson et al. (2010a, 2010b). We specifically targeted the two plastid regions that were most informative in our previous studies on chloridoid grasses (Peterson et al., 2010a, 2010b, 2011, 2012, 2014a, 2014b, 2014c, 2015).

Phylogenetic analyses

We used Geneious 6.1.6 (Kearse et al., 2012) for contig assembly of bidirectional sequences of *rpl32-trnL*, *rps16-trnK*, and ITS regions, and Muscle (Edgar, 2004) to align consensus sequences and adjust the final alignment. We identified models of molecular evolution for the cpDNA and nrDNA regions using jModeltest (Posada, 2008) and applied maximum-likelihood (ML) and Bayesian searches to infer overall phylogeny. The combined data sets were partitioned in accordance with the number of the markers used. Nucleotide substitution models selected by Akaike's Information Criterion, as implemented in jModelTest v.0.1.1, were specified for each partition (Table 1). The ML analysis was conducted with GARLI 0.951 (Zwickl, 2006). The ML bootstrap analysis used 1000 replicates with 10 random addition sequences per replicate. The tree file from the ML result was read into PAUP

Table 1 Characteristics of the three regions, *rpL32-trnL, rps16-trnK* and ITS, and parameters used in Bayesian analyses indicated by Akaike Information Criterion (AIC)

|	*rpL32-trnL*	*rps16-trnK*	Combined plastid data	ITS	Overall
Total aligned characters	862	1045	1907	740	2647
Number of variable characters	240	233	473	409	882
Sequencing success (%)	94.1	87.7	91.0	84.4	88.8
Number of new sequences	175 (95.7%)	157 (91.3%)	336 (93.5%)	151 (83.4%)	483 (90.1%)
Overall number of sequences	183	172	355	181	536
Likelihood score (-lnL)	3375.6	3338.6		8310.2	
Number of substitution types	6	6		6	
Model for among-site rate variation	gamma	gamma		gamma	
Substitution rates	2.8066 4.0117 1.0000 2.8066 4.0117 1.0000	0.3943 1.8438 0.3943 1.0000 1.8438 1.0000		0.6974 1.7278 1.5970 0.5049 3.8447 1.0000	
Character state frequencies	0.3854 0.1203 0.1274 0.3669	0.3252 0.1358 0.1395 0.3995		0.1279 0.1279 0.1279 0.1279	
Proportion of invariable sites	0.0	0.0		0.248	
Substitution model	TPM3uf+G	TPM2uf+G		SYM+I+G	
Gamma shape parameter (α)	0.841	0.683		0.950	
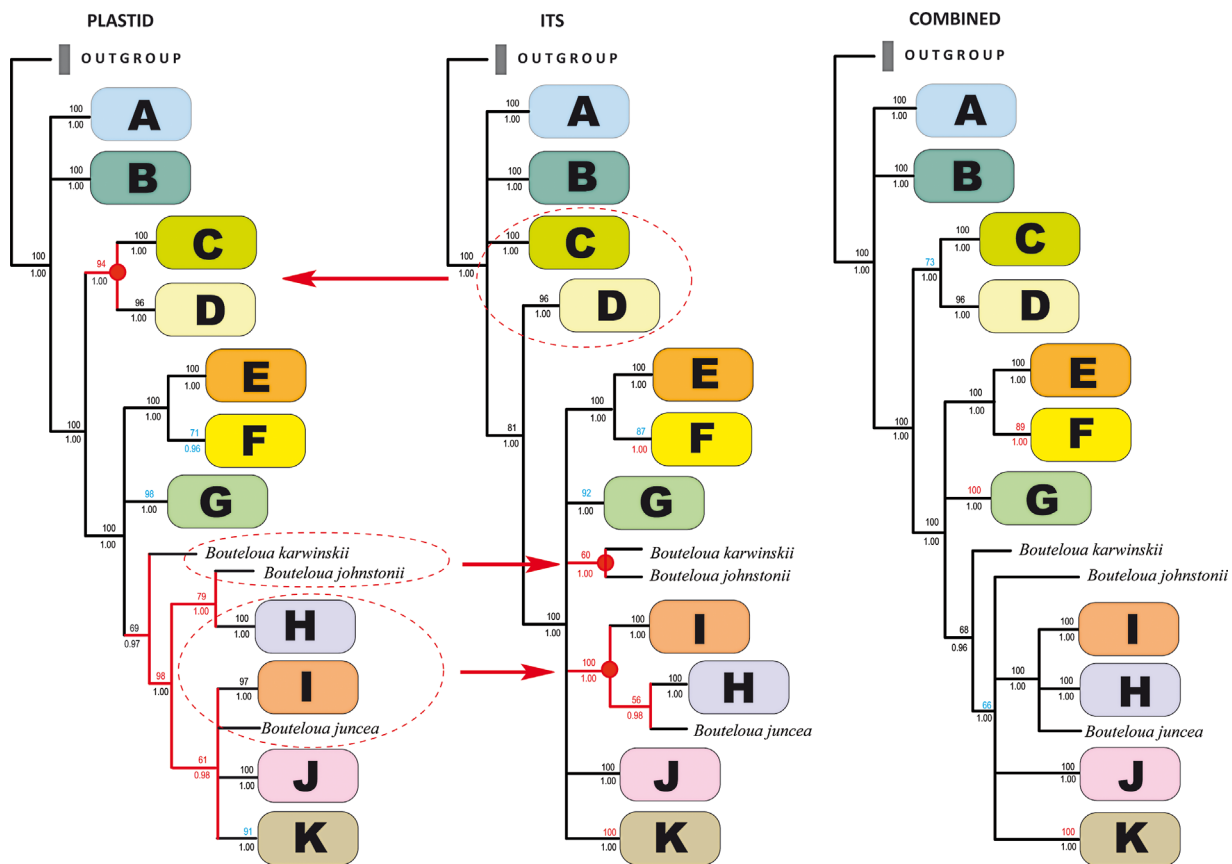
Fig. 1. A summary comparison of the plastid, ITS, and combined plastid maximum-likelihood trees. Letters indicate the clades discussed in the text; numbers above branches are bootstrap values and numbers below branches are posterior probabilities; arrows indicate the formation of a new clade not found in alternative phylogeny (dashed red outline); red numbers and branches indicate higher support values than other phylogenies; blue numbers indicate lower bootstrap values than other phylogenies.

where the majority-rule consensus tree was constructed. Bootstrap (BS) values of 90%−100% were interpreted as strong support, 70%−89% as moderate, and 50%−69% as weak (Peterson et al., 2010a, 2010b).

Bayesian posterior probabilities (PP) were estimated using a parallel version of the MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) where the run of eight Markov chain Monte Carlo iterations was split between an equal number of processors. Bayesian analysis was initiated with random starting trees and was initially run for four million generations, sampling once per 100 generations. The analysis was run until the value of the standard deviation of split sequences dropped below 0.01 and the potential scale reduction factor was close to or equal to 1.0. The fraction of the sampled values discarded as burn in was set at 0.25. Posterior probabilities (PP) of 0.95−1.00 were considered to be strong support.

Assessment of incongruence and data combining strategy
The incongruence length difference (ILD) test (Farris et al., 1994) assessed for possible incongruence between plastid and nrDNA ITS sequence data using WinClada v.1.00.08 (Nixon, 2002), with 1000 replications with remaining default parameters. Invariant characters were removed from the data sets prior to performing ILD tests. We considered $P < 0.01$

(Cunningham, 1997; Pirie et al., 2008) as evidence of significant incongruence. The resulting plastid and ITS topologies were also inspected for conflicting nodes (see Fig. 1) with $\geq$ 80% bootstrap support (BS) and/or posterior probabilities (PP) $\geq$ 0.95. If no supported conflict was found, plastid and ITS sequences were combined. Where conflicting topologies were found, the datasets for inconsistently placed taxa were duplicated in the matrix. One set of the taxon was represented by the corresponding plastid sequences only, the other taxon set by only ITS sequences. The remaining positions for the truncated datasets were then coded as missing data. We use this 'taxon duplication' approach (Pirie et al., 2008; Pelser et al., 2010) to resolve a phylogenetic tree minimizing the diffusing effects of taxa with strongly supported incongruence between the plastid and ITS data, and to represent their alternative placements in relation to the remaining phylogenetic groups among which relationships are congruent (see Fig. 2). The individual ITS and combined plastid trees are found in the supplement (Figs. S1, S2).

Phylogenetic reconstruction is challenging when plastid and nuclear data bear conflicting signals. Based on the identified incongruence between plastid and nuclear tree topologies it appears that an adequate representation of the phylogeny of *Bouteloua* required two separate trees
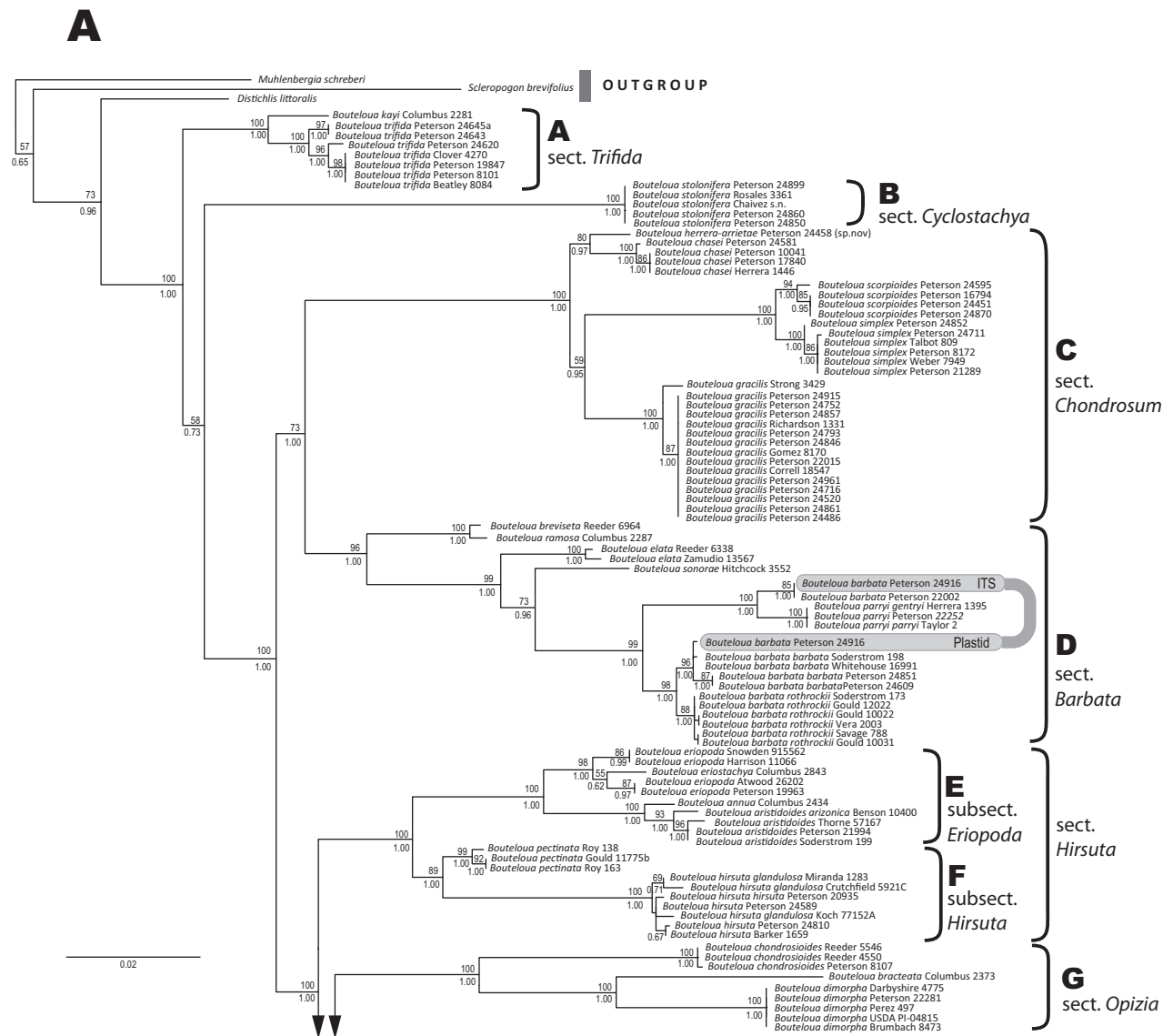
A

OUTGROUP

Muhlenbergia schreberi
Scleropogon brevifolius
Distichlis littoralis

Bouteloua kayi Columbus 2281
Bouteloua trifida Peterson 24645a
Bouteloua trifida Peterson 24643
Bouteloua trifida Peterson 24620
Bouteloua trifida Clover 4270
Bouteloua trifida Peterson 19847
Bouteloua trifida Peterson 8101
Bouteloua trifida Beatley 8084
A sect. *Trifida*

Bouteloua stolonifera Peterson 24899
Bouteloua stolonifera Rosales 3361
Bouteloua stolonifera Chaivez s.n.
Bouteloua stolonifera Peterson 24860
Bouteloua stolonifera Peterson 24850
B sect. *Cyclostachya*

Bouteloua herrera-arrietae Peterson 24458 (sp.nov)
Bouteloua chasei Peterson 24581
Bouteloua chasei Peterson 10041
Bouteloua chasei Peterson 17840
Bouteloua chasei Herrera 1446

Bouteloua scorpioides Peterson 24595
Bouteloua scorpioides Peterson 16794
Bouteloua scorpioides Peterson 24451
Bouteloua scorpioides Peterson 24870
Bouteloua simplex Peterson 24852
Bouteloua simplex Peterson 24711
Bouteloua simplex Talbot 809
Bouteloua simplex Peterson 8172
Bouteloua simplex Weber 7949
Bouteloua simplex Peterson 21289

Bouteloua gracilis Strong 3429
Bouteloua gracilis Peterson 24915
Bouteloua gracilis Peterson 24752
Bouteloua gracilis Peterson 24857
Bouteloua gracilis Richardson 1331
Bouteloua gracilis Peterson 24793
Bouteloua gracilis Peterson 24846
Bouteloua gracilis Gomez 8170
Bouteloua gracilis Correll 18547
Bouteloua gracilis Peterson 24961
Bouteloua gracilis Peterson 24716
Bouteloua gracilis Peterson 24520
Bouteloua gracilis Peterson 24861
Bouteloua gracilis Peterson 24486
C sect. *Chondrosum*

Bouteloua breviseta Reeder 6964
Bouteloua ramosa Columbus 2287
Bouteloua elata Reeder 6338
Bouteloua elata Zamudio 13567
Bouteloua sonorae Hitchcock 3552

Bouteloua barbata Peterson 24916   ITS
Bouteloua barbata Peterson 22002
Bouteloua parryi gentryi Herrera 1395
Bouteloua parryi Peterson 22252
Bouteloua parryi parryi Taylor 2

Bouteloua barbata Peterson 24916   Plastid
Bouteloua barbata barbata Soderstrom 198
Bouteloua barbata barbata Whitehouse 16991
Bouteloua barbata barbata Peterson 24851
Bouteloua barbata barbata Peterson 24609
Bouteloua barbata rothrockii Soderstrom 173
Bouteloua barbata rothrockii Gould 12022
Bouteloua barbata rothrockii Gould 10022
Bouteloua barbata rothrockii Vera 2003
Bouteloua barbata rothrockii Savage 788
Bouteloua barbata rothrockii Gould 10031
D sect. *Barbata*

Bouteloua eriopoda Snowden 915562
Bouteloua eriopoda Harrison 11066
Bouteloua eriostachya Columbus 2843
Bouteloua eriopoda Atwood 26202
Bouteloua eriopoda Peterson 19963
Bouteloua annua Columbus 2434
Bouteloua aristidoides arizonica Benson 10400
Bouteloua aristidoides Thorne 57167
Bouteloua aristidoides Peterson 21994
Bouteloua aristidoides Soderstrom 199
E subsect. *Eriopoda*

Bouteloua pectinata Roy 138
Bouteloua pectinata Gould 11775b
Bouteloua pectinata Roy 163
Bouteloua hirsuta glandulosa Miranda 1283
Bouteloua hirsuta glandulosa Crutchfield 5921C
Bouteloua hirsuta hirsuta Peterson 20935
Bouteloua hirsuta Peterson 24589
Bouteloua hirsuta glandulosa Koch 77152A
Bouteloua hirsuta Peterson 24810
Bouteloua hirsuta Barker 1659
F subsect. *Hirsuta*

sect. *Hirsuta*

Bouteloua chondrosioides Reeder 5546
Bouteloua chondrosioides Reeder 4550
Bouteloua chondrosioides Peterson 8107
Bouteloua bracteata Columbus 2373
Bouteloua dimorpha Darbyshire 4775
Bouteloua dimorpha Peterson 22281
Bouteloua dimorpha Perez 497
Bouteloua dimorpha USDA PI-04815
Bouteloua dimorpha Brumbach 8473
G sect. *Opizia*

0.02

Fig. 2. A, B, Maximum-likelihood tree inferred from combined plastid (*rpl32-trnL* and *rps16-trnK*) and ITS sequences using taxon duplication to show incongruent clades. Letters indicate the clades discussed in the text and these include our classification; numbers above branches are bootstrap values and numbers below branches are posterior probabilities; scale bar = 0.02 substitutions/site.

(Figs. S1, S2). However, the combination of data has an obvious advantage; it can provide better backbone support for nodes through the use of plastid data, and it efficiently improves resolution for terminal nodes within the main phylogenetic groups by using ITS sequences, i.e., different areas of the tree can be addressed most efficiently using these two types of data. Additionally, the ITS signal can be used to resolve issues of reticulate evolutionary history among species, an insight not provided by plastid data.

Network analysis

To investigate relationships among the species of *Bouteloua* we constructed a phylogenetic split network for ITS (Fig. 3) using the neighbor-net algorithm (Bryant & Moulton, 2004) implemented in SplitsTree version 4.11.3 (Huson &

Bryant, 2006). Uncorrected p-distances were used to weight the splits. We use the splitstree to detect putative cases of ancient hybridization among well-defined lineages with supported conflicting positions. Samples with identical sequences were removed from our analysis for a clearer presentation.

Results

Phylogenetic analyses

Ninety percent (483/536) of the sequences in our study are newly reported here and in GenBank, 15% (91/627) are missing, and 10% (53/536) are existing GenBank accessions (Appendix S1). Total aligned characters for individual regions
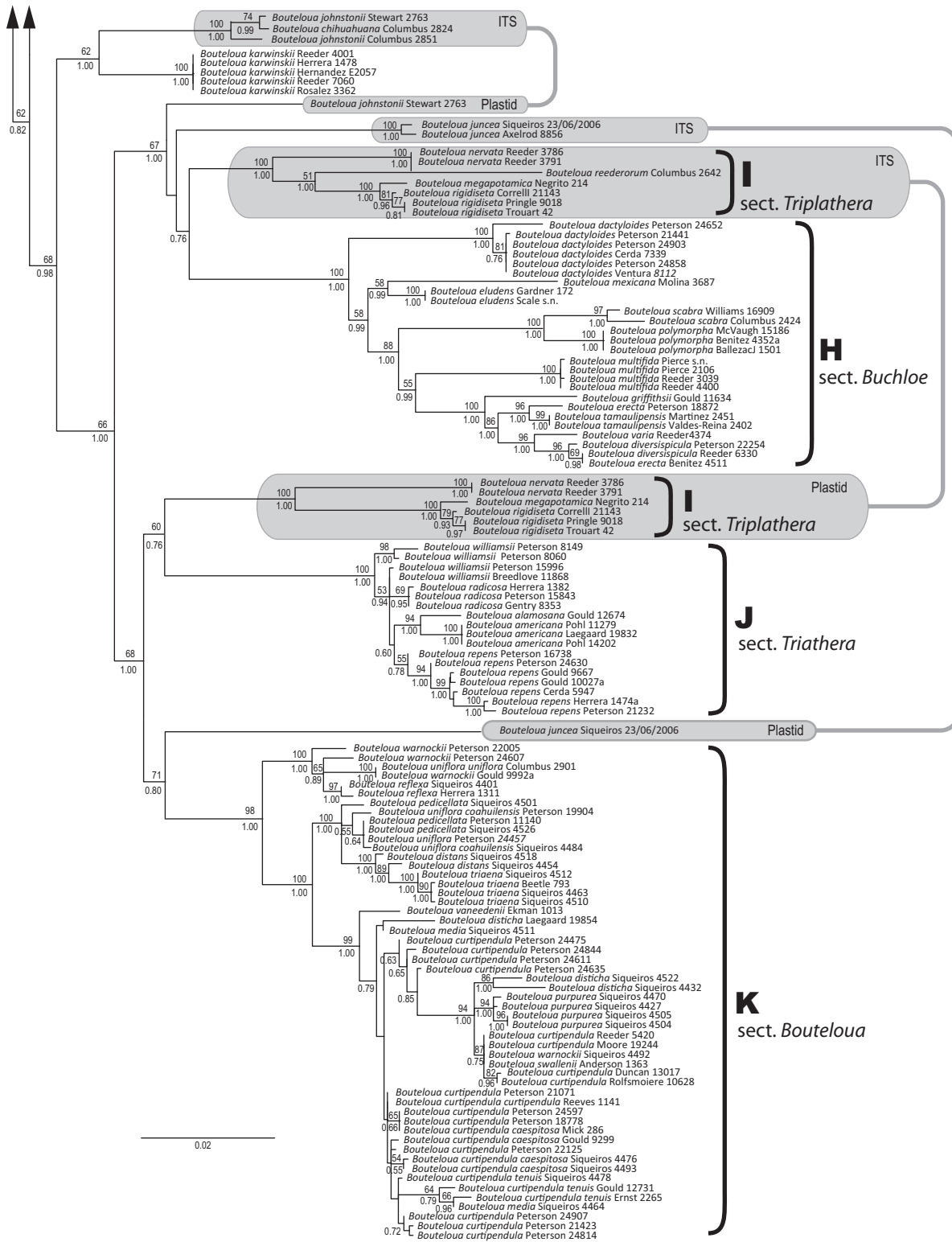
Fig. 2. Continued

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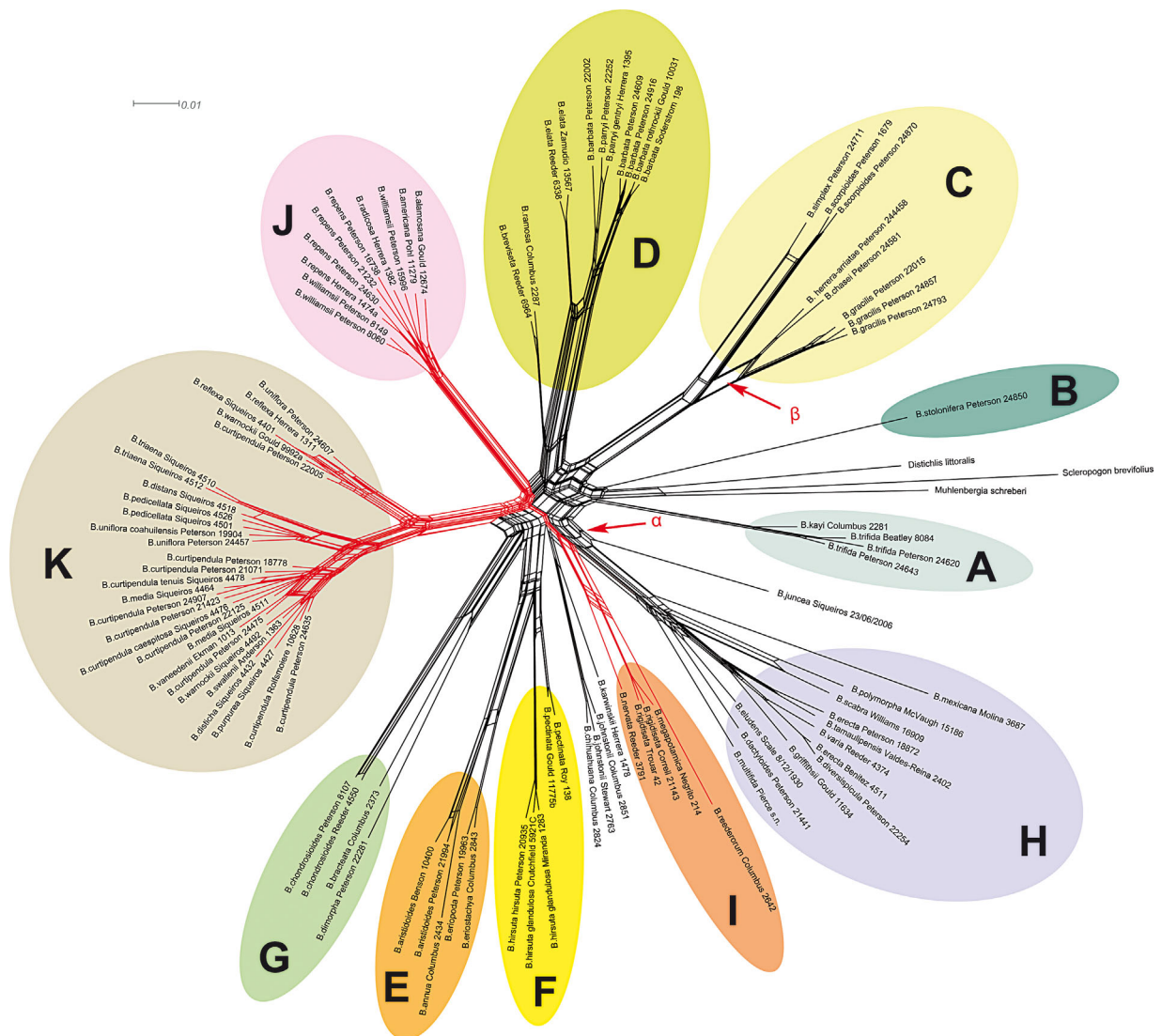*J. Syst. Evol.* 53 (4): 351–366, 2015

Fig. 3. Split network of nuclear ITS data for *Bouteloua*. Letters represent the major clades discussed in the text; α and β indicate incompatible splits; red branches indicate a different alignment not found in the plastid analysis; scale bar = 0.01 substitutions/site.

are noted in Table 1. Plastid *rpl32-trnL* and *rps16-trnK* had the highest sequencing success of 94.1% and 87.7%, respectively whereas recovery of ITS was 84.4%.

Incongruence between the ITS and combined plastid phylogram

ITS and plastid phylogenies rendered similar patterns overall. However, despite a high number of compatible characters between the two datasets (Table 1), the ITS phylogeny lacked backbone structure, recovering only four (BS ≥ 80, and/or PP ≥ 0.95) internal clades (excluding crown nodes for the labeled clades) out of nine found in the plastid phylogeny. Additionally, ITS data suggested two new rearrangements not found in the plastid phylogeny (Fig. 1): *B. johnstonii* Swallen aligns with *B. karwinskii* (E. Fourn.) Griffiths (BS = 60, PP = 1.00), whereas *B. juncea* (Desv. ex P. Beauv.) Hitchc. and "I" phylogenetic group form a strongly supported clade (BS = 100, PP = 1.00) with "H" group breaking alignment with

"J" and "K" groups found in the plastid phylogeny (BS = 61, PP = 0.98). Relationships among these taxa is not further resolved in ITS phylogeny whereas plastid data render strong support (BS = 98, PP = 1.00) for the clade including *B. johnstonii*, *B. juncea*, and groups "H", "I", "J" and "K", which is sister (BS = 69, PP = 0.97) to *B. karwinskii*. Among primarily diverged lineages plastid data support separation of "A" and "B" group prior to separation of the major clade (BS = 100, PP = 1.00) including "C–D" clade (BS = 94, PP = 1.00) and a clade including all remaining taxa (BS = 100, PP = 1.00). ITS data are not congruent with this arrangement and place "C" group in a clade with "A", "B", and all remaining taxa as single phylogenetic unit (BS = 81, PP = 1.00).

The combined analysis (Fig. 2) reinforces the phylogenetic pattern as suggested by plastid analysis overriding ITS signal in cases of inconclusive placements of "C" group, as well as supporting the position of *B. karwinskii* as sister to the clade including *B. johnstonii*, *B. juncea*, and "H–K" groups. However,

the H, I, and *B. juncea* clade is identical to the ITS signal. To avoid the confounding effect by combining incompatible data, we applied in this case a 'taxon duplication' approach seen in the final phylogenetic tree (Fig. 2), where ITS and plastid sequences are inserted separately for group "I" [*B. nervata* Swallen, *B. megapotamica* (Spreng.) Kuntze, *B. rigidiseta* (Steud.) Hitchc., without *B. reederorum* because of -missing plastid signal], *B. johnstonii*, *B. juncea*, and a single sample of *B. barbata* Lag. (*Peterson 24916*). Our final phylogenetic tree (Fig. 2) constructed using the 'taxon duplication' approach represents both alternative positions for these taxa. Noticeably, some clades which included ITS or plastid samples (Fig. 2) received lower bootstrap support than similar clades in separate plastid and ITS trees. This is true for joint H–K clade, which includes *B. juncea* and plastid subset of sequences of *B. johnstonii*, where BS value dropped from 98% in plastid analysis to 66% in combined analyses. Likewise, for H–I–*B. juncea* (ITS only) clade (including the plastid subset of sequences of *B. johnstonii*), there is a reduction of the BS value from 100% in separate ITS and plastid tree (Fig. 1, without *B. johnstonii*) to 67% in combined tree (Fig. 2) where the 'taxon duplication' approach was used. However, the posterior probability values for all of these nodes remained unchanged (PP = 1.00), suggesting that Bayesian inference is a less biased predictor of phylogenetic accuracy than the bootstrapping method (Alfaro et al., 2003) when there is a significant amount of missing data.

Given that the ILD test was $P = 0.0099$ for the overall combined analysis and $P = 0.5347$ when conflicting taxa were excluded (group "I", *B. johnstonii*, *B. juncea*, and one sample of *B. barbata*; 99% confidence level), it failed to reject the null hypothesis of congruence between the ITS and plastid data sets, so the datasets were combined.

Phylogenetic tree of Bouteloua

The ML tree from the combined plastid (*rpl32-trnL* and *rps16-trnK*) and ITS regions (Fig. 2) is well resolved and depicts a strongly supported monophyletic *Bouteloua* (BS = 100, PP = 1.00) that includes ten strongly supported (BS = 96–100, PP = 1.00; labeled A–E, G–K) clades and one moderately supported clade (BS = 89, PP = 1.00; F). Our subgeneric classification for *Bouteloua* is presented in Table 2. In order of divergence, the *B. kayi* Warnock–*B. trifida* Thurb. clade A (first split, *B.* sect. *Trifida* P.M. Peterson, Romasch. & Y. Herrera) and four individuals of *B. stolonifera* Scribn. clade B [second split, *B.* sect. *Cyclostachya* (Reeder & C. Reeder) P.M. Peterson, Romasch. & Y. Herrera] form the basal lineages (see Table 2). This grade is sister to two lineages, one containing clades C + D (BS = 73, PP = 1.00) and the other containing clade E–K (BS = 100, PP = 1.00). Clade C [*B.* sect. *Chondrosum* (Desv.) Benth. & Hook.f.] contains *B. chasei* Swallen–*B. gracilis*–*B. scorpioides* Lag.–*B. simplex* Lag.–*B. herrera-arrietae*, sp. nov. (see Fig. 4) and clade D (*B.* sect. *Barbata* P.M. Peterson, Romasch. & Y. Herrera) contains *B. barbata*–*B. breviseta* Vasey–*B. elata* Reeder & C. Reeder–*B. parryi* (E. Fourn.) Griffiths–*B. ramosa* Scribn. ex Vasey-*B. sonorae* Griffiths. In order of divergence, the *B. annua* Swallen–*B.aristidoides* (Kunth) Griseb.–*B. eriopoda* (Torr.) Torr.–*B. eriostachya* (Swallen) Reeder–*B. hirsuta* Lag.–*B. pectinata* Feath. clade (first split, BS = 100, PP = 1.00) is dividable into two clades: E (*B.* sect. *Hirsuta* subsect. *Eriopoda* P.M. Peterson, Romasch. & Y.

Herrera): *B. annua*–*B.aristidoides*–*B. eriopoda*–*B. eriostachya* (BS = 100, PP = 1.00) and F (*B.* sect. *Hirsuta* P.M. Peterson, Romasch. & Y. Herrera subsect. *Hirsuta*): *B. hirsuta*–*B. pectinata* (PP = 89, PP = 1.00). *Bouteloua bracteata* (McVaugh) Columbus–*B. chondrosoides* (Kunth) Benth. ex S. Watson–*B. dimorpha* Columbus. Clade G [*B.* sect. *Opizia* (J. Presl) P.M. Peterson, Romasch. & Y. Herrera] (second split) form a grade and are sister to a weakly supported (BS = 62, PP = 1.00) *B. chihuahuana* (M.C. Johnst.) Columbus–*B. johnstonii*–*B. karwinskii* and weakly supported (BS = 66, PP = 1.00) H–K clade. Clade H [*B.* sect. *Buchloe* (Engelm.) P.M. Peterson, Romasch. & Y. Herrera] contains *B. dactyloides*–*B. diversispicula* Columbus–*B. eludens* Griffiths–*B. erecta* (Vasey & Hack.) Columbus–*B. griffithsii* Columbus–*B. mexicana* (Scribn.) Columbus–*B. multifida* (Griffiths) Columbus–*B. polymorpha* (E. Fourn.) Columbus–*B. scabra* (Kunth) Columbus–*B. tamaulipensis* G.J. Pierce ex D. Pacheco & Columbus–*B. varia* (Swallen) Columbus and clade I [*B.* sect. *Triplathera* (Spreng.) P.M. Peterson, Romasch. & Y. Herrera] contains *B. megapotamica*–*B. nervata*–*B. reederorum*–*B. rigidiseta*. Clade J [*B.* sect. *Triathera* (Desv.) Benth. & Hook. f.] contains *B. alamosana* Vasey–*B. americana* (L.) Scribn.–*B. radicosa* (E. Fourn.) Griffiths –*B. williamsii* Swallen and is sister to clade K (*B.* sect. *Bouteloua*), containing *B. curtipendula*–*B. distans* Swallen–*B. disticha* (Kunth) Benth.–*B. media* (E. Fourn.) Gould & Kapadia–*B. pedicellata* Swallen–*B. purpurea* Gould & Kapadia–*B. reflexa* Swallen–*B. swallenii*–*B. triaena* (Trin. ex Spreng.) Scribn.–*B. uniflora* Vasey–*B. vaneedenii* Pilg.–*B. warnockii* Gould & Kapadia. Backbone support for clades H–K is mostly weak.

Network Analysis

The ITS network (Fig. 3) shows a radiative rather than tree-like pattern for *Bouteloua*. The lineage "I" appears to be significantly diverged from "K" and "J" lineages (these marked in red) with which it aligns in plastid analysis. A number of incompatible spits between "I" and "H" lineages are indicated (see α arrow), and these suggest ancient hybridizations. The same is true for *B. juncea* which aligns with "I", "J", and "K" groups in the plastid analysis, but which exhibits incompatible splits with "H" lineage in our network. Additionally, the network revealed the putative hybrid origin of *B. chasei* and *B. herrera-arrietae* (see β arrow, Fig. 3), which was not apparent in the ML trees.

Discussion

Our phylogenetic tree (Fig. 2), topologically, is highly similar to a previous phylogeny (Fig. 5 in Columbus et al., 1998) based on ITS sequences wherein *B. kayi*–*B. trifida* (first split, our clade A), *B. stolonifera* (second split, our clade B), and *B. chasei*–*B. gracilis*–*B. scorpioides*–*B. simplex* (third split, our clade C) were basal forming a grade to the remaining species of *Bouteloua* (Columbus et al., 1998). However, our plastid markers are more variable than *trnL-F* used by Columbus (1999a) and Columbus et al. (2000), and when used in combination with ITS we have obtained a better resolved phylogeny with strong support for 11 clades. Our phylogeny verifies the species composition found in Columbus et al. (1998) of *B. barbata*–*B. breviseta*–*B. elata*–*B. parryi*–*B. ramosa* (our clade D), *B. chihuahuana*–*B. johnstonii*–*B. karwinski*, *B. annua*–

Table 2 A proposed subgeneric classification of *Bouteloua* based on combined plastid and nuclear ITS DNA analysis. Letters in **bold** correspond to clades **A–K**, in Figs. 1 and 2; *indicates species not examined in this study; chromosome numbers are given in brackets [].

Bouteloua Lag.

Incertae sedis: *B. chihuahuana* (M.C. Johnst.) Columbus, *B. johnstonii* Swallen, *B. karwinskii* (E. Fourn.) Griffiths

B. sect. *Barbata* P.M. Peterson, Romasch. & Y. Herrera, Type: *B. barbata* Lag. (**D**): *B. barbata* [2*n* = 20, 40], *B. breviseta* Vasey [2*n* = 40], *B. elata* Reeder & C. Reeder [2*n* = 20], *B. parryi* (E. Fourn.) Griffiths [2*n* = 20], *B. ramosa* Scribn. ex Vasey [2*n* = 40], and *B. sonorae* Griffiths.

B. sect. *Bouteloua*, Type: *B. curtipendula* (Michx.) Torr. ≡ *Chloris curtipendula* Michx. (**K**): *B. curtipendula* [2*n* = 20, 40, 42, 45–103] *B. distans* [2*n* = 20], *B. disticha* [2*n* = 20], *B. juncea* (Desv. ex P. Beauv.) Hitchc., *B. media* [2*n* = 20], *B. pedicellata* [2*n* = 20], *B. purpurea* [2*n* = 40], *B. reflexa* [2*n* = 20], *B. swallenii*, *B. triaena* [2*n* = 20], *B. uniflora* [2*n* = 20], *B. vaneedenii*, and *B. warnockii* [2*n* = 20, 40].

B. sect. *Buchloe* (Engelm.) P.M. Peterson, Romasch. & Y. Herrera, Type: *Buchloe dactyloides* (Nutt.) Engelm. ≡ *Sesleria dactyloides* Nutt. (**H**): *B. dactyloides* [2*n* = 20, 40, 56, 60], *B. diversispicula* [2*n* = 20, 40, 60], *B. eludens* [2*n* = 20], *B. erecta* [2*n* = 20, 40], *B. griffithsii* [2*n* = 20], *B. mexicana* [2*n* = 20], *B. multifida* [2*n* = 20], *B. polymorpha*, *B. scabra* [2*n* = 20], *B. tamaulipensis* [2*n* = 20], and *B. varia* [2*n* = 20].

B. sect. *Chondrosum* (Desv.) Benth. & Hook.f., Type: *B. simplex* Lag. (**C**): *B. chasei* Swallen [2*n* = 40], *B. gracilis* (Kunth) Lag. ex Griffiths [2*n* = 20, 40, 42, 45, 50, 60, 61, 77, 84], *B. scorpioides* Lag. [2*n* = 20, 40], *B. simplex* [2*n* = 20, 40], and *B. herrera-arrieata* P.M. Peterson & Romasch.

B. sect. *Cyclostachya* (Reeder & C. Reeder) P.M. Peterson, Romasch. & Y. Herrera, Type: *B. stolonifera* Scribn. (**B**): *B. stolonifera* [2*n* = 60].

B. sect. *Hirsuta* P.M. Peterson, Romasch. & Y. Herrera, Type: *B. hirsuta* Lag. (**E, F**):

B. subsect *Eriopoda* P.M. Peterson, Romasch. & Y. Herrera, Type: *B. eriopoda* (Torr.) Torr. ≡ *Chondrosum eriopoda* Torr. (**E**): *B. annua* Swallen [2*n* = 20], *B. aristidoides* (Kunth) Griseb. [2*n* = 40], *B. eriopoda* [2*n* = 20], and *B. eriostachya* (Swallen) Reeder [2*n* = 60].

B. subsect. *hirsuta* (**F**): *B. hirsuta* [2*n* = 20, 21, 22, 24, 26, 28, 30, 32, 34, 36, 40, 42, 43, 44, 45, 46, 48, 50, 52, 53, 54, 56, 58, 60], *B. pectinata* Feath. [2*n* = 20], and **B. quiriegoensis* Beetle.

B. sect. *Opizia* (J. Presl) P.M. Peterson, Romasch. & Y. Herrera, Type: *B. dimorpha* Columbus ≡ *Opizia stolonifera* J. Presl (**G**): *B. bracteata* (McVaugh) Columbus, *B. chondrosoides* (Kunth) Benth. ex S. Watson [2*n* = 20, 22, 40], and *B. dimorpha* Columbus [2*n* = 40, 43].

B. sect. *Triathera* (Desv.)Benth. & Hook. f., Type: *B.americana* (L.) Scribn. ≡ *Triathera americana* (L.) Desv. ≡ *Aristida americana* L. (**J**): *B. Vasey* [2*n* = 40, 60], *B. americana* [2*n* = 40], *B. radicosa* (E. Fourn.) Griffiths [2*n* = 20, 60], *B. repens* (Kunth) Scribn. & Merr. [2*n* = 20, 21, 22, 23, 40, 60], and *B. williamsii* Swallen [2*n* = 20].

B. sect. *Trifida* P.M. Peterson, Romasch. & Y. Herrera, Type: *B. trifida* Thurb. (**A**): *B. kayi* Warnock and *B. trifida* [2*n* = 20]. B. sect. *Triplathera* (Spreng.) P.M. Peterson, Romasch. & Y. Herrera, Type: *Eutriana multiseta* Nees [= *Bouteloua megapotamica* (Spreng.) Kuntze] (**I**): *B. megapotamica*, *B. nervata* [2*n* = 40], *B. reederorum* [2*n* = 40], and *B. rigidiseta* [2*n* = 40].

aristidoides–*B. eriopoda*–*B. eriostachya* (our clade E), *B. hirsuta*–*B. pectinata* (our clade F), *B. bracteata*–*B. chondrosoides*–*B. dimorpha* (our clade G), *B. dactyloides*–*B. diversispicula*–*B. eludens*–*B. mexicana*–*B. multifida*–*B. polymorpha*–*B. scabra*–*B. varia* (our clade H, in part without *B. erecta*, *B. griffithsii*, and *B. tamaulipensis*), *B. nervata*–*B. reederorum*–*B. rigidiseta* (our clade I without *B. megapotamica*), B. alamosana–*B. americana*–*B. radicosa*–*B. williamsii* (our clade J), and *B. curtipendula*–*B. media*–*B. triaena*–*B. uniflora*–*B. warnockii* (our clade K, in part without *B. distans*, *B. disticha*, *B. pedicellata*, *B. purpurea*, *B. reflexa*, *B. swallenii*, and *B. vaneedenii*). We discuss each of our 11 clades separately.

Even though we choose to recognize 11 well supported clades and erect a subgeneric classification for these (Table 2, see Taxonomy section below), some species are still unplaced.

It would be premature to place the clade of *B. chihuahuana*–*B. johnstonii* and *B. karwinskii* in a section or subsection since they are not strongly supported as sister (BS = 62, PP = 1.00), and are apparently involved in a past hybridization event. Further study is required to sort out their evolutionary history.

Clade A
Bouteloua kayi, an endemic known only from calcareous rocky slopes of Boquilla Canyon in southern Brewster County, Texas, was thought by Gould (1975, 1980) to be closely related to the more wide-ranging, *B. trifida* (Espejo Serna et al., 2000; Wipff, 2003). We confirm his hypothesis in our study. Morphologically, *B. kayi* can be separated from *B. trifida* by its acuminate-lobed lemmas (not lobed in the latter species), but otherwise

Fig. 4. *Bouteloua herrera-arrietae* P.M. Peterson & Romasch. [*P.M. Peterson, K. Romaschenko & J. Valdés Reyna 24458* (US)]. **A,** Habit. **B,** Sheath, ligule, and blade. **C,** Panicle branch. **D,** Glumes. **E,** Florets. **F,** Lower glume. **G,** Upper glume. **H,** Lower lemma, ventral view. **I,** Lower lemma, dorsal view. **J,** Palea, lateral view. **K,** rudiment (second sterile floret). **L,** Third sterile floret. **M,** palea, lodicules, ovary, and stamens. **N,** Palea, lodicules, ovary, and stamen filaments. **O,** Lodicules and ovary. Drawn by Alice Tangerini.

the species are quite similar (Wipff, 2003). Correll & Johnston (1970) indicated that *B. kayi* was "probably only a form of *B. trifida*." Columbus (1996) found lemma epidermal characters to be sufficiently different between *B. kayi* and *B. trifida*, and our data clearly support recognition of these two species as distinct lineages. Significant genetic variation occurs within *B. trifida* and in the past the species was divided into two varieties, with *B. trifida* var. *burkii* (Scribn. ex S. Watson) Vasey ex L.H. Dewey having densely hairy and shorter-awned lemmas (Wipff, 2003). Our tree does not support this since nearly glabrous and hairy lemma individuals align in the

derived lineage. We describe a new section below to include these two species.

Clade B

Based on possessing staminate and pistillate plants with different spikelet morphologies and a hyaline ligule (most species of *Bouteloua* have a ciliate fringe of hairs), Reeder & Reeder (1963a) erected *Cyclostachya* Reeder and C. Reeder to include *Bouteloua stolonifera*. They suggested that *B. stolonifera* had undergone a long evolutionary history from the time that unisexuality first appeared and up to that

time no diclinous species had been reported in *Bouteloua*. Our tree indicates that *B. stolonifera* is an isolated lineage (longest branch indicating most indels) and we place it in a monotypic section below.

Clade C

Bouteloua chasei, B. gracilis, B. scorpioides, B. simplex, and *B. herrera-arrietae,* sp. nov., form a clade we place in *B.* sect. *Chondrosum.* We interpret the type of this section to be *B. simplex,* although it is clear that the genus *Chondrosum* Desv. was based on *Chondrosum procumbens* P. Durand. In his monograph of the grama grasses, Griffiths (1912) accepted *Bouteloua procumbens* (P. Durand) Griffiths as the common North American species and placed the 'heavy spiked form' from South America in *B. simplex.* Hitchcock (1935) later placed *B. procumbens* as a synonym of *B. simplex,* and this has been followed by modern agrostologists (Veldkamp, 2001). There appears to be some morphological basis for separating these two forms since the South American specimens commonly have two or more inflorescence branches at the terminal end of the culm (Griffiths, 1912). We regret not including a sample of *B. simplex* from South America in our study to test whether there is a genetic basis for segregating these two forms.

Clayton et al. (2006) still accepts *Chondrosum* as a viable genus, stating in Clayton & Renvoize (1986) that "*Chondrosum* bears an outward resemblance to *Ctenium* Panz. (now placed in subtribe Cteniinae, see Peterson et al., 2014a), and evidently is related to Chloridinae." We recognize *Bouteloua* sect. *Chondrosum* s.s. to include five species that have densely pilose fertile lemmas (3.5–5.5 mm long) with hairs located along the margins and veins (at least on the lower ½), and 3-awned apices (awns 1–3 mm long) (Herrera Arrieta et al., 2004). Our tree supports recognition of a new species, sister to *B. chasei.* The new species differs from *B. chasei* in having densely lanate upper glumes with whitish, basally flattened hairs (glabrous to sparsely pilose with terete hairs in *B. chasei*), straight flat (involute and arcuate in *B. chasei*) leaf blades with abaxial appressed hairs (glabrous in *B. chasei*), and 2.5–3.2 mm long upper glumes (3–3.5 mm long in *B. chasei*).

Clade D

This clade, also recovered by Columbus et al. (1998) includes at least six species, with significant genetic variation occurring within *B. barbata.* *Bouteloua parryi* is characterized by having papillose-based hairs along the midveins of the upper glumes and florets bearing long (2–3 mm) anthers whereas *B. barbata* and its varieties, var. *rothrockii* (Vasey) Gould, var. *sonorae* (Griffiths) Gould, and var. *barbata,* can have a few papillose-based hairs on the blades and sheaths but never on the glumes and shorter anthers usually less than 1.3 mm long (Wipff, 2003). This is contrary to Esparza Sandoval & Herrera Arrieta (1996) and Herrera Arrieta et al. (2004), where *B. barbata* var. *rothrockii* was thought to have papillose-based hairs along the midveins of the upper glumes and rachis. We believe these researchers sampled a specimen of *B. parryi* mistaking it for *B. barbata* var. *rothrockii.* In our phylogeny (Fig. 2A) it is clear that the interpretation of these taxa can be puzzling. The strongly supported clade of *B. parryi* (BS = 100, PP = 1.00) is sister to an accession of *B. barbata* (*Peterson 22002*). Sister to these is another accession of *B. barbata*

(*Peterson 24916*). The sample of *Peterson 22002* has an ITS and plastid haplotype similar to *B. parryi* whereas *Peterson 24916* has an ITS haplotype of *B. barbata* var. *barbata* and a plastid haplotype similar to *B. parryi.* The latter event is probably the result of chloroplast capture. We are unable to identify any morphological characters to differentiate these two accessions of *B. barbata* from *B. parryi.* Our single accession of *B. barbata* var. *sonorae* (*Hitchcock 3552*) is clearly separated from other varieties of *B. barbata* and *B. parryi.* Morphologically, it is a stoloniferous perennial that we believe warrants recognition at the species level as originally described by Griffiths (1912).

Bouteloua breviseta–B. ramosa and *B. elata* form a grade with the remaining species in the *B. barbata* clade. Even though our study reveals little genetic variation between *B. breviseta* and *B. ramosa,* these two species can be easily separated morphologically (Reeder & Reeder, 1980). *Bouteloua breviseta* has short or elongate scaly rhizomes, internodes with a white chalky bloom, and inflorescence branches that are narrow and erect, whereas *B. ramosa* has knotty, semi-woody culm bases (not rhizomatous), no conspicuous bloom, and nodding, arcuate inflorescence branches (Reeder & Reeder, 1980). *Bouteloua elata,* from central and southern Mexico, is the tallest member of the *B. barbata* complex having culms up to 1.4 m tall, and papillose-based hairs along the midveins of the glumes and rachis similar to *B. parryi* (Reeder & Reeder, 1963b). However, *B. elata* differs from *B. parryi* by being a stout, tall perennial with leaf blades up 20–50 cm long (5–15 mm long in *B. parryi*) (McVaugh, 1983). We include the species of this clade in *B.* sect. *Barbata.*

Clade E

Based on similar features of morphology, leaf blade anatomy, and lemma micromorphology, Columbus (1999b) hypothesized that *B. annua, B. aristidoides, B. eriopoda,* and *B. eriostachya* shared a recent common ancestor. Our phylogeny supports this conclusion and members of this clade have 2–20 appressed spikelets per inflorescence branch with a rachis axis that extends 2–12 mm beyond insertion of the terminal spikelet, and the base of the rachis is hairy to lanate (Swallen, 1935; Columbus, 1999b; Wipff, 2003; Herrera Arrieta et al., 2004). The *B. eriopoda* clade can be divided into two subclades: an annual clade in which *B. annua* is sister to three specimens of *B. aristidoides,* and a perennial clade with *B. eriopoda* and *B. eriostachya.* Our single sample of *Bouteloua eriostachya* (*Columbus 2843*) supports the inclusion of this taxon as a variety of *B. eriopoda,* as originally proposed by Swallen (1943) aligning in a grade between two pairs of *B. eriopoda* samples, all in a strongly supported clade (Fig. 2A, BB = 96, PP = 1.00). We hesitate to argue for varietal status since we have only an ITS sequence and lack plastid sequences for *B. eriostachya.* Reeder (1967) suggested that *B. eriostachya* was strictly caespitose and not stoloniferous (as in *B. eriopoda*) but Columbus (1999b) indicated that greenhouse grown specimens of *B. eriostachya* developed stolons.

In our tree (Fig. 2A) within *B. aristidoides, B. aristidoides* var. *arizonica* M.E. Jones is sister to three samples of *B. aristidoides* var. *aristidoides.* Morphologically, the latter variety has longer inflorescence branches 1.5–3.5 cm long (0.5–1.6 cm long in

typical var.) with 6–10 (2–5 in typical var.) spikelets (Wipff, 2003).

A possible synapomorphy for clades E and F is the 2–12 mm rachis extension found beyond the terminal spikelets on each inflorescence branch (Herrera Arrieta et al., 2004). Therefore, we describe clade E–F as a section below, and recognize clade E and clade F, individually as subsections.

Clade F

The *B. hirsuta* clade contains two taxa, *B. hirsuta* and *B. pectinata*, sometimes placed in the same species (Wipff & Jones, 1996). However, our phylogeny shows a long branch separating seven samples of *B. hirsuta* (BS = 100, PP = 1.00) from three samples of *B. pectinata* (BS = 99, PP = 1.00) indicating quite a few indels. *Boutloua hirsuta* generally has shorter, decumbent (versus erect in *B. pectinata*) culms 15–40 cm tall (versus 40–75 cm) with only three nodes (versus 4–6), and lacks a tuft of hairs at the base of the rudimentary floret (Wipff & Jones, 1996). Both species have inflorescence branches with pectinately inserted spikelets along a rachis that extend 5–10 mm beyond the terminal spikelet. In our tree there appears to be little genetic variation to separate *B. hirsuta* var. *glandulosa* (Cerv.) Gould from *H. hirsuta* var. *hirsuta*.

Clade G

Reeder & Reeder (1966) were first to report gynodioecy in *B. chondrosoides,* which is sister to *B. bracteata* and *B. dimorpha* in our tree, the latter two species formally treated in *Opizia* J. Presl, a genus with monoecious and dioecious plants (McVaugh, 1983; Kinney et al., 2007). Presl (1830) first described the genus *Opizia* as having a single species (*Opizia stolonifera* J. Presl) with hermaphrodite flowers, and dicliny was not noticed in this taxon until Bentham & Hooker (1883) described the spikelets as unisexual with monoecious and dioecious individuals. Columbus et al. (1998) and Kinney et al. (2007) recovered the *B. bracteata–B. chondrosoides–B. dimorpha* clade and found *B. karwinskii* to be sister. In our analyses, *B. karwinskii* is never a sister to the *B. bracteata–B. chondrosoides–B. dimorpha* clade but it does appear in a strongly supported clade with *B. chihuahuana*, *B. johnstonii*, and *B. karwinskii*, and in a larger E–K clade in the ITS, plastid, and combined trees. All three species have hispid to pilose upper glumes 3–6.5 mm long and hispid to pilose, indurate or leathery lemmas 2–6.5 mm long (McVaugh, 1983; Wipff, 2003). We include these three species in section *Opizia*.

Clade H

Historically, the species in this clade were placed in six different genera (*Bouteloua*, *Buchloe*, *Cathestecum*, *Griffithsochloa* G.J. Pierce, *Pentarrhaphis*, and *Soderstromia*), primarily to emphasize the diclinous habit. Columbus et al. (1998) and Kinney et al. (2007) recovered eight and six, respectively, of the 11 species we place in clade H. For the first time, we include *B. erecta*, *B. griffithsii*, and *B. tamaulipensis* along with *B. dactyloides*, *B. diversispicula*, *B. eludens*, *B. mexicana*, *B. multifida*, *B. polymorpha*, *B. scabra*, and *B. varia* in a strongly supported clade (Fig. 2B). All the species of this clade except *B. scabra* (lower florets perfect, upper florets sterile or staminate), have been determined as diclinous, including

B. eludens where the proximal florets of each spikelet are described as pistillate with unawned, mucronate lemmas, and the second florets are perfect with three hairy (hispid) awns (Griffiths, 1912, see Plate 78). *Bouteloua eludens* is a typical gynomonoecious species, (type Ia in Soreng & Keil, 2004) but was indicated as a monoclinous species in Kinney et al. (2007). The species of this clade have 2–4-flowered spikelets with prominently three-nerved lemmas that usually are awned or with bifid apices (Herrera Arrieta et al., 2004, 2008). We recognize these 11 species in section *Buchloe*.

Clade I

Columbus et al. (1998) and Kinney et al. (2007) found *B. rigidiseta* to be sister to the diclinous, *B. nervata–B. reederorum* pair in their molecular trees. We include the South American, *B. megapotamica* for the first time. It also is a member of clade I, forming a lineage with three samples of *B. rigidiseta*. As earlier indicated, the plastid and ITS trees are incongruent with respect to the placement of the I clade where *B.* sect. *Triplathera* forms a strongly supported clade (BS = 100, PP = 1.00) with H species and *B. juncea* in the ITS and combined tree (Fig. 1) and forms a weakly supported clade (BS = 68, PP = 1.00) with J–K species (Fig. 2B). *Bouteloua megapotamica* and *B. rigidiseta* share a few morphological features, such as being low 10–50 cm tall perennials (stoloniferous in *B. megapotamica*) with 2–8 inflorescences branches that contain 2–10 spikelets (Wipff, 2003; Sede, 2012). *Bouteloua reederorum* is sister to *B. megapotamica–B. rigidiseta* in our tree (Fig. 2B) but we cannot be sure where *B. reederorum* lies because it is found on a long branch, and we lack the plastid signal in our analysis.

Clade J

Griffiths (1912), Gould (1969, 1980), and Columbus et al. (1998) all recognized the *B. repens* complex which our data corroborate in a strongly supported clade that includes *B. alamosana, B. americana, B. radicosa, B. repens, and B. williamsii* (*B.* sect. *Triathera*). As seen in the ITS network (Fig. 3, J), this clade shows a long branch and a highly radiative pattern. The morphological concept used to circumscribe *B. repens* is large and previous taxonomists have segregated *B. bromoides* Lag., *B. filiformis* (E. Fourn.) Griffiths, *B. heterostega* (Trin.) Griffiths, and *B. pubescens* Pilg. out of *B. repens* (Griffiths, 1912; Gould, 1969, 1980). The following leaf anatomical characters have been attributed to the *B. repens* complex: sclerenchymatous girders usually associated with all vascular bundles, the girders narrower that the bundle outer sheaths, prickles usually present of the adaxial costal zones, and small papillae (Columbus, 1996). The species in this clade usually have spikelets with a perfect proximal floret with 3-veined lemmas with the nerves extending as mucros or short-awns (the central awn usually the longest); staminate upper florets with long-awns and central and lateral awns at least as long as the lower floret (Griffiths, 1912; Gould, 1969). *Bouteloua americana* is the exception in having only a lower, perfect floret and a sterile, long-awned rudiment.

Species delimitation among the samples in the J clade was problematic, and at least three ploidy levels (2n = 20, 40, 60) occur within *B. repens.* Two samples from Chihuahua, Mexico (*Peterson 8060 & 8147*) reside in a strongly supported clade

(BS = 98, PP = 1.00) sister to the remaining species in clade J (Fig. 2B). We tentatively place them in *B. williamsii* rendering this species polyphyletic since the other two samples (*Peterson 15996* from Zacatecas and *Breedlove 11868* from Chiapas) lie in a weakly supported (BS = 53, PP = 0.94) trichotomy, suggesting multiple origins. This is the first report of *B. williamsii* north of Zacatecas.

Clade K
Gould & Kapadia (1964) included 12 species in their morphologically variable, *B. curtipendula* complex (*B.* sect. *Bouteloua*), and it has been hypothesized that hybridization has played a major role in the evolution of this group (Gould, 1980; Siqueiros-Delgado, 2001, 2007; Siqueiros-Delgado et al., 2013). We include for the first time, the Central and South American, *B. swallenii* in this complex. Additionally, based on our plastid signal, we include *B. juncea* in the *B. curtipendula* complex, bringing the total to 13 species.

The *B. curtipendula* complex can be distinguished by having 12–80 pendulant, deciduous branches per inflorescence, relatively few 1–9 (–16) spikelets per branch, and spikelets that fall intact with the branch axis (Siqueiros-Delgado et al., 2013). Anatomically, is has round and peripheral chloroplasts within the Kranz cells in leaves and culms, except for *B. juncea* (Columbus, 1996; Siqueiros-Delgado, 2007), and by displaying an anatomically and biochemically intermediate NAD-ME/PCK C_4 pathway (Hattersley & Browning, 1981; Prendergast et al., 1987). Since all morphological features used to circumscribe the species in this complex were found to be homoplasious (Siqueiros-Delgado et al., 2013) we will not discuss morphological features differentiating among species. In our overall combined tree (Fig. 2B) *B. purpurea* (4 samples, BS = 94, PP = 1.00), *B. reflexa* (2 samples, BS = 97, PP = 1.00), and *B. triaena* (4 samples, BS = 100, PP = 1.00) appear monophyletic, whereas all other species are polyphyletic, or are represented by a single sample (*B. swallenii* and *B. vaneedenii*). However, *B. swallenii* is a member of the *B. purpurea* clade in the ITS tree (see Fig. S1), *B. purpurea* is not monophyletic in the plastid tree (see Fig. S2), and there is only a single sample of *B. reflexa* in the plastid tree. Therefore, we come to the identical conclusion as Sequeiros-Delgado et al. (2013), that only *B. triaena* in sect. *Bouteloua* is monophyletic.

Taxonomy

Bouteloua herrera-arrietae P.M. Peterson & Romasch., **sp. nov.** (Fig. 4). Type: MEXICO. Nuevo León. Municipio Galeana, Sierra Madre Oriental, 5 km E of San Roberto on Hwy 62 towards Galeana (24.6900°N, 100.1856°W), 2007 m, 13 Sep 2012, *P.M. Peterson, K. Romaschenko & J. Valdés Reyna 24458* (Holotype: US-3668950; isotype: ANSM).

Diagnosis—*Bouteloua herrera-arrietae* differs from *B. chasei* in having densely lanate upper glumes with whitish, basally flattened hairs (glabrous to sparsely pilose with terete hairs in *B. chasei*), straight flat (arcurate and involute in *B. chasei*) leaf blades with abaxial appressed hairs (glabrous in *B. chasei*), and 2.5–3.2 mm long upper glumes (3–3.5 mm long in *B. chasei*).

Description—Caespitose perennials. Culms 28–36 (–45) cm tall, erect, terete near base, nodes dark purplish, glabrous below, only 1 node above the base; internodes minutely pubescent between the veins, otherwise glabrous. Leaf sheaths 5–8 cm long, shorter than the internodes above, mostly glabrous and minutely pubescent between the veins, sometimes with a few appressed hairs when immature, the hairs 0.4–1.2 mm long, summit often with hairs near the margins, the hairs often up to 2 mm long; ligules 0.2–0.4 mm long, a ciliate line of hairs, apex truncate; blades 3–8 cm long, 1.2–2.3 mm wide, flat to involute, apically acuminate, often with a few appressed hairs abaxially when immature, otherwise minutely pubescent between the veins abaxially and pubescent adaxially. Panicle axes 2–8 cm long, with 1–3 (–4) branches, these racemosely inserted; branches 1.5–2.6 cm long with 32–50 spikelets, arcuate, persistent, terminating in a spikelet, each spikelet nearly sessile, pectinately inserted in 2 rows along a flattened rachis; disarticulation above the glumes. Spikelets 3.2–4.2 mm long with 1 perfect (lower) and 2 rudimentary florets (above); lower glumes 2–2.6 mm long, hyaline to membranous, narrowly lanceolate, pubescent along the midveins below, apex acuminate; upper glumes 2.5–3.2 mm long (excluding the mucro), membranous, lanceolate, purplish, densely lanate with whitish, basally flattened hairs, apex often mucronate; perfect lemmas 3.5–4.1 mm long, membranous to chartaceous, ovate, pilose, apex 3-awned and bifid, the narrowly acuminate teeth about 1 mm long between the central and lateral awns, the awns 1–2.2 mm long, the central longer than the lateral; paleas 3–3.7 mm long, 2-veined, pilose between the nerves; rudiment 3-awned, the awns about 3 mm long, equal, densely pilose in a ring at the insertion of the awns, between awns are two or three small hyaline scales; upper floret consisting of two flabellate-lobed, membranous structures; stamens 3, anthers 1.2–1.7 mm long, yellow; lodicules ca. 0.3 mm long, 2, membranous; ovary glabrous with two styles and two stigmas. Caryopsis not seen.

Distribution and habitat—*Boutloua herrera-arrietae* is known only from the type locality in the Sierra Madre Oriental, Mexico where it occurs on calcareous rocky hills with *Pinus cembroides* Zucc., *Yucca carnerosana* (Trel.) McKelvey, *Agave striata* Zucc., *Lycium* sp., *Bouteloua scopioides*, and *Aristida pansa* Wooton & Standl.

Etymology—The new species is named for Dr. Yolanda Herrera Arrieta (born 1954), a well-known agrostologist who has completed a revision of *Bouteloua* in Mexico and many fine floristic treatments of the grasses in Chihuahua, Durango, Mexico, and Zacatecas.

Bouteloua section *Barbata* P.M. Peterson, Romasch. & Y. Herrera, **sect. nov.** – Type: *Bouteloua barbata* Lag., Varied. Ci. 2 (4): 141. 1805

Description—Annuals or perennials, caespitose or rhizomatous, rarely stoloniferous. Culms 1–140 cm tall, erect, sometimes geniculate; internodes glabrous, occasionally glaucus. Leaves mostly basal, occasionally cauline; sheaths glabrous to scabridulous; ligules 0.1–0.5 mm long, ciliate fringe of hairs; blades 1–50 cm long, 0.7–4 mm wide, flat, folded or involute, glabrous, scabrous, sometimes with papillose-based hairs. Inflorescences 0.7–60 cm long with 1–20 branches; branches 1–8 cm long with 30–100 spikelets, straight to recurved-arcuate, rachis sometimes with pappilose-based hairs (*B. parryi*), axes terminating in a spikelet; disarticulation

above the glumes. Spikelets pectinately inserted with 1 basal perfect floret and 1 or 2 rudimentary florets; upper glumes 1.5–4 mm long, 1-veined, occasionally with pappilose-based hairs (*B. parryi*); lemmas 2–4 mm long, 3-veined and 3-awned, sparingly hairy to pilose or villous especially on the lower 1/2, awns 1–4 mm long, the central awns flanked by two membranous lobes.

Species included: *B. barbata*, *B. breviseta*, *B. elata*, *B. parryi*, *B. ramosa*, *B. sonorae.*

Bouteloua section **Buchloe** (Engelm.) P.M. Peterson, Romasch. & Y. Herrera, **comb. nov. & stat. nov.** ≡ *Buchloe* Engelm., Trans. Acad. Sci. St. Louis 1: 432, pl. 12 & 14, f. 1 bis 17. 1859–Type: *Buchloe dactyloides* (Nutt.) Engelm. (≡ *Sesleria dactyloides* Nutt.).

Species included: *B. dactyloides*, *B. diversispicula*, *B. eludens*, *B. erecta*, *B. griffithsii*, *B. mexicana*, *B. multifida*, *B. polymorpha*, *B. scabra*, *B. tamaulipensis*, and *B. varia.*

Bouteloua section **Cyclostachya** (Reeder & C. Reeder) P.M. Peterson, Romasch. & Y. Herrera, **comb. nov. & stat. nov.** ≡ *Cyclostachya* Reeder & C. Reeder, Bull. Torrey Bot. Club 90: 195. 1963–Type: *Cyclostachya stolonifera* (Scribn.) Reeder & C. Reeder (≡ *Bouteloua stolonifera* Scribn.).

Species included: *B. stolonifera.*

Bouteloua section **Hirsuta** P.M. Peterson, Romasch. & Y. Herrera, **sect. nov.** Type: *Bouteloua hirsuta* Lag., Varied. Ci. 2 (4): 141. 1805.

Description—Caespitose annuals or perennials, sometimes stoloniferous and shortly rhizomatous. Inflorescences branches with a flattened rachis extending 2–12 mm beyond the insertion of the terminal spikelet; spikelets appressed or pectinately inserted along the branches.

Bouteloua subsection **Eriopoda** P.M. Peterson, Romasch. & Y. Herrera, **subsect. nov.** – Type: *Bouteloua eriopoda* (Torr.) Torr., Pacif. Railr. Rep. 4(5): 155. 1857 (≡ *Chondrosum eriopodum* Torr.).

Description—Caespitose annuals or perennials, sometimes stoloniferous and shortly rhizomatous. Culms 6–60 (–75) cm tall, erect or geniculate; internodes glabrous, sometimes lanate pubescent especially below. Leaf sheaths glabrous above; ligules 0.2–0.6 mm long, ciliate fringe of hairs; blades 1–10 cm long, 0.5–2 mm wide, flat, folded or involute, glabrous to pubescent, sometimes upper surface and margins with papillose-based hairs. Inflorescences 2–18 cm long with 2–15 branches; branches 0.5–5 cm long with 2–20 spikelets; rachis axis extending 2–12 mm beyond insertion of the terminal spikelet, the base hairy to lanate. Spikelets appressed inserted with 1 basal perfect floret and 1 rudimentary floret (sometimes lacking the basal spikelet), greenish; upper glumes 4–9 mm long, 1-veined, glabrous or pubescent to lanate; lemmas 6–9.5 mm long, 3-veined, 3-awned or mucronate, apex bifid, the awns (if present) 1–4 mm long.

Species included: *B. annua, B. aristidoides, B. eriopoda, B. eriostachya.*

Bouteloua subsection **Hirsuta**

Description—Caespitose perennials, sometimes stoloniferous. Culms 15–75 cm tall, erect or decumbent; internodes glabrous or densely hairy with papillose-based hairs. Leaf sheaths glabrous, pilose near ligule; ligules 0.2–0.5 mm long,

ciliate fringe of hairs; blades 5–30 cm long, 1–2 mm wide, flat or involute, papillose-based hairs common, especially near the margins below. Inflorescences 10–45 cm long with 1–6 branches; branches 15–45 mm long with 25–50 spikelets; rachis axis extending 5–10 mm beyond the terminal spikelet, adaxially hairy or hairy on both surfaces. Spikelets pectinately inserted with 1 basal perfect floret and 1 or 2 rudimentary florets, green to dark purple; upper glumes 3–6 (–6.5) mm long, 1-veined, with well-developed papillose-based hairs; lemmas 2–6 mm long, pubescent, 3-veined and 3-awned or mucronate, apex bifid, the awns 1–2.5 mm long; rachilla segments subtending the second floret glabrous or hairy.

Species included: *B. hirsuta, B. pectinata.*

Bouteloua section **Opizia** (J. Presl) P.M. Peterson, Romasch. & Y. Herrera, **comb. nov. & stat. nov.** ≡ *Opizia* J. Presl, Reliq. Haenk. 1(4–5): 293, pl. 41, f. 1–11. 1830–Type: *Opizia stolonifera* J. Presl (≡ *Bouteloua dimorpha* Columbus).

Species included: *B. bracteata, B. chondrosoides, B. dimorpha.*

Bouteloua section **Trifida** P.M. Peterson, Romasch. & Y. Herrera, **sect. nov.** – Type: *Bouteloua trifida* Thurb., Proc. Amer. Acad. Arts 18: 177. 1883.

Description—Caespitose perennials, occasionally short rhizomatous. Culms 5–50 cm tall, erect to geniculate, slender, wiry; lower internodes glabrous. Leaves basal; sheaths glabrous to scabridulous; ligules 0.2–0.5 mm long, ciliate fringe of hairs often somewhat membranous near margins; blades 0.7–20 cm long, 0.5–1.5 (–2) mm wide, flat or involute, upper surface scabrous, margins occasionally with papillose-based hairs basally. Inflorescences 3–11 cm long with 2–20 branches; branches 7–20 mm long with (6–) 8–24 (–32) spikelets; rachis axes terminating in a spikelet; disarticulation above the glumes. Spikelets appressed to pectinately inserted with 1 basal perfect floret and 1 rudimentary floret, often reddish-purple; glumes 1.7–4 mm long, 1-veined, acute or bidentate; lower lemmas 1.2–7 mm long, 3-veined and 3-awned, glabrous, appressed pubescent along the veins or densely appressed pubescent on lower 2/3, awns 2.2–6.6 mm long, the central awns flanked by two membranous acuminate lobes or not lobed.

Species included: *B. kayi, B. trifida.*

Bouteloua section **Triplathera** (Endl.) P.M. Peterson, Romasch. & Y. Herrera, **comb. nov. & stat. nov.** ≡ *Eutriana* [unranked] *Triplathera* Endl., Gen. Pl. 94. 1836–Type: *Eutriana multiseta* Nees [= *Bouteloua megapotamica* (Spreng.) Kuntze (≡ *Pappophorum megapotamicum* Spreng.)].

Species included: *B. megapotamica, B. nervata, B. rigidiseta, B. reederorum.*

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12159/suppinfo:

Appendix S1. List of specimens sampled. Taxon, voucher (collector, number, and where the specimen is housed), country of origin, state or province, and GenBank accession number for DNA sequences *rps16-trnK, rpl32-trnL* and ITS regions (**bold** indicates new accession); a dash (−) indicates missing data.

Fig. S1. Maximum-likelihood tree inferred from ITS sequences. Letters indicate the clades discussed in the text and these include our classification; numbers above branches are bootstrap values and numbers below branches are posterior probabilities; scale bar = 0.04 substitutions/site.

Fig. S2. Maximum-likelihood tree inferred from combined plastid (*rpl32-trnL* and *rps16-trnK*) sequences. Letters indicate the clades discussed in the text and these include our classification; numbers above branches are bootstrap values and numbers below branches are posterior probabilities; scale bar = 0.005 substitutions/site.