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RESEARCH ARTICLE

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A Hyb-Seq phylogeny of *Boechera* and related genera using a combination of Angiosperms353 and Brassicaceae-specific bait sets

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Abstract

Premise: Although *Boechera* (Boechereae, Brassicaceae) has become a plant model system for both ecological genomics and evolutionary biology, all previous phylogenetic studies have had limited success in resolving species relationships within the genus. The recent effective application of sequence data from target enrichment approaches to resolve the evolutionary relationships of several other challenging plant groups prompted us to investigate their usefulness in *Boechera* and Boechereae.

Methods: To resolve the phylogeny of *Boechera* and closely related genera, we utilized the Hybpiper pipeline to analyze two combined bait sets: Angiosperms353, with broad applicability across flowering plants; and a Brassicaceae-specific bait set designed for use in the mustard family. Relationships for 101 samples representing 81 currently recognized species were inferred from a total of 1114 low-copy nuclear genes using both supermatrix and species coalescence methods.

Results: Our analyses resulted in a well-resolved and highly supported phylogeny of the tribe Boechereae. Boechereae is divided into two major clades, one comprising all western North American species of *Boechera*, the other encompassing the eight other genera of the tribe. Our understanding of relationships within *Boechera* is enhanced by the recognition of three core clades that are further subdivided into robust regional species complexes.

Conclusions: This study presents the first broadly sampled, well-resolved phylogeny for most known sexual diploid *Boechera*. This effort provides the foundation for a new phylogenetically informed taxonomy of *Boechera* that is crucial for its continued use as a model system.

KEYWORDS

angiosperms, *Boechera*, Boechereae, Brassicaceae, Hyb-Seq, phylogenomics, supermatrix

Because of its small genome (~200 MB), close evolutionary relationship with *Arabidopsis* (Mitchell-Olds, 2001; Song et al., 2006; Kliver et al., 2018; Hendriks et al., 2023), and prevalence of diploid apomixis (Schranz et al., 2005; Beck et al., 2012), the genus *Boechera* (Brassicaceae) has become a subject of intense research interest. It has emerged as a model system in several research fields, including ecological and evolutionary genomics (Mitchell-Olds, 2001; Schranz et al., 2005, 2007; Rushworth et al., 2011, 2022; Li et al., 2017; Wagner and Mitchell-Olds, 2018), climate change studies (Anderson and Gezon, 2015; Anderson et al., 2015; Colautti et al., 2017; Bemmels and Anderson, 2019; Hamann et al., 2021), and the genetics of apomixis (Lovell et al., 2013; Brukhin et al., 2019; Carman et al., 2019; Mandáková et al., 2020).

Nikolai M. Hay and Michael D. Windham contributed equally to the manuscript.

However, some of the same characteristics that have drawn the attention of researchers to *Boechera* have also made it very difficult to reconstruct evolutionary relationships within the genus. Its presumed rapid rate of evolution (Rushworth et al., 2011; Li et al., 2017), prodigious production of reproductively competent apomictic hybrids (Beck et al., 2012; Alexander et al., 2015; Li et al., 2017), and subtle morphological distinctions among taxa (Figure 1; Windham and Al-Shehbaz, 2006, 2007a, 2007b; Al-Shebaz and Windham, 2010; Windham et al., 2016, 2022), as well as the remarkable incongruence it displays between its plastid and nuclear gene trees (Alexander et al., 2013), have all combined to undermine previous attempts to produce a well-supported phylogeny of *Boechera*.

Members of *Boechera* have traveled a long and winding road to reach their current taxonomic position. The genus was first segregated from *Arabis* in 1975, based on the discovery that some North American species ascribed to *Arabis* had a base chromosome number of $x = 7$ rather than $x = 8$ (Löve and Löve, 1975). This transfer was summarily rejected by most taxonomists working on the Brassicaceae at the time (e.g., Rollins and Rüdenberg, 1977; Rollins, 1993; Mulligan, 1995; Welsh, 2003), though Weber (1982) and Dorn (2001) accepted the new genus and swiftly published many new combinations in *Boechera*. The polyphyletic nature of *Arabis* s.l. became clear in the late 1990s as early phylogenetic studies of the Brassicaceae began to proliferate (Galloway et al., 1998). More in-depth analyses established that the morphological similarities between *Arabis* s.s. and the North American species assigned to *Boechera* were due to evolutionary convergence, not shared ancestry (Koch et al., 1999, 2000, 2001, 2003; Heenan et al., 2002; O'Kane and Al-Shehbaz, 2003). Based on these findings, Al-Shehbaz (2003) transferred most of the remaining North American *Arabis* taxa (32 species) to *Boechera* and subsequently (Al-Shehbaz et al., 2006) circumscribed a new tribe, Boechereae, to reflect its significant divergence from Arabideae. Based primarily on published molecular data, Al-Shehbaz et al. (2006) added six other morphologically disparate genera to Boechereae, including *Anelsonia*, *Cusickiella*, and *Phoenicaulis* (transferred from Arabideae), *Nevada* and *Polyctenium* (from Smelowskieae), and *Sandbergia* (from Sisymbrieae). Two additional genera (*Borodinia* and *Yosemitea*) were subsequently segregated from *Boechera* by Alexander et al. (2013).

As reaffirmed by Mandáková et al. (2020), the tribe Boechereae includes nine genera. Seven of these (*Anelsonia*, *Cusickiella*, *Nevada*, *Phoenicaulis*, *Polyctenium*, *Sandbergia*, and *Yosemitea*) comprise just one or two species and are (aside from a few localities across the Canadian border) confined to the western United States (Figure 2). The genus *Borodinia* currently includes eight species (Alexander et al., 2013); seven of these are restricted to eastern North America and one is endemic to Siberia and coastal northeast Asia. *Boechera* is, by far, the most species-rich and widespread genus in the tribe. Al-Shebaz and Windham (2010) recognized 109 species in the *Flora of North America*, but the *Boechera* Microsatellite Website (Li et al., 2017) listed >480 genetically distinct taxa. The vast majority of these are endemic to the western United States, with a few of the more common taxa extending north to central Alaska and the Northwest Territories and east along the U.S.-Canadian border to the Great Lakes, Quebec, and Newfoundland (Figure 2). One species (*B. holboellii*) occurs in Greenland, while another (*B. falcata*) is endemic to Siberia.

No clear morphological synapomorphies define the Boechereae, which is not surprising given that the tribe was assembled by uniting species and genera from across Brassicaceae (Al-Shehbaz et al., 2006). Nearly all characters historically used to circumscribe mustard tribes are variable within Boechereae. Members of the tribe range from long-lived, suffrutescent perennials to biennials and facultative annuals, from small, spreading mats to single-stemmed plants nearly a meter tall. Basal leaves range from entire to repand or lyrate, and cauline leaves can be auriculate or not. Petals range from white to lavender, purple, yellow, or rose, and trichomes can be absent, simple, bifurcate, or dendritic with 3–14 terminal branches. Fruits can be either siliques or silicles, and angustiseptate or latiseptate. Seeds are arranged in one or two rows within a locule, and the cotyledons can be accumbent or incumbent. Information regarding the distribution of many of these character states within Boechereae can be found in the generic key published by Alexander et al. (2013).

Despite the lack of clear morphological synapomorphies, Boechereae do share a unique cytogenetic signature, a derived base chromosome number of $x = 7$ (Mandáková et al., 2015, 2020). Morphological synapomorphies also have not been recognized for *Boechera*, which accounts for >95% of the taxonomic diversity in the tribe. As currently circumscribed, *Boechera* comprises 83 sexual diploids and >400 documented apomictic hybrids (Li et al., 2017). Nearly 100 of the latter are diploid apomicts, which are otherwise extremely rare among flowering plants. These apomictic diploid hybrids are capable of pollinating sympatric sexual species, resulting in numerous triploid hybrids with three distinct parental genomes. A few of these triploids have gone on to hybridize with additional sexual taxa to form occasional apomictic tetraploids that contain four different genomes (M. D. Windham et al., unpublished data). Rampant hybridization and apomixis, coupled with the failure to identify such hybrids and remove them from phylogenetic analyses, have clearly contributed to the lack of progress in reconstructing relationships within *Boechera*.

To date, only four molecular phylogenetic studies haveincluded a diverse array of Boechereae species. As part of a global Brassicaceae phylogeny, Bailey et al. (2006) included six species (representing four genera) of Boechereae in their 10-locus supermatrix analysis and an additional 24 samples in their nuclear ITS study. For the first *Boechera*-focused phylogeny, Kiefer et al. (2009) included 67 Boechereae samples in their ITS analysis and generated plastid *trnL-F* sequences for 41 of these. Kiefer and Koch (2012) had, by far, the most comprehensive taxonomic representation; their ITS study included 911 samples of

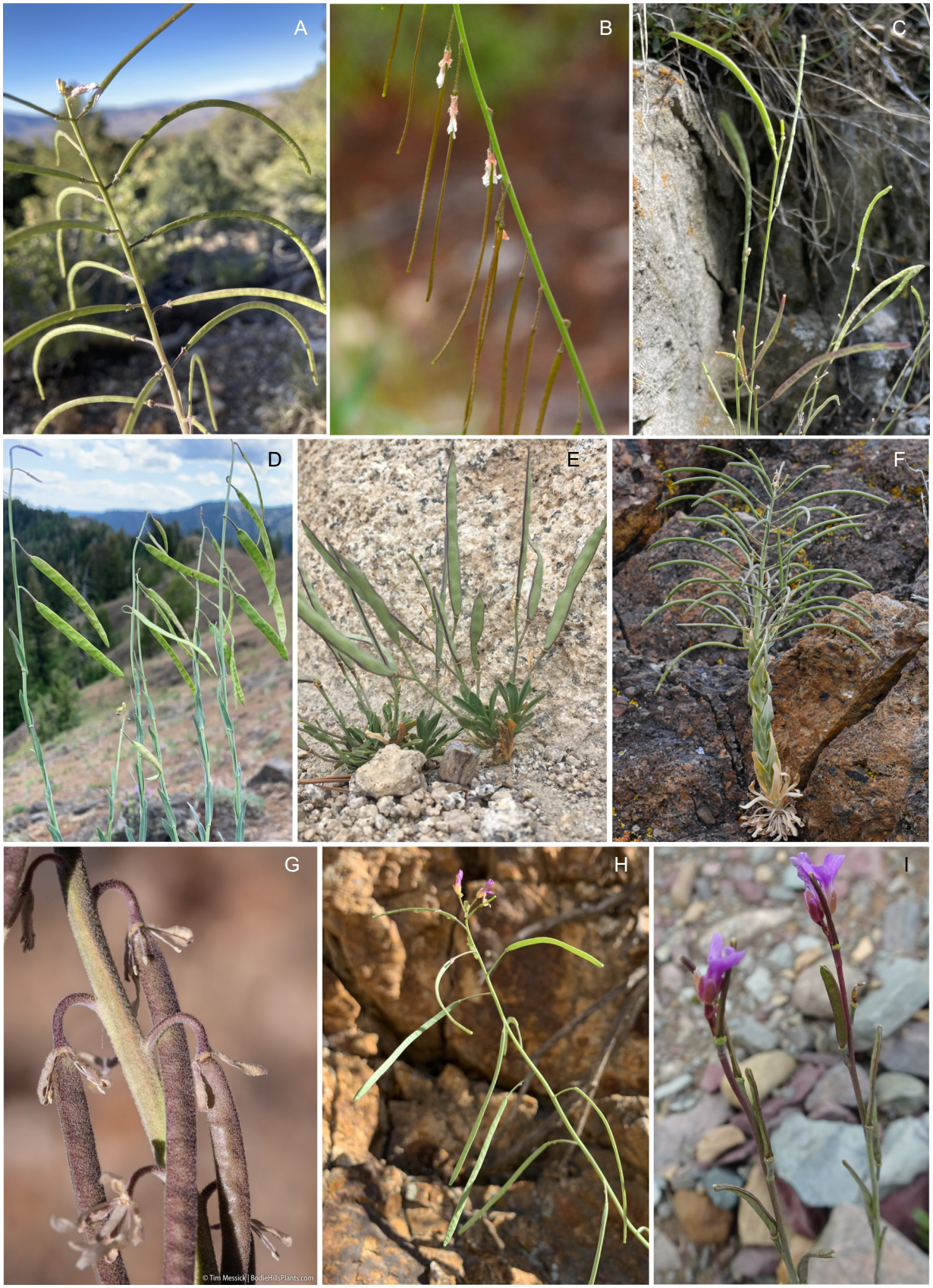

FIGURE 1 (See caption on next page).

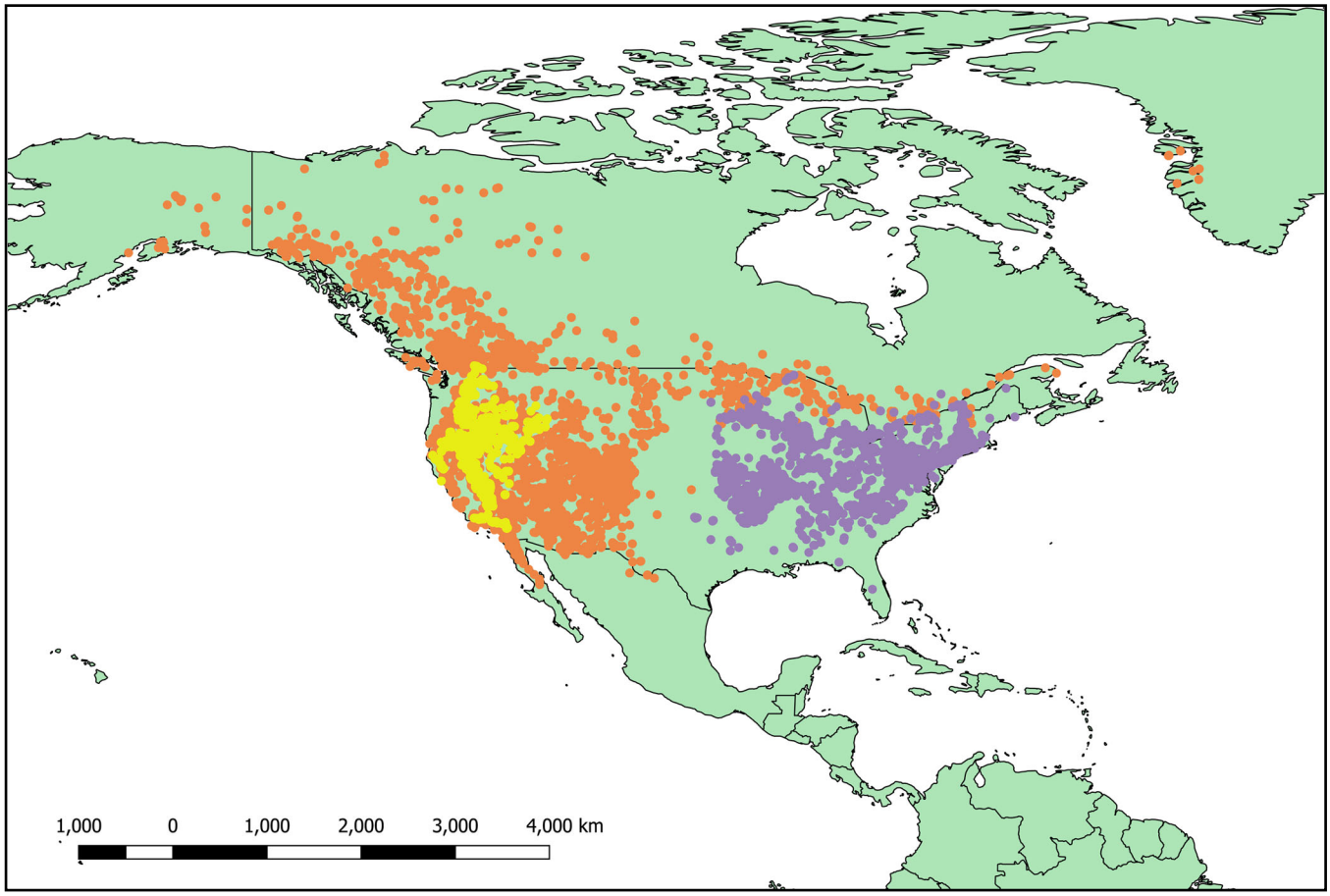

FIGURE 2 North American range of the tribe Boechereae from GBIF (GBif.org, 2022; accessed 22 July 2022): *Boechera* (orange dots), *Borodinia* (purple dots), all other genera in the tribe (yellow dots). Location data were curated to remove obviously erroneous records.

Boechereae and sequence data from two single-copy nuclear loci (At2g25920 and At3g18900) that were analyzed for 210 and 111 individuals, respectively. Most recently, Alexander et al. (2013) analyzed a total of 82 samples based on ITS, seven low-copy nuclear loci, and sequences from two plastid genes. This was the first study to explicitly exclude polyploids and hybrids from the analyses.

In their supermatrix analysis of 10 plastid and nuclear loci, Bailey et al. (2006) found strong support for Boechereae, and for the closely related Halimolobeae. These tribes formed a well-supported polytomy with a group of genera (including *Crucihimalaya*) that were, at the time, assigned to the Camelineae. The ITS-only analysis of Bailey et al. (2006) was less informative, adding additional elements of Camelineae and Physarieae to the polytomy. In the study by Kiefer et al. (2009), the *trnL-F* analysis supported the monophyly of Boechereae, nested within Halimolobeae; tribal relationships were unresolved in their ITS analysis. Phylogenetic analyses of the massive Kiefer and Koch (2012) data set, which included hundreds of samples representing 66% of the species recognized in the *Flora of North America* (Al-Shebaz and Windham, 2010), yielded largely unresolved trees. In total, Bailey et al. (2006), Kiefer et al. (2009), and Kiefer and Koch (2012) identified fewer than a dozen well-supported relationships within Boechereae and *Boechera*.

The seven-locus nuclear analysis of Alexander et al. (2013) provided significantly better resolution, strongly supporting the monophyly of Boechereae, as well as its sister relationship with Halimolobeae (the latter represented by a single species). Western North American *Boechera* was also identified as monophyletic, with all other Boechereae genera and species clustering (without support) sister to it. Within the latter group, the eastern North American

FIGURE 1 Select taxa of *Boechera* illustrating morphological diversity: (**A**) *B. sparsiflora* (Nutt.) Dorn, © Sean Carson, permission requested, iNaturalist. (**B**) *B. retrofracta* (Graham) Á. Löve & D. Löve, © Julia Carr, iNaturalist. (**C**) *B. microphylla* (Nutt.) Dorn, © crothfels, iNaturalist. (**D**) *B. suffrutescens* (S. Watson) Dorn, © Tynan Ramm-Granberg, iNaturalist. (**E**) *B. platysperma* (A. Gray) Al-Shehbaz, © Corey Lange, iNaturalist. (**F**) *B. shockleyi* (Munz) Dorn, © Chloe and Trevor Van Loon, iNaturalist. (**G**) *B. puberula* (Nutt.) Dorn, © Tim Messick, iNaturalist. (**H**) *B. perennans* (S. Watson) W.A. Weber, © Eric Hough, iNaturalist. (**I**) *B. paupercula* (Greene) Windham & Al-Shehbaz, © Thomas Koffel, iNaturalist.

species previously assigned to *Boechera* (Al-Shehbaz, 2003; Al-Shebaz and Windham, 2010) formed a weakly supported clade that included the type species of *Borodinia*. As a result, Alexander et al. (2013) proposed new combinations for these taxa under the older generic name. Another member of this unsupported group (formerly called *Boechera repanda*) was found to be sufficiently divergent from *Boechera* to warrant recognition as a new genus (*Yosemitea*). Alexander et al. (2013) identified several additional, well-supported species groups within *Boechera*, but their study was unable to resolve deeper generic and intergeneric relationships.

In *Boechera*, limitations of the labor-intensive method of cloning and sequencing individual low-copy nuclear genes left important questions about evolutionary relationships unanswered (Alexander et al., 2013). The Hyb-Seq methods set out in Weitemier et al. (2014) successfully combined hybrid enrichment of low-copy nuclear genes with high-throughput sequencing and de novo assembly. The utility and viability of Hyb-Seq increased dramatically with the development of HybPiper (Johnson et al., 2016), a streamlined pipeline that allowed for the de novo assembly and recovery of Hyb-Seq loci, and the Angiosperms353 universal bait set (Johnson et al., 2019), designed to enrich for a set of low-copy nuclear genes found across all angiosperms. At about the same time, a set of 764 Brassicaceae-specific genes was developed by Nikolov et al. (2019), resulting in the first well-resolved tribe-level phylogeny for Brassicaceae. Hyb-Seq methods are effective in recovering loci from herbarium specimens, even specimens >200 yr old (Hart et al., 2016; Brewer et al., 2019; Forrest et al., 2019). These developments, along with thousands of genotyped *Boechera* herbarium specimens (Li et al., 2017), coalesced here into an opportunity to finally answercrucial questions about evolutionary relationships within *Boechera*.

Our study builds on the work of Alexander et al. (2013), with slightly expanded taxonomic representation and the addition of 1114 low-copy nuclear loci. The main goals were to resolve generic relationships within Boechereae and establish a broadly sampled, robust phylogeny of the model genus *Boechera*. To minimize the impact of hybridization on the analyses, we utilized a strict "diploids-first" approach (Beck et al., 2010), excluding any *Boechera* samples exhibiting cytogenetic or microsatellite evidence of polyploidy. Leveraging established correlations between microsatellite heterozygosity and reproductive mode (Beck et al., 2012), we selected only known or inferred sexual diploid samples from among the 5000+ individuals currently included in the *Boechera* Microsatellite Website (Li et al., 2017).

MATERIALS AND METHODS

Taxon sampling

We sampled 98 individuals (Appendix S1), representing all nine genera in the tribe Boechereae, as well as seven species representing five genera in the tribe Halimolobeae, which has been shown by previous analyses to be the sister group of Boechereae (Beilstein et al., 2008; Couvreur et al., 2010; Alexander et al., 2013; Nikolov et al., 2019; Hendriks et al., 2023). All *Boechera*, *Borodinia*, and *Nevada* samples included in the study were inferred to be sexual diploids, based on data available in the *Boechera* Microsatellite Website (Li et al., 2017; https://sites.biology.duke.edu/windhamlab/). Hyb-Seq data for most of the remaining samples were obtained through collaboration with Hendriks et al. (2023), who provided both sampling guidance and outgroup data for this study. One species of Crucihimalayeae and two species of Arabidopsideae were utilized as outgroups (Appendix S1); data for the latter were downloaded from the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra).

Marker selection

To increase the number of available nuclear loci, we combined two previously published probe sets following Hendriks et al. (2021). The bait sets used were Angiosperm353 (A353; Johnson et al., 2019; Baker et al., 2021), comprising 80,000 probes; and a Brassicaceae-specific bait set (B764; from Hendriks et al., 2023; adapted from Nikolov et al., 2019) comprising 40,000 probes (both from Arbor Biosciences, Ann Arbor, Michigan, USA). Considering the slight overlap between A353 and B764, probes targeted a total of 1117 nuclear genes.

Library preparation, target enrichment, and sequencing

Our library preps, target enrichment and sequencing protocols followed Hendriks et al. (2021). DNA was extracted from 88 herbarium specimens previously genotyped and analyzed (Li et al., 2017) using the DNeasy PowerPlant Pro Kit (Qiagen, Hilden, Germany), and subsequent purification of extracts used the DNeasy PowerClean CleanUp Kit (Qiagen) (Appendix S1). Nine taxa were extracted as part of Hendriks et al. (2023), and two existing samples were taken from Alexander et al. (2013) (Appendix S1). Samples were each uniquely indexed using New England Biolabs single- and dual-index adapters (New England Biolabs, Ipswich, Massachusetts, USA) according to the manufacturer's specifications. Genomic libraries were generated using NEBNext Ultra II FS kit (New England Biolabs) according to the production manual (E7805L kit, version 5.0). A fragmentation time of 5–10 min and 6–7 cycles of PCR amplification were used. Target capture was performed using a mixed bait method as described in Hendriks et al. (2021). To conserve baits, the target sequence hybridization reactions were performed in pools of ~30 libraries each, based on their total molecular weight. In the hybridization reactions, we used a ratio of 1 part B764:2 parts A353, and added unenriched libraries at a

ratio of 1:1 to facilitate chloroplast skimming. Sequencing was performed by Novogene (Beijing, China) with either an Illumina MiSeq Micro or a 96-sample multiplexed lane of HiSeq 4000 with 150 bp pair-end reads (Appendix S1). The raw-read sequence files were uploaded to NCBI's SRA under BioProjects PRJNA700668. Samples from Hendriks et al. (2023) were processed at Naturalis Biodiversity Center (Netherlands) and are found under NCBI BioProjects PRJNA806513 and PRJNA678873 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA806513?reviewer=756ti1639u9t7d630vi1q9kmr5).

Assembly and alignment

Raw multiplexed data were separated into index-specific samples and trimmed to remove adapters and low-quality data using Trimmomatic version 0.36 (Bolger et al., 2014) with the parameters ILLUMINACLIP: <AdapterFastaFile>: 2:30:10:2:true, LEADING: 10, TRAILING: 10, SLIDINGWINDOW: 4:20, MINLEN: 40. Data quality was assessed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). The trimmed reads were transferred to the HybPiper version 1.3.1 pipeline (Johnson et al., 2016) to assemble the loci. To increase loci recovery for the A353 baits, we used the "mega353" nonstandard gene references files described in Hendriks et al. (2023). To allow for loci compatibility for the B764 baits, we used the same concatenated reference file described in Hendriks et al. (2023). Off-target reads from the addition of unenriched libraries were used to reconstruct 84 chloroplast genes using coding sequences from *B. stricta* (NCBI RefSeq NC_049599.1). To assemble the loci, Hybpiper version 1.3.1 uses BWA version 0.7.16a (Li and Durbin, 2009), SPAdes version 3.14.1 (Bankevich et al., 2012), and GNU Parallel (Tange, 2011). Hybpiper 1.3.1 was executed three times, once for each of the probe sets and once for plastid genes. Only gene sequences were used to construct loci. All genes were aligned with MAFFT version 7.273 (Katoh and Standley, 2013) using the L-INS-I method.

Phylogenetic analyses

Two different analytical approaches were used in this study, a supermatrix maximum-likelihood phylogeny approach and a species-coalescent tree-based approach. To construct the supermatrix tree, the gene alignments were combined into a supermatrix using FASconCAT-G (https://github.com/PatrickKueck/FASconCAT-G) with default parameters. The supermatrix alignment was trimmed to reduce gaps and missing data using trimmAl version 1.2 (Capella-Gutiérrez et al., 2009) with the -automated1 setting. For tree construction, we utilized RAxML-NG version 1.1.0 (Kozlov et al., 2019) with 1000 bootstrap replicates and a GTR+F+R model with the FBE command; all other settings were left as default. Support values reported in Figure 3 are maximum likelihood bootstrap (BS) percentages. Support values were also calculated in IQTREE (Nguyen et al., 2015; Hoang et al., 2018; Minh et al., 2020b), utilizing ultrafast bootstraps with a GTR+F+R model with 1000 replicates.

For the coalescent approach, RAxML-NG was run with 100 bootstrap replicates with a GTR+F+R model on all genes. Species-coalescent tree-based inference was performed using ASTRAL-III version 5.7.3 (Zhang et al., 2018; Appendix S3). Concordance factors for both site and gene trees were calculated in IQTREE (Minh et al., 2020a; Appendices S2 and S3). The same supermatrix maximum-likelihood (ML) phylogeny utilized for the nuclear data was also applied to the plastid data (Appendix S4). FASconCAT-G was used to create the supermatrix of gene alignments. For tree construction we utilized RAxML-NG version 1.1.0 (Kozlov et al., 2019) with 1000 bootstrap replicates and a GTR+F+R model with the FBE command; all other settings were left as default. Phylogenetic trees were relabeled in R version 4.1.2 (R Core Team, 2022) utilizing the package ape 5.6-2 (Paradis and Schliep, 2019) and phylotools (https://github.com/helixcn/phylotools). Final trees were visualized using FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

Target-capture sequencing data sets

Of the 1117 targeted low-copy nuclear genes, we recovered 1114 from across all 98 samples. With the A353 probe set, we recovered 350 genes (278,804 bp) for 94.7% of the total target length (294,516 bp). For the B764 bait set, we recovered all targeted genes (916,943 bp) for an average of 99.7% of the total target length (919,712 bp). Locus recovery was better than that of Hendriks et al. (2023), most likely due to the relative phylogenetic proximity of tribe Boechereae to *Arabidopsis*, one of the genomic sources for the development of both bait sets (A353 and B764).

Nuclear phylogeny for tribe Boechereae

We utilized both a maximum-likelihood supermatrix method implemented in RAxML-NG and a coalescent gene-tree-based approach using ASTRAL-III to reconstruct well-supported nuclear phylogenies (see above; Appendices S2 and S3). Node support was high (≥90 ML bootstrap, ≥0.95 ASTRAL-III local posterior probabilities) across almost the entire topology, with most clades, especially within *Boechera*, being fully supported. There is, however, significant gene-tree discordance across the phylogeny, especially within *Boechera*. The site concordance factors (sCF) range from 0.54 to 95.9, and gene concordance factors (gCF) range from 34.9 to 90.6 (Appendices S2 and S3). There is notably more discordance along the backbone of the phylogeny than is observed near branch tips or among the outgroups.

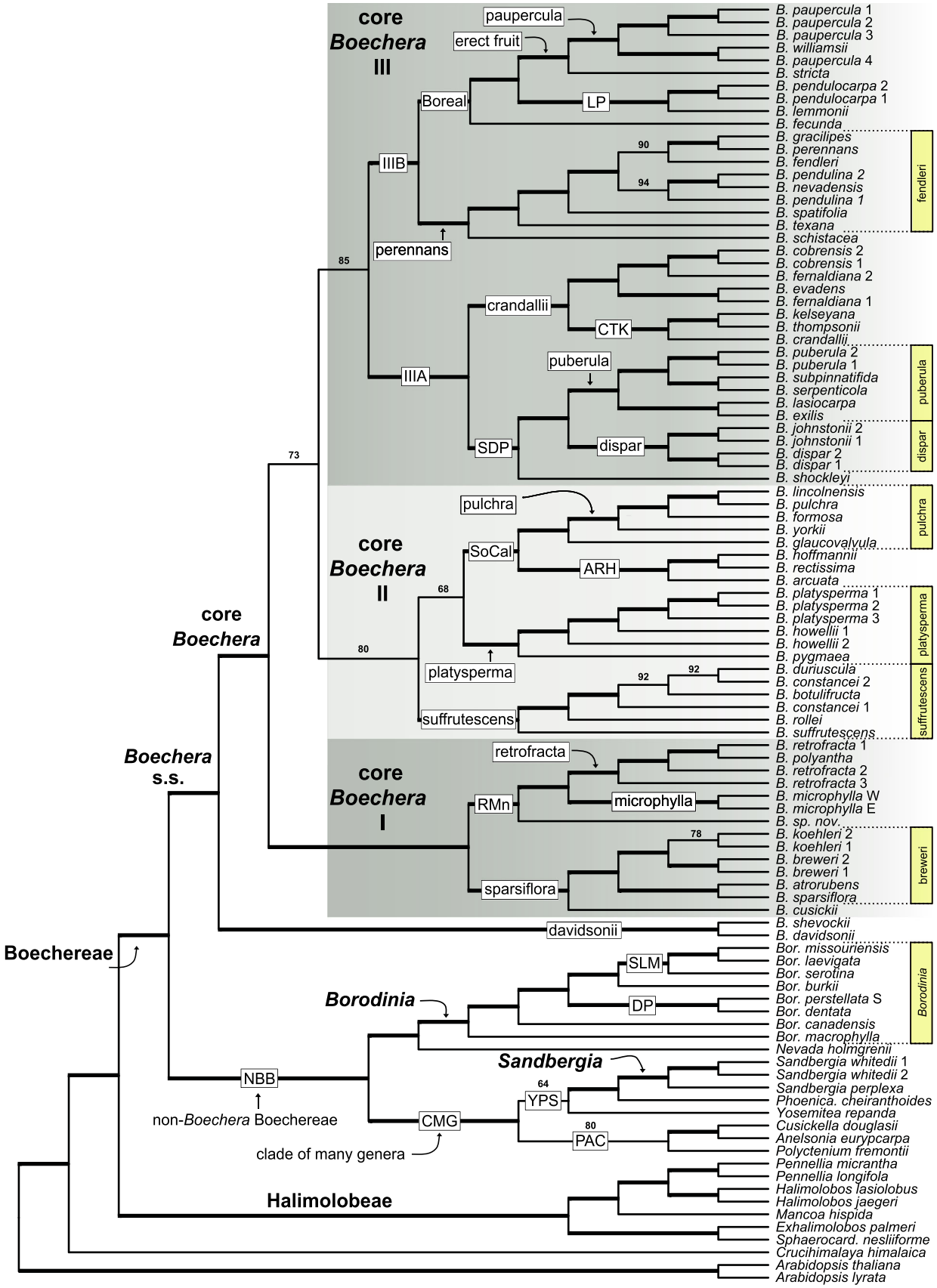

FIGURE 3 RAxML-NG supermatrix species phylogeny of the tribe Boechereae from analysis of data retrieved from Angiosperm353 and Brassicaceae764 probe sets. Nodes with ≥95 bootstrap support have thickened bars. Maximum likelihood bootstrap values are indicated for those nodes with <95% bootstrap support. Each of the clades recognized and discussed in the text are labeled in white boxes along branches. Labels at the far right in yellow boxes circumscribe "species groups" identified by Alexander et al (2013). Abbreviations are explained in the text.

We find strong support for the monophyly of both Boechereae and *Boechera*, as well as for the other genera of the tribe represented by two or more samples (*Borodinia* and *Sandbergia*). The non-*Boechera* Boechereae (NBB clade; Figure 3) form an unnamed but strongly supported clade sister to *Boechera* s.s. The two major non-*Boechera* lineages, *Borodinia+Nevada* and the "clade of many genera" (CMG), are both well-supported. All the relationships within the *Borodinia+Nevada* clade are strongly supported, with *Nevada* sister to *Borodinia* and the Siberian *B. macrophylla* sister to the eastern North American species of *Borodinia* (Figure 3). The CMG shows full support for *Cusickella +Anelsonia* and *Phoenicaulis+Sandbergia*, with 80% BS for the placement of *Polyctenium* as sister to *Cusickella +Anelsonia*, and low support (64% BS) for the placement of *Yosemitea* as sister to *Phoenicaulis+Sandbergia*. Internally, the genus *Boechera* shows maximum support across most nodes, with only one bootstrap support value <70%. Notably, the lower support values in the phylogeny are found along the *Boechera* backbone.

Plastid phylogeny for tribe Boechereae

Our plastid sampling included all 98 individuals represented in the nuclear phylogeny (Appendix S1). We recovered 84 plastid genes (69,492 bp) comprising 98.5% of the total target length (70,558 bp). Analysis of these loci yielded a phylogeny with strongly skewed support values (Appendix S4). Relationships among the outgroups are well-supported and largely congruent with the nuclear phylogeny. However, there is almost no support for relationships within the ingroup, and the few Boechereae branches that are supported by the plastid analysis are mostly in conflict with the many well-supported relationships observed in the nuclear data (Appendix S5).

DISCUSSION

Plastid-nuclear discordance

Despite excluding recent hybrids from the sampling, our study found notable incongruence between nuclear- and plastid-based phylogenies(Appendix S5), which has been documented repeatedly in Brassicaceae (Koch and Matschinger, 2007; Beilstein et al., 2008; Nikolov et al., 2019; Mabry et al., 2020; Hendriks et al., 2023), and within tribe Boechereae in particular (Kiefer et al., 2009; Alexander et al., 2013). Recent research by Forsythe et al. (2020) and Guo et al. (2021) on *Arabidopsis* and close relatives led them to hypothesize that rampant hybridization and introgression are the underlying causes of this cytonuclear discordance. This is consistent with the observation by Hendriks et al. (2023) that across Brassicaceae the prevalence of hybridization, and the degree of discordance, increases among more recently diverged lineages (i.e., the tips of the tree), of which tribe Boechereae is a prime example.

Alexander et al. (2013) found no support in the plastid tree for the monophyly of the two largest genera (*Boechera* and *Borodinia*), or even for individual species represented by more than one sample. In fact, the few groups that were supported bore no resemblance to either the traditional taxonomy based on morphology or to the revised species circumscriptions supported by microsatellite DNA analyses (Li et al., 2017). By contrast, the seven-locus nuclear DNA analysis of Alexander et al. (2013) was highly congruent with the species boundaries suggested by other data sets, leading the authors to choose the nuclear tree as a more accurate representation of the evolutionary history of Boechereae.

Similar patterns of plastid-nuclear discordance are apparent in our expanded sampling of the tribe, which compared phylogenetic reconstructions based on 1114 nuclear loci to those inferred from the analysis of 84 plastid loci (Appendix S4). Again, the highly supported nuclear tree (Figure 3) was remarkably congruent with taxon circumscriptions derived from other data sets, while the topology of the poorly supported plastid tree appeared almost random by comparison (Appendix S4). Because potential explanations for this discordance are the subject of ongoing research (T. Mandáková et al., unpublished data), we focus entirely on the results from our nuclear DNA data set in the following discussion.

Support for nuclear tree

We recovered strong support at most nodes across the tree, regardless of the approach taken (Figure 3; Appendices S2 and S3). In the following discussion, we consider all maximum likelihood bootstrap values >95% as supported and discuss only values that are below this threshold (Figure 3); all support values are shown in the supplemental figures (Appendices S2 and S3). Despite the high maximum likelihood bootstrap (BS) using RAxML-NG, and the similarly local posterior probabilities (LPP) generated from ASTRAL-III, there is considerable gene-tree and site discordance in the phylogeny (Appendices S2 and S3). As noted by Minh et al. (2020a), BS, sCF, and gCF each measure different things (sCF measures concordance of sites to the species tree and gCF measures concordance of sites to the gene tree), and their values need not be correlated. In the nuclear phylogeny (Appendices S2 and S3), discordance is particularly obvious at the 11 nodes that have <95% bootstrap support, but discordance is apparent at some other nodes as well. Low gCFs can result from at least two distinct issues: a lack of information (decisiveness) in most genes considered, and/or well-supported conflict between loci (e.g., incomplete lineage sorting [ILS] and hybrid lineages). When the information content per gene is low for the node, a very low gCF can result, and this is likely to be observed in conjunction with a short branch and potential for ILS and/or random resolution. By contrast, a simple hybrid scenario with decisive gene trees for each alternative

resolution could have much higher gCFs and be associated with longer branches.

The proportion of decisive characters supporting a node using a sCF represents the sum of all character support (not the sum of gene support) observed for a set of four terminals versus alternative resolution for a set of four taxa (Minh et al., 2020a). When sCF falls <33%, the parsimonious resolution of the four terminals being considered should conflict with the resolution being tested (e.g., ML or Bayesian; Minh et al., 2020a). Some of the lowest sCF values in the nuclear phylogeny (Appendices S2 and S3), approach, but do not breach, the 33% threshold. Thus, both BS and sCF broadly support the resolution presented in Figure 3, while some extremely low gCFs, associated with very short branches, appear unable to address the phylogenetic resolution due to lack of information (rather than clearly conflicting signal). Thus, the lower BS and sCF, as well as the extremely low gCF for a few nodes, are more likely to be associated with limited information content (and potential ILS/random resolution) at the gene level, rather than with the impact of deep hybridization among terminals.

Intertribal relationships

Both Hendriks et al. (2023) and the present study recognize a monophyletic Boechereae (represented in our analysis by 91 samples) that is supported as sister to the Halimolobeae (represented here by seven species assigned to five genera). A sister relationship between these tribes has been inferred by most previous nuclear phylogenies of Brassicaceae with appropriate sampling (Beilstein et al., 2008; Couvreur et al., 2010; Alexander et al., 2013; Nikolov et al., 2019; Hendriks et al., 2023), and Mandáková et al. (2020) have conducted detailed analyses of the chromosomal changes that occurred between these two tribes that diverged over the past 4 million yr (Hendriks et al., 2023).

Generic relationships within Boechereae

Boechereae includes nine genera, all of which were represented in our analyses, as well as in Alexander et al. (2013) and Hendriks et al. (2023). In all three studies, the deepest divergence within Boechereae separated *Boechera* s.s. from the non-*Boechera* Boechereae (NBB) clade (Figure 3). *Boechera* s.s. consistently formed a robust clade, but there was no statistical support for the monophyly of NBB in Alexander et al. (2013), where generic relationships within were mostly unresolved. The only exceptions were strong support (95% BS) for a sister relationship between *Anelsonia* and *Cusickiella* and the hint of a possible association between *Borodinia* and *Nevada* (63% BS) (Alexander et al., 2013).

The nuclear supermatrix tree (Figure 3) shows significant progress in our understanding of generic relationships among the NBB. The deepest divergence among members of this clade separate *Borodinia*+*Nevada* from the other six genera belonging to the "clade of many genera" (CMG). This geographically intriguing association between *Nevada* (a rare, monospecific, Great Basin endemic) and *Borodinia* (a genus with several widely distributed species found in eastern North America and Siberia) was well-supported (97% BS) by Hendriks et al. (2023) as well. The CMG clade itself was well-supported in both studies, but relationships among the constituent genera in our study were less resolved.

The earliest divergence within the CMG clade (Figure 3) resulted in two clades that were also recovered by Hendriks et al. (2023): *Polyctenium*, *Anelsonia*, and *Cusickiella* (PAC clade) and *Yosemitea*, *Phoenicaulis*, and *Sandbergia* (YPS clade). Although the topologies produced by both studies agree on the circumscriptions of the PAC and YPS clades, they are incongruent with respect to relationships recovered within these groups.

Within the PAC clade (Figure 3), our analysis fully supports a sister relationship between *Anelsonia* and *Cusickiella*, a topology also well-supported (95% BS) by the seven-locus nuclear matrix of Alexander et al. (2013). By contrast, Hendriks et al. (2023) inferred a closer relationship (93% BS) between *Polyctenium* and *Cusickiella*. Similarly, within the YPS clade, our phylogenetic tree fully supported a sister relationship involving *Phoenicaulis* and *Sandbergia*, whereas the Hendriks et al. (2023) analysis inferred a closer relationship (94% BS) between *Yosemitea* and *Sandbergia*. The reasons for these conflicting results are unclear at this time, but it is hoped that more detailed and innovative investigations of our 1114 locus data set will lead to improved resolution in the future.

Relationships within *Borodinia*

As recircumscribed by Alexander et al. (2013), the largely eastern North American genus *Borodinia* included eight species: *B. burkii*, *B. canadensis*, *B. dentata*, *B. laevigata*, *B. macrophylla* (the type species and sole Asian representative), *B. missouriensis*, *B. perstellata*, and *B. serotina*. Six of these were included in the nuclear phylogeny of Alexander et al. (2013), and seven were included in a study by Kiefer et al. (2009). Our analysis included samples for all currently accepted species of *Borodinia*. The nuclear tree (Figure 3) recovered full resolution among these species.

Despite the broad taxon sampling of all three studies, there is little basis for comparison between the nuclear supermatrix tree (Figure 3) and the Kiefer et al. (2009) parsimony analysis. The latter relied solely on sequence data from ITS and resulted in few well-supported hypotheses of interspecific relationships. *Boechera* s.s. and *Nevada* appeared nested among the species herein assigned to *Borodinia* (with all but *B. macrophylla* called *Boechera* by Kiefer et al., 2009). This led the authors to suggest that *Borodinia* and *Nevada* should be subsumed within *Boechera*, a premature conclusion given that their ITS tree lacked support for the paraphyly of *Borodinia* s.l. Despite the general lack of resolution, there is some agreement

between the Kiefer et al. (2009) ITS analysis and our supermatrix nuclear tree (Figure 3) in that they both recognize two interspecific relationships within *Borodinia* s.l.: a group comprising *B. laevigata*, *B. missouriensis*, and *B. serotina* (SLM clade) and another with *B. dentata* and *B. perstellata* (DP clade).

The seven-locus nuclear analysis of Alexander et al. (2013) yielded a topology for *Borodinia* that was largely congruent with our tree (Figure 3). Although relationships among the three constituent taxa of our SLM clade were unresolved in Alexander et al. (2013), the group itself was supported. In the Alexander et al. (2013) analysis, *B. dentata* was moderately supported (81% BS) as the closest relative of the SLM clade, which is concordant with our topology, given that neither *B. burkii* (sister to SLM) nor *B. perstellata* (sister to *B. dentata*) was studied by Alexander et al. (2013). The only supported incongruence between our *Borodinia* topology and that of Alexander et al. (2013) was a sister relationship between *B. macrophylla* and *B. canadensis* (89% BS), with this clade being sister to SLM+*B. dentata*. In our analysis (Figure 3), these two species are sequentially sister to the SLM+DP clade.

Relationships within *Boechera* s.s.

Congruent with the nuclear topology of Alexander et al. (2013), our tree resolves a monophylic *Boechera* s.s. and identifies members of the *B. davidsonii* clade as sister to all other *Boechera* (Figure 3). Our study is the first to include *B. shevockii*, a recent segregate of *B. davidsonii*, which differs in its smaller stature and sparsely puberulent (vs. glabrous) stems and leaves (Windham and Al-Shehbaz, 2006). Not surprisingly, considering their morphological similarity and taxonomic history, these two taxa (here recognized as the *B. davidsonii* clade) are strongly supported as closest relatives. The *B. davidsonii* clade has been found only in California, Nevada, and Oregon, USA, where it overlaps extensively with genera of the NBB clade. Members of the *B. davidsonii* clade are separable from other species of *Boechera* s.s. in lacking branched hairs (being either glabrous or sparsely puberulent with simple trichomes), in this regard bearing a closer resemblance to the genus *Nevada*.

Aside from the initial branch segregating *B. davidsonii*, the nuclear strict-consensus tree of Alexander et al. (2013) resolved few nodes along the backbone of the *Boechera* s.s. phylogeny. Resolution improved at shallower nodes, however, with most species represented by more than one sample recovered as monophyletic and seven multispecies clades recovered with moderate to strong support. In a major step forward, our phylogeny (Figure 3) provides greatly improved resolution, with only one internal branch showing bootstrap support <70% and 61/70 (87%) of internal branches with ≥95% BS support. Our analysis indicates that core *Boechera* (i.e., the sister group to the *B. davidsonii* clade) comprises three major lineages. Core *Boechera* I is strongly supported as monophyletic, but support for its sister relationship to the other two major core lineages is lower (73% BS). Core *Boechera* II and III are each moderately supported as monophyletic (80% and 85% BS, respectively). The composition of these newly circumscribed clades and their relationships to previously recognized groups are discussed below.

Core Boechera *I*

Despite being the least speciose lineage of core *Boechera*, this group encompasses three morphologically disparate clades, as well as an isolated sexual diploid taxon that appears to be a new species. The earliest divergence within core *Boechera* I separates the RMn lineage (which includes three distinctive sexual diploid lineages, here designated the *B. retrofracta* clade, the *B. microphylla* clade, and *Boechera* sp. nov.) from the *B. sparsiflora* clade (Figure 3). The *B. sparsiflora* clade represents an expanded version of the well-supported *breweri* group initially recognized by Alexander et al. (2013). Our inclusion of a sample of *B. koehleri* from the type locality (*B. koehleri* 1) helped resolve the relationship between this taxon and *B. breweri*, and the addition of *B. atrorubens* to our analysis supported a close affinity between it and *B. sparsiflora*. In Alexander et al. (2013), *B. cusickii* appeared as sister to the *breweri* group, though with no support. Our analysis strongly supports this relationship, with the combined lineage here designated the *B. sparsiflora* clade (see also Table 1).

Boechera retrofracta *clade*

Within the RMn lineage, the *B. retrofracta* clade is the most widely distributed, with a range extending from southern California and Colorado north to Alaska and east across Canada and the northern United States to Quebec (Al-Shebaz and Windham, 2010). Adoption of the epithet *retrofracta* for this lineage is relatively recent, and the constituent taxa were commonly treated as synonyms or varieties of *Arabis* (*Boechera*) *holboellii* in the older literature (Rollins, 1941, 1993; Mulligan, 1995). The type of *B. holboellii* (Windham and Al-Shehbaz, 2006) was excluded from our study because recent microsatellite DNA analyses indicated that it arose through hybridization between *B. retrofracta* and *B. lemmonii* apomictic triploids (M. D. Windham et al., unpublished data). Alexander et al. (2013) included a specimen identified as *B. retrofracta*, but it was subsequently confirmed to be *B. exilis*, a superficially similar taxon belonging to the distantly related *B. puberula* clade in core *Boechera* III (Schilling et al., 2018). Our analyses included three samples of *B. retrofracta*: (1) a typical specimen collected from Ontario, (2) a collection with sparingly branched trichomes from Saskatchewan previously identified as *B. collinsii*, and (3) a specimen from Idaho representing western North American populations referred to *B. holboellii* var. *secunda* by Holmgren et al., (2005).

TABLE 1 Geographic and morphological notes for select clades within core *Boechera*.

Lineage within core *Boechera*	Geographic distribution	Comments on morphological traits
Core *Boechera* I		
B. sparsiflora clade	From California and northern Utah to southern British Columbia; greatest diversity found in the southern Cascade Range.	Separable from most other *Boechera* by their short, stout, ascending pedicels with mostly simple, spreading trichomes and relatively long, often arcuate fruits (Figure 1A).
B. retrofracta clade	Most widely distributed clade of *Boechera*, with a range extending from southern California and Colorado north to Alaska and east across Canada and the northern United States to Quebec (Al-Shehbaz and Windham, 2010).	Though there are many *Boechera* taxa with down-curved pedicels, only five consistently exhibit slender, appressed, geniculate pedicels: *B. retrofracta*, *B. polyantha* and the sample herein designated *Boechera* sp. nov. (all in the RMn clade of core Boechera I), *B. rectissima* (ARH clade in core *Boechera* II), and *B. exilis* (*B. puberula* clade in core *Boechera* III). Given the distribution of these taxa across the phylogenetic tree, we hypothesize that this distinctive combination of pedicel features has arisen several times during the evolution of the genus (Figure 1B).
B. microphylla clade	The broader *B. microphylla* clade (including the typical apomictic hybrid) ranges from northern Utah and Nevada to western Montana and southern British Columbia.	Members of this lineage can be distinguished from other sexual diploid *Boechera* by their slender, ascending, glabrous pedicels, mat-forming habit, and very small leaves (Figure 1C).
Core *Boechera* II		
B. suffrutescens clade	From the southern Sierra Nevada of California to Mt. Adams in southern Washington, with populations scattered across the mountains of Oregon and northern Nevada into central Idaho. None of the six sexual diploid members of the clade recognized by Morin et al. (2018) are common, and three (the disparate populations of *B. constancei* s.l. and *B. rollei*) are serpentine endemics of conservation concern.	As noted by Morin et al. (2018), members of this clade are distinguished from most other *Boechera* by their wide (2.5–6 mm) pendent fruits containing a single row of broadly winged (0.3–1.5 mm) seeds (Figure 1D).
B. platysperma clade	Aside from isolated populations in the San Bernardino and San Gabriel Mountains of southern California, this clade is largely confined to the Sierra Nevada and southern Cascades, with scattered populations in the Coast Ranges of California and Oregon.	Members of this clade are distinguished from most other *Boechera* by their wide (3–7 mm) ascending fruits containing a single row of broadly winged (0.8–2.5 mm) seeds. The plants (and even the fruits themselves) look very similar to those of the related *B. suffrutescens* clade, but the two lineages are easily distinguished by mature fruit orientation (ascending in the *B. platysperma* clade vs. pendent in the *B. suffrutescens* clade) (Figure 1E).
Core *Boechera* III		
B. dispar clade	One of the most narrowly distributed lineages of *Boechera*, with the three species (all relatively rare) almost entirely restricted to desert and semi-desert habitats in southern California.	With their divaricate-ascending fruits and basal leaves with a dense covering of minute, 6–14-rayed trichomes, the species of this clade most closely resemble *B. shockleyi*. However, they differ from that species in having fewer (1–10 vs. 14–60), non-auriculate (vs. auriculate) cauline leaves, fewer (4–20 vs. 20–70) flowers per inflorescence, and strictly uniseriate (vs. sub-biseriate) seeds (Al-Shehbaz and Windham, 2010).
B. puberula clade	The two relatively common species (*B. exilis* and *B. puberula*) are concentrated in and around the Great Basin in Nevada, southern Oregon, and western Utah; the three rare taxa occur along the periphery of this region in the Klamath and North Coast Ranges of California and Oregon (*B. serpenticola* and *B. subpinnatifida*) and the northern Wasatch Mountains. of Utah (*B. lasiocarpa*).	With their closely pendent to reflexed fruits that are usually puberulent, members of this clade are most like *B. polyantha* (core *Boechera* I) and taxa of the *B. pulchra* clade (core *Boechera* II). In the *B. puberula* clade, however, the young ovaries are glabrous and usually (but not always) become puberulent as they mature. By contrast, the ovaries of *B. polyantha* and members of the *B. pulchra* clade are densely pubescent from the start. The *B. puberula* clade is further distinguished from the *B. pulchra* clade by having a single longitudinal row of seeds (vs. two parallel rows) in well-developed fruits (Figure 1G).

(Continues)

TABLE 1 (Continued)

Lineage within core *Boechera*	Geographic distribution	Comments on morphological traits
B. crandallii clade	None of the species occur west of the Sierran-Cascade axis, and one subgroup (CTK) is essentially confined to the Colorado Plateau and the southern Rocky Mountains.	Most taxa have ascending fruits, but widely pendent fruits are characteristic of *B. cobrensis* and *B. kelseyana* (Windham et al., 2016). Most members of the clade are mat-forming perennials with narrow (often linear) basal leaves, but *B. kelseyana* and *B. thompsonii* are common exceptions. Fortunately, trichomes can be useful for diagnosing the group when combined with the features discussed above. For example, all taxa belonging to the *B. crandallii* clade have proximal portions of fertile stems moderately pubescent (i.e., stem surfaces visible) with mostly 3–7-rayed trichomes (Al-Shehbaz and Windham, 2010). This distinguishes them from all other clades with ascending, small-seeded fruits, which are either glabrous (*B. davidsonii* clade), have sparsely hirsute stems with simple and 2–3-rayed trichomes (*B. sparsiflora* and *B. microphylla* clades), or have densely pubescent stems with more highly divided (6–14-rayed) trichomes (*B. dispar* clade).
B. perennans clade	Distributed from west Texas and Baja California, north to central Nevada and Wyoming, and especially prevalent and diverse in semi-desert habitats in Arizona and New Mexico (Windham, 2023).	Distinguished from other *Boechera* by having pendent, glabrous fruits with small (<2 mm) seeds, ciliate basal leaves, and moderately hirsute lower stems showing various combinations of simple and 2–4-rayed trichomes. *Boechera nevadensis* is unusual in having ± horizontal fruits and largely glabrous leaves and stems (Al-Shehbaz and Windham, 2010) (Figure 1H).

Among the three samples identified as *B. retrofracta*, evolutionary divergence appears to have proceeded from southwest to northeast. This is congruent with the hypothesis of Dobeš et al. (2004), who postulated that this taxon (referred to as *Arabis holboellii* at the time) originated in western North America (where all its close relatives occur) and spread to the eastern portions of the continent after deglaciation of the area during the early Holocene. Nested among the three *B. retrofracta* samples (sister to *retrofracta* 1) is our only collection of *B. polyantha* (Figure 3). Often treated as a synonym of *Boechera* (*Arabis*) *holboellii* var. *secunda* (see Holmgren et al., 2005; represented in our analysis by *retrofracta* 3), this taxon was treated as a separate species by Windham and Al-Shehbaz (2006), based on its distinctly pubescent fruits.

While the topology of our phylogenetic tree (Figure 3) suggests that the taxonomy of the *B. retrofracta* clade needs revision, the group itself is relatively distinctive when apomictic hybrids with other species are excluded. As the epithet *retrofracta* indicates, members of this clade have fruiting pedicels abruptly downcurved (geniculate) at the base such that the slender pedicels (and usually the fruits) are appressed to the rachises (Figure 1B; see also Table 1).

Boechera microphylla *clade*

The *B. retrofracta* clade is fully supported as sister to the newly recognized *B. microphylla* clade (Figure 3). *Boechera microphylla* was excluded from Alexander et al. (2013) because the samples available at the time were known to be apomictic diploid hybrids (Beck et al., 2012). We have since identified the two allopatric sexual diploid parents, one (*B. microphylla* E) most abundant around the headwaters of the Snake River in Wyoming and the other (*B. microphylla* W) concentrated along the lower portion of the river in western Idaho and Oregon (M. D. Windham et al., unpublished data). These form the monophyletic *B. microphylla* clade in the nuclear analysis (Figure 3; see also Table 1).

Boechera *sp. nov.*

The last member of core *Boechera* I to be discussed is the species herein labeled "*Boechera* sp. nov." Rollins (1941) originally included this taxon within *Arabis holboellii* var. *retrofracta* but later recognized it as a separate species, *A. hastatula* (Rollins, 1993). Recent microsatellite DNA analyses (M. D. Windham et al., unpublished data) have shown that what is now called *B. hastatula* includes three subtly distinct taxa: a very rare sexual diploid as well as apomictic diploid and triploid hybrids between this diploid and *B. retrofracta*. Because the type collection of *A. hastatula* represents the triploid hybrid, the sexual diploid requires a new name. This species currently is known only from the western rim of Hells Canyon in Oregon and most closely resembles *B. retrofracta*. However, *Boechera* sp. nov. has glabrous stems, a character state absent from *B. retrofracta*.

Morphological features for core Boechera *I*

Fruit orientation is one of the most useful features for species identification in *Boechera*, and it figures prominently in floristic keys to the genus (Dorn, 2001; Holmgren et al., 2005; Al-Shebaz and Windham, 2010; Weber and Wittmann, 2012; Windham, 2023). However, this character exhibits extensive homoplasy at deeper nodes in the phylogeny, reducing its value for evolutionary reconstruction and the circumscription of larger clades. Core *Boechera* I provides a good example of this, with the major subgroups having either ascending fruits (the *B. sparsiflora* and *B. microphylla* clades; Figure 1) or strongly reflexed fruits (the *B. retrofracta* clade [Figure 1] and *Boechera* sp. nov.). Although core *Boechera* I is well-supported in our analyses, previous researchers have not hypothesized a close relationship among these taxa. There are, however, two earlier studies that provide circumstantial evidence for the existence of core *Boechera* I. An exploratory RADseq analysis by Jordon-Thaden et al. (2020) showed strong support for a sister relationship between *B. retrofracta* and *B. sparsiflora* despite their disparate fruit orientations (*B. microphylla* and *Boechera* sp. nov. were not sampled). ITS sequence analyses by Kiefer and Koch (2012) also appear to provide additional support for core *Boechera* I. That study identified a possible synapomorphy for the group (ITS type H), which was "mostly found in *B. retrofracta*, *B. microphylla* and its varieties defined by Rollins (1993) as well as *B. sparsiflora*" (Kiefer and Koch, 2012).

Core Boechera *II*

Although relatively weakly supported (80% BS; Figure 3), core *Boechera* II brings together four subclades largely congruent with lineages recovered by Alexander et al. (2013). The *B. suffrutescens* clade is portrayed as sister to the rest of Core *Boechera* II, but its proposed sister lineage constitutes a weakly supported (68% BS) group, effectively forming a polytomy encompassing the *B. suffrutescens*, *B. platysperma*, and Southern California (SoCal) clades. Both the *B. platysperma* and SoCal lineages are supported, and the SoCal clade is further subdivided into the newly recognized ARH clade (consisting of *B. arcuata*, *B. rectissima*, and *B. hoffmannii*) and a clade equivalent to the *pulchra* group of Alexander et al. (2013).

Boechera suffrutescens *clade*

As circumscribed by Alexander et al. (2013), the *suffrutescens* group included three sexual diploid taxa (*B. constancei*, *B. rollei*, and *B. suffrutescens*), with *B. constancei* and *B. suffrutescens* supported as closest relatives. Subsequent analyses of microsatellite DNA data by Morin et al. (2018) identified three additional sexual diploids, consisting of a genetically divergent population cluster within *B. constancei* (labeled *constancei* 2) and two largely allopatric segregates of *B. suffrutescens* (*B. botulifructa* and *B. duriuscula*). All six sexual diploids, now attributed to the *B. suffrutescens* clade, were included in our supermatrix analysis (Figure 3). *Boechera suffrutescens* s.s. is fully supported as sister to all other members of the *B. suffrutescens* clade. Although this appears to conflict with the relationships portrayed by Alexander et al. (2013), true *B. suffrutescens* was not included in that study (i.e., the sample referred to there as this taxon is now recognized as *B. duriuscula*). In the lineage sister to *B. suffrutescens* s.s., *B. rollei* was sister to a resolved lineage containing the remaining four taxa. Although relationships within this group were only moderately supported, the topology suggests that neither *B. constancei* s.l. nor *B. suffrutescens* s.l. is monophyletic. In these closely related species, this may be the result of ongoing introgression in the central Sierra Nevada, potential evidence of which was noted in the microsatellite analyses of Morin et al. (2018). See also Table 1.

Boechera platysperma *clade*

The strongly supported (98% BS) *B. platysperma* group of Alexander et al. (2013) encompassed three sexual diploid taxa (*B. howellii*, *B. platysperma*, and *B. pygmaea*), with *B. howellii* and *B. platysperma* fully supported as closest relatives. Each of these taxa was represented in that study by a single sample, augmented here by three additional specimens (two of *B. platysperma* and one of *B. howellii*) chosen to expand geographic coverage of the two most widely distributed taxa. Relationships among all six *B. platysperma* clade samples included in our analysis are fully resolved (Figure 3), with *B. pygmaea* sister to all others, and with the three samples of *B. platysperma* resolved as monophyletic. Interestingly, the two samples of *B. howellii* did not form a monophyletic group; they were, instead, resolved as sequentially sister to *B. platysperma* s.l. Our *B. platysperma* clade coincides with the circumscription of the *B. platysperma* group recognized by Alexander et al. (2013). See also Table 1.

SoCal clade

The SoCal clade comprises two strongly supported but morphologically diverse lineages, the members of which are primarily found in southern California. The newly recognized ARH clade includes three currently recognized species (Figure 3). Two of these, *B. arcuata* and *B. rectissima*, exhibited a sister relationship in Alexander et al. (2013). However, inclusion of the rare Channel Island endemic *B. hoffmannii* in our sampling altered this topology, resolving *B. arcuata* as sister to *B. rectissima* plus *B. hoffmannii* (Figure 3).

Taxa belonging to the ARH clade occur primarily west of the Great Basin Divide, in watersheds draining directly into the Pacific Ocean, with *B. arcuata* distributed from the

Mexican border north to the central Sierra Nevada and *B. rectissima* extending from the San Bernardino Mountains of California north to the vicinity of Crater Lake, Oregon. So far, we have been unable to identify any non-molecular synapomorphies for the ARH clade due to the extreme morphological disparities among its species. *Boechera rectissima* is the most divergent, with relatively short (4–10 vs. 8–45 mm), abruptly reflexed (vs. divaricate-ascending) fruiting pedicels, much smaller (3–4 × 0.7–1.2 vs. 8–14 × 1.5–4 mm) petals, and mostly simple (vs. mostly 2–7-rayed) trichomes (Al-Shebaz and Windham, 2010). The narrowly endemic *B. hoffmannii* is distinguished from the more widespread *B. arcuata* by its glabrous (vs. pubescent) fruiting pedicels and wider (2.5–3 vs. 1.5–2.2) fruits.

Boechera pulchra *clade*

The last lineage of core *Boechera* II to be addressed comprises the species ascribed to the "*Boechera pulchra* group." Alexander et al. (2013) applied this name to a moderately supported clade consisting of five taxa branching in the following order: *B. glaucovalvula*, *B. yorkii*, *B. formosa*, *B. pulchra*, and *B. lincolnensis*. Our analysis included one sample of each and recovered the same relationships (Figure 3). Alexander et al. (2013: 202) characterized the *pulchra* group as encompassing "five species with pendent to reflexed fruits occurring in the Mojave, Great Basin, and Colorado Plateau regions." This is insufficient to distinguish the group from the *B. puberula* clade of core *Boechera* III, and we narrow the circumscription of our *B. pulchra* clade by excluding the Mohave Desert endemic *B. glaucovalvula*. This has little impact on the geographic distribution of the clade, which remains most diverse in the deserts of southern California. *Boechera glaucovalvula* is morphologically divergent from the other four taxa, with wider (5–8 vs. 1.6–4 mm) glabrous fruits and broadly winged (1.8–2.5 vs. 0.07–0.65 mm) seeds (Al-Shebaz and Windham, 2010), and its exclusion from the *B. pulchra* clade greatly simplifies the diagnosis of this lineage. Their pendent (occasionally reflexed), consistently pubescent fruits separate them from all other species except *B. polyantha* (core *Boechera* I) and members of the *B. puberula* clade (core *Boechera* III). Species belonging to the *B. pulchra* clade are distinguished from these superficially similar taxa by having two parallel rows (vs. a single longitudinal row) of seeds in the fruits.

Core Boechera *III*

This moderately supported (85% BS) clade encompasses just over 50% of the sexual diploid taxa attributed to *Boechera* s.s. (Figure 3). The first evolutionary split in the group produced two clades (IIIA and IIIB) of approximately equal size, but neither shows clear morphological synapomorphies. Clade IIIA further split into two equally diverse fully resolved lineages (the SDP and *B. crandallii* clades).

Boechera shockleyi/B. dispar/B. puberula (*SDP*) *clade*

The earliest-diverging taxon in the SDP clade is *B. shockleyi*, the relationships of which were unresolved in Alexander et al. (2013). This species, resolved here as sister to the combined *B. dispar*/*B. puberula* clade, is largely confined to dolomite outcrops in the Great Basin region of Nevada, Utah, and southern California. *Boechera shockleyi* is one of the most distinctive species in the genus, exhibiting prominent rosettes of basal leaves with a dense covering of minute, 7–12-rayed trichomes, strongly overlapping cauline leaves, and relatively long (4.5–11 cm), curved, divaricate-ascending fruits with sub-biseriate seeds (Figure 1F; Al-Shebaz and Windham, 2010).

Following the divergence of *B. shockleyi* (Figure 3), the other members of the SDP clade split into two lineages (the *B. dispar* and *B. puberula* groups), consistent with the results of Alexander et al. (2013). In the latter study, the *B. dispar* group comprised a well-supported (93% BS) clade of three species (*B. dispar*, *B. johnstonii*, and *B. parishii*), each represented by a single sample. Although we failed to obtain data for *B. parishii*, our analysis included paired samples of the other two (Figure 3), with each species represented by a typical collection (labeled "1") and a heterotypic synonym (i.e., a former species-level segregate labeled "2").

Both *B. dispar* s.l. and *B. johnstonii* s.l. are monophyletic in our analysis, and there is little doubt that their close relationship to *B. parishii* portrayed by Alexander et al. (2013) will be upheld by further analyses. Thus, the circumscription of our *B. dispar* clade exactly coincides with the *B. dispar* group of Alexander et al. (2013). See also Table 1. This clade is resolved as sister to the slightly more diverse *B. puberula* clade (Figure 3). Alexander et al. (2013) were the first to recognize a group with this approximate circumscription, but the situation is complicated by the misidentification of two of the seven samples they analyzed. Their samples labeled "*B. retrofracta*" and "*B. subpinnatifida*" were subsequently corrected to *B. exilis* and *B. puberula* subsp. *puberula*, respectively (Schilling et al., 2018). The *puberula* group recognized by Alexander et al. (2013) was fully supported, but, with the name changes indicated above, one species (*B. subpinnatifida*) remained unsampled and relationships among the other four were unresolved.

The *B. puberula* clade was studied by Schilling et al. (2018), who presented a genotype-by-sequencing (GBS) analysis that included 45 samples representing all known taxa. Their results were largely congruent with ours, showing a sister relationship between the *B. puberula* and *B. arida* morphs of *B. puberula* (labeled "1" and "2" in our analysis; Figure 3), which are sister to a clade composed of *B. serpenticola* and *B. subpinnatifida* (both of which were monophyletic). The only topological differences between

Schilling et al.'s (2018) GBS-based analysis and our phylogeny involved the relationships of *B. exilis* and *B. lasiocarpa*. They resolved *B. exilis* as sister to the rest of the *B. puberula* clade, whereas our tree indicates that *B. exilis* and *B. lasiocarpa* together form a clade sister to all other taxa. Schilling et al. (2018) provided county-level maps showing the geographic distributions of taxa belonging to the *B. puberula* clade. See also Table 1 and Figure 1.

Boechera crandallii *clade*

The sister group to the SDP clade is a newly recognized lineage herein referred to as the *B. crandallii* clade (Figure 3). Its first evolutionary split separates the CTK clade from the remaining taxa, with *B. crandallii* sister to a lineage comprising *B. thompsonii* and *B. kelseyana*. Based on the strength of these relationships and the morphological similarities of the taxa, Windham et al. (2022) treated the latter two taxa as subspecies of *B. crandallii*. Alexander et al. (2013) identified a fully supported clade consisting of *B. pallidifolia* (=*B. thompsonii* in our study), *B. lignifera* (=*B. kelseyana*), and *B. villosa* (not included in our sampling; also treated as a subspecies of *B. crandallii* by Windham et al., 2022), but *B. crandallii* appeared on an isolated branch in a polytomy encompassing all of core *Boechera*.

The sister clade to CTK comprises three taxa recognized as species in the *Boechera* treatment in *Flora of North America* (Al-Shebaz and Windham, 2010). *Boechera fernaldiana* is not monophyletic in our analysis; a typical, purple-flowered sample from Nevada (labeled "1" in Figure 3) is sister to the recently segregated *B. evadens*, but a white-flowered collection from eastern Utah (labeled "2") is sister to *B. cobrensis*. By contrast, the two samples of *B. cobrensis* ("1" from Oregon and "2" from Wyoming) do form a monophyletic group despite their considerable (875 km) geographic separation.

Aside from *B. retrofracta* (core *Boechera* I), the *B. crandallii* clade has the most easterly distribution of any of the clades discussed so far. None of the species occur west of the Sierran-Cascade axis, and one subgroup (CTK) is essentially confined to the Colorado Plateau and the southern Rocky Mountains. Although there are some characteristics shared by all members of the *B. crandallii* clade (e.g., glabrous fruits with a single row of relatively small [<2 mm] seeds), these character states are not exclusive to this group. The failure of previous research to recognize the broader *B. crandallii* clade likely stems from its morphological diversity in several key characters. See also Table 1.

Like its sister group in core *Boechera* III, clade IIIB is split into two diverse lineages (the *B. perennans* and "Boreal" clades; Figure 3). These two groups are easily separated morphologically, and their geographic distributions and habitat preferences are similarly divergent. While taxa belonging to the *B. perennans* clade occupy primarily warm, semiarid environments south of 43°N latitude, species of the Boreal clade favor cooler, moister habitats and extend north to at least 66°N. In regions where the two clades appear to overlap broadly (i.e., Colorado and Utah), they segregate by elevation, with the *B. perennans* occupying lower elevations as opposed to the high-elevation affinity of the Boreal clade. In regions of overlap they rarely form mixed populations. Most species of the Boreal clade have ascending to erect fruits, readily distinguished from the pendent (rarely horizontal) fruits characteristic of the *B. perennans* clade (Alexander et al., 2015). The two species of the Boreal clade with horizontal or pendent fruits (*B. lemmonii* and *B. pendulocarpa*, respectively) are easily differentiated from their hirsute congeners in the *B. perennans* clade by an abundance of minute, highly divided trichomes on their basal leaves.

Boechera perennans *clade*

As circumscribed herein, the *B. perennans* clade represents a slightly expanded version of the supported (93% BS) *B. fendleri* group recognized by Alexander et al. (2013, 2015). With one exception (*B. pendulina*), all *B. fendleri* group taxa represented in Alexander et al. (2013) by more than one sample were strongly supported as monophyletic. However, relationships among several of the species were not adequately resolved by the seven-locus nuclear data set (Alexander et al., 2013). Our analysis deepens our understanding of this group, providing full resolution for all branches and no hard incongruence with Alexander et al. (2013).

In both studies, *B. texana* is well-supported as sister to all other taxa in the *B. fendleri* group (*sensu* Alexander et al., 2013) and *B. spatifolia* is equally well-supported as the next lineage. Our analysis (Figure 3) splits the remaining taxa into two groups: (1) a well-supported (94% BS) clade including one sample of *B. nevadensis* and two specimens identified as *B. pendulina*, and (2) a clade with slightly lower support (90% BS) comprising single samples of *B. fendleri*, *B. gracilipes*, and *B. perennans*. In the first group, *B. pendulina* is not resolved as monophyletic. Instead, *B. nevadensis* is nested within it, sister to a sample from Wyoming (here labeled "*B. pendulina* 2" = *B.* "*wyomingensis*" in Alexander et al., 2015). In the second group, *B. gracilipes* and *B. perennans* are fully supported as sister taxa, a relationship also recovered by Alexander et al. (2013) but with weak support.

In Alexander et al. (2013), the *B. fendleri* group formed a polytomy with two species pairs: *B. stricta*/*B. williamsii* and *B. schistacea*/*B. oxylobula*. The first pair is morphologically quite divergent from the *B. fendleri* group and here is shown to belong to the Boreal clade of *Boechera* discussed below. However, *B. schistacea* and *B. oxylobula* are difficult to distinguish from certain members of the *B. fendleri* group (especially *B. pendulina*), and *B. schistacea* is sister to the *B. fendleri* group in our analysis (Figure 3). Although this study does not include data for *B. oxylobula*, there is little doubt that this species is closely related to the *B. fendleri* group as circumscribed by Alexander et al. (2013, 2015). Based on

morphological similarities and our current understanding of relationships, we combine *B. schistacea* and *B. oxylobula* with the *B. fendleri* group, referring to this expanded lineage as the *B. perennans* clade (Figure 3). See also Table 1.

Boreal clade

Boechera fecunda is sister to all other taxa in the Boreal clade and is a globally rare sexual diploid that was not included in Alexander et al. (2013). This species is restricted to calc-silicate soil outcrops in western Montana between 1326 and 2438 m elevation (Song and Mitchell-Olds, 2007). It is one of the most distinctive species in the genus, immediately recognizable by its erect-ascending fruits that are persistently pubescent (Al-Shebaz and Windham, 2010).

Following the divergence of *B. fecunda*, the remaining taxa of the Boreal clade split into two species groups: the LP clade and the "erect fruit" clade (Figure 3). The geographic distributions of these two groups are very similar, extending from Alaska, the Northwest Territories, and Saskatchewan south to about 36°N latitude in the high mountains of California and northern New Mexico. The LP clade (consisting of *B. lemmonii* and *B. pendulocarpa*) is easily differentiated from its sister group by having horizontal to pendent (vs. erect) fruits. The primary morphological feature shared by the two species of the LP clade is the strong dimorphism of the pubescence on different parts of the plant. In particular, the basal leaves are densely covered with minute, highly dissected trichomes, whereas the lower stems are sparsely to moderately pubescent with larger, less dissected hairs. *Boechera lemmonii* is most easily distinguished from other sexual diploids by its unique combination of short (≤6 mm) pedicels, horizontal fruits arranged in one-sided (secund) infructescences, and purplish sepals (Al-Shebaz and Windham, 2010).

The sister group to the LP lineage is here referred to as the erect fruit clade. All members of this clade have fruiting pedicels that lie parallel to the rachis (and appressed to it), with the apex of the fruit oriented toward the shoot apical meristem. As noted previously, fruit orientation is one of the most useful characters for species identification in *Boechera*. However, the value of the character for evolutionary reconstruction is limited by extensive homoplasy (as discussed previously in the RMn, ARH, and *B. crandallii* clades) as well as our inability to identify discrete character states. Most *Boechera* species show fruit orientations that occupy a portion of the continuum between "appressed-erect" and "appressed-reflexed."

The results presented in Figure 3 suggest that the uncommon appressed-erect fruit type was derived relatively recently within *Boechera* and may have had just two independent origins. Outside the Boreal clade, there is just one sexual diploid species (*B. pygmaea*) characterized as having "fruits erect to ascending, often appressed to rachis" (Al-Shebaz and Windham, 2010: 401). This taxon is part of the *B. playsperma* clade (in which all other members have ascending fruits), and its unusually short (2–7 mm) pedicels may contribute to the perception that some individuals have appressed-erect fruits. Within the Boreal clade, the fruits of *B. fecunda* are also described as erect to ascending and often appressed (Al-Shebaz and Windham, 2010: 377). Given the data available, there is no way to determine whether appressed-erect fruits originated in the stem lineage of the Boreal clade (followed by a reversal in the LP clade) or evolved independently in *B. fecunda* and the erect fruit clade.

Boechera stricta (previously known as *Arabis drummondii*) is sister to all other members of the erect fruit clade. It is widespread in the mountains and boreal regions of western North America and has been a primary focus of research within *Boechera* (Song et al., 2006; Windsor et al., 2006; Schranz et al., 2007, 2009; Manzaneda et al., 2010; Lee and Mitchell-Olds, 2013; Lee et al., 2014; Naithani et al., 2014; Anderson and Gezon, 2015; Anderson et al., 2011, 2015; Salmela et al., 2016; Heo et al., 2018; Lee et al., 2018; Rojek et al., 2018; Wagner and Mitchell-Olds, 2018; Bemmels and Anderson, 2019; Olsen et al., 2019; Wang et al., 2019; Hamann et al., 2021; Lin et al., 2021; Liang et al., 2022). It is one of the most distinctive species in the genus, easily separated from all other sexual diploids by its unique pubescence. Although most parts of the plant are glabrous, the basal leaves always have at least some appressed, sessile, 2-rayed (i.e., malpighiaceous) trichomes aligned parallel to the leaf midrib (Al-Shebaz and Windham, 2010).

The sister group of *B. stricta* comprises five samples assigned to the newly recognized *B. paupercula* clade (Figure 3). Four of these represent geographically scattered populations of *B. paupercula* s.l., including three collections previously misidentified as *B. lyallii* (a name typified by hybrids between *B. paupercula* s.l. and *B. stricta*; M. D. Windham et al., unpublished data). The sample labeled *B. paupercula* 1 (from the type locality of *B. paupercula* in the southern Sierra Nevada of California) is resolved as sister to *B. paupercula* 2 (southwest Oregon), and this lineage is, in turn, sister to *B. paupercula* 3 (western Idaho). However, *B. paupercula* 4 (central Idaho) is fully supported as sister to *B. williamsii* (western Wyoming), making *B. paupercula* s.l. paraphyletic. In Alexander et al. (2013), *B. williamsii* was weakly supported as sister to *B. stricta*, and this species pair was part of a polytomy that included paired samples of *B. paupercula* s.l. (misidentified as *B. lyallii*), as well as most other samples of core *Boechera* III. As circumscribed herein, the *B. paupercula* clade ranges from the high mountains of California and Wyoming north to the Yukon Territory in Canada. Members of this lineage are distinguished from *B. stricta* by their lack of malpighiaceous trichomes on the basal leaves and from all other sexual diploids by their strictly erect, glabrous fruits that are <3 mm wide (Figure 1I).

CONCLUSIONS

Our phylogenetic analyses are the first to fully elucidate relationships within *Boechera* and related genera. Largely congruent with results from the seminal seven low-copy

nuclear loci study by Alexander et al. (2013), they greatly improve our understanding of relationships in the group by resolving all polytomies and providing good support for the backbone of the tree. Our study supports (1) the separation of Boechereae into two major clades (the non-*Boechera* Boechereae [NBB clade] and *Boechera* s.s.), (2) the transfer (by Alexander et al., 2013) of the eastern North American/East Asian species of "*Boechera*" to the genus *Borodinia*, and (3) the recognition of several major lineages within *Boechera* s.s.

The hyperdiverse *Boechera* s.s. is strongly supported as monophyletic and provides yet another example of an "*Amborella* syndrome" (Larsén et al., 2022), in which an unusual, species-poor lineage (in this case, *B. davidsonii* s.l.) is sister to the remainder of the genus, which comprises three core clades. Each of the core *Boechera* clades are further subdivided into reasonably well-defined species lineages that are now amenable to phylogenetically based taxonomic revision (M. D. Windham et al., unpublished data). Our phylogeny has already opened the door to a host of additional research projects, including analyses of interclade hybridization and niche differentiation (N. M. Hay et al., unpublished data), *Boechera* karyotype and repeatome evolution and plastid-nuclear discordance (T. Mandáková et al., unpublished data), and the evolution of woodiness in the Brassicaceae Tree of Life (K. P. Hendriks et al., unpublished data). We encourage others to tap into this rich data source as *Boechera* comes closer to realizing its full potential as a model system for plant science.

AUTHOR CONTRIBUTIONS

This study was conceived of and organized by M.D.W. and C.D.B. Data collection and analyses were conducted by N.M.H. First drafts were written by N.M.H, K.M.P., and M.D.W.; all authors discussed the results and contributed to the final manuscript. N.M.H. was responsible for all data curation, and for Figure 2 and Appendices S2–S4. K.M.P. and M.D.W. were responsible for Figures 1 and 3 and Appendix S5.

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DATA AVAILABILITY STATEMENT

All sequencing data has been deposited at the NCBI Sequence Read Archive (PRJNA700668).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Voucher data, DNA extraction, and sequencing information for each sample used in this study, including the NCBI SRA accession IDs.

Appendix S2. RAxML-NG supermatrix species phylogeny of the tribe Boechereae from analysis of data retrieved from Angiosperm353 and Brassicaceae764 probe sets, with gene concordance factors (gCF) and site concordance factors (sCF) indicated. Concordance factors were calculated with IQTREE2 and are displayed with the maximum likelihood bootstrap values, gCF, and sCF.

Appendix S3. Coalescent species tree with gene concordance factors (gCF) and site concordance factors (sCF) of Boechereae from Angiosperm353 and Brassicaceae764 using RAxML-NG and ASTRAL-III. Concordance factors were calculated with IQTREE2 and are displayed with the local posterior probabilities (from ASTRAL-III), gCF, and sCF.

Appendix S4. RAxML-NG supermatrix species phylogeny with maximum likelihood bootstrap support values for the tribe Boechereae from analysis of data retrieved from a plastid target file derived from the published *B. stricta* chloroplast genome.

Appendix S5. Comparison between nuclear and plastid topologies resulting from this study. Well-supported (≥95% BS) branches have thickened bars. Red lines connect identical samples in the two phylogenies.

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