REVIEW & INTERPRETATION

Research Gaps and Challenges in the Conservation and Use of North American Wild Lettuce Germplasm

Aleš Lebeda,* Eva Křístková, Miloslav Kitner, Ľuboš Majeský, Ivana Doležalová, Colin K. Khoury, Mark P. Widrlechner, Jinguo Hu, Daniel Carver, Harold A. Achicanoy, and Chrystian C. Sosa

ABSTRACT

The North American crop wild relatives (CWR) of lettuce (*Lactuca* L.) represent an underexplored pool of genetic diversity of potential value to breeding programs. The 10 species belong to three different groups: a native clade including at least six allotetraploid species [*L. biennis* (Moench) Fernald, *L. canadensis* L., *L. floridana* (L.) Gaertn., *L. graminifolia* Michx., *L. hirsuta* Muhl. ex Nutt., and *L. ludoviciana* (Nutt.) Riddell], a diploid clade with one species [*L. tatarica* (L.) C. A. Mey. subsp. *pulchella* (Pursh) Stebbins], and a clade related to the cultivated taxon (*L. sativa* L.) with three non-native species (*L. saligna* L., *L. serriola* L., and *L. virosa* L.). In this review, we examine the role of herbarium and genebank holdings in taxonomic and other foundational studies, as well as for germplasm exploration and use. We compile the state of knowledge on the ranges of lettuce CWR in North America, modeling the potential distributions of the species and assessing their ex situ and (for native species) in situ conservation status. We categorize seven of the species as high priority for further conservation and three as medium priority, with none currently considered low priority or sufficiently conserved. Further, we review morphological, phenological, genetic diversity, and pest and disease information with regard to North American species. We conclude by outlining the critical gaps and describing a way forward for addressing challenges in the conservation and use of North American wild lettuce germplasm.

A. Lebeda*, E. Křístková, M. Kitner, and Ľ. Majeský, Dep. of Botany, Faculty of Science, Palacký Univ. in Olomouc, 78371 Olomouc, Czech Republic; I. Doležalová, Crop Research Institute, Centre of the Region Hana for Biotechnological and Agricultural Research, Šlechtitelů 29, 78371 Olomouc, Czech Republic; C.K. Khoury and D. Carver, USDA-ARS, National Laboratory for Genetic Resources Preservation, 1111 South Mason St., Fort Collins, CO, 80521, USA; C.K. Khoury, H.A. Achicanoy, and C.C. Sosa, International Center for Tropical Agriculture (CIAT), Km 17, Recta Cali-Palmira, Apartado Aéreo 6713, 763537 Cali, Colombia; M.P. Widrlechner, Departments of Horticulture and of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011, USA; J. Hu, USDA-ARS, Washington State University, Pullman, WA 99164-6402, USA; D. Carver, Natural Resource Ecology Laboratory, Colorado State Univ., Fort Collins, CO 80524-1499, USA; C.C. Sosa, Grupo de investigación en Evolución, Ecología y Conservación EECO, Programa de Biología, Facultad de Ciencias Básicas y Tecnologías, Universidad del Quindío, Armenia, Colombia. Received 31 May 2019. Accepted 4 Oct. 2019. Assigned to Associate Editor Jorge da Silva.*Corresponding author (ales.lebeda@upol.cz).

Abbreviations: AFLP, amplified fragment length polymorphism; CA50, circular area of 50-km radius; CGN, Centre for Genetic Resources, the Netherlands; CWR, crop wild relatives; ERSex, ecological representativeness score ex situ; ERSin, ecological representativeness score in situ; FCSc-mean, final combined conservation score (mean); FCSex, final conservation score ex situ; FCSin, final conservation score in situ; GBIF, Global Biodiversity Information Facility; GISH, genomic in situ hybridization; GRIN, Germplasm Resources Information Network; GRSex, geographical representativeness score ex situ; GRSin, geographical representativeness score in situ; IPK, Leibniz Institute of Plant Genetics and Crop Plant Research; ITS, internal transcribed spacer; LGCS, USDA *Lactuca* Germplasm Collection—Salinas; LGRCD, *Lactuca* Genetic Resources Collection, University of California, Davis; NPGS, US National Plant Germplasm System; PGRC, Plant Gene Resources of Canada; SRS, sampling representativeness score; SSR, simple sequence repeat; WIEWS, World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture; WRPIS, Western Regional Plant Introduction Station.

CROP WILD RELATIVES (CWR) represent a large pool of genetic diversity from which to draw new allelic variation required in breeding programs (Maxted et al., 2006). Crop wild relatives have been extremely valuable in adapting crop varieties

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to changing pest and disease pressures, farming practices, market demands, and climatic conditions (Dempewolf et al., 2017). Unfortunately, CWR are a threatened resource (Kell et al., 2012) and measures need to be taken to protect them, both in the wild and in genebanks (Castañeda-Álvarez et al., 2016; Khoury et al., 2019b; Vincent et al., 2019). Limited resources for conservation management demand careful planning to prioritize those taxa in most urgent need of conservation (Kell et al., 2017; Magos Brehm et al., 2017). In this paper, we review how wild North American *Lactuca* species, both native and non-native, may contribute to the breeding of lettuce varieties and where efforts must be concentrated to harness their potential value.

The genus *Lactuca* is composed of >100 species, the majority occur in Asia (51 species) and Africa (43 species), some others in Europe (17) (Lebeda et al., 2004b). From North America, up to 12 species have been described (Lebeda et al., 2007, 2019; Kilian et al., 2017). Unfortunately, from a taxonomic viewpoint, the genus has not been the subject of a modern monograph (Lebeda et al., 2019). However, useful perspectives on the biosystematics and phylogeny of North American *Lactuca* species have recently been published (Jones et al., 2018).

Within North America, native *Lactuca* species are widely distributed from Canada south to Florida and Mexico. These species are, in most cases, biennial (Lebeda et al., 2019). *Lactuca* species native to North America likely arose relatively recently, in the Pliocene (Kilian et al., 2017). Six of them [*L. biennis* (Moench) Fernald, *L. canadensis* L., *L. floridana* (L.) Gaertn., *L. graminifolia* Michx., *L. hirsuta* Muhl. ex Nutt., and *L. ludoviciana* (Nutt.) Riddell] are known to be allotetraploids ($x = 17$) (Strother, 2006; Jones et al., 2018).

Additionally, three weedy, Old World species (*L. saligna* L.*,* *L. serriola* L., and *L. virosa* L.) are found in North America (Lebeda et al., 2012), as well as the domesticate, cultivated lettuce (*L. sativa* L.) (Lebeda et al., 2007). All these species were introduced to the New World by European settlers (Lebeda et al., 2019), with the introduction of *L. sativa* possibly dating back to Columbus's second voyage of 1494 (Hedrick, 1972). Two additional taxa, *L. terrae-novae* Fern. and *L. tatarica* (L.) C.A. Mey. subsp. *pulchella* (Pursh) Stebbins, are included in the "Synonymized Check List of the Vascular Flora of the United States, Canada and Greenland" (Kartesz, 1994).

Cultivated lettuce is among the most important and popular leafy vegetables, used in salads in most parts of the world (Lebeda et al., 2007, 2019). It is one of the most morphologically and genetically diverse vegetables, having distinct horticultural types (crisphead, cos [romaine], butterhead, leaf, Latin, stem [stalk], red leaf, baby lettuce, and oilseed), which differ in head shape and size, the shape, size, and texture of the leaves, stem length, and seed size (Lebeda et al. 2007; Simko et al., 2014a, 2014b). All these forms except for two (stem and oilseed) are typically consumed raw (Lebeda et al., 2019).

As one of the earliest domesticated vegetables (up to 10,800 yr ago), lettuce belongs to a core group of ancient crops (Hancock, 2012; Zhang et al., 2017). Lettuce is considered to be of polyphyletic origin and most likely was selected from the genepool of *L. serriola* (Lebeda et al., 2007; Kuang et al., 2008) [most probably *L. serriola* f. *integrifolia* (Gray) S.D. Prince & R.N. Carter], followed by simultaneous introgressions of traits from other closely related taxa (Lebeda et al., 2019). We suspect that certain other species (*L. azerbaijanica* Rech. f., *L. altaica* Fisch. & C.A. Mey., *L. aculeata* Boiss. & Kotschy, and *L. scarioloides* Boiss.) (Zohary, 1991; Koopman et al., 1998; Lebeda et al., 2007) played roles in the evolution of cultivated lettuce (Lebeda et al., 2019). Based on recent findings, the center of diversity of these related *Lactuca* species is in southwestern Asia (i.e., eastern Turkey, Armenia, and northwestern Iran) (Kuang et al., 2008), and the origin of lettuce may also spring from this region, especially the Euphrates–Tigris valley (Zohary and Hopf, 1993).

Although considerable progress has been achieved both in the areas of fundamental research on *Lactuca* germplasm and its practical applications during the last 35 yr (Lebeda et al., 2007, 2009, 2014, 2019), the study of *Lactuca* species occurring in North America (Strother, 2006; Lebeda et al., 2012, 2019) has been relatively neglected. As part of a recent volume reviewing the status of CWR in North America (Greene et al., 2019), the first comprehensive overview of wild and weedy North American *Lactuca* was published (Lebeda et al., 2019). Building on the findings of that review, here we highlight the most important research gaps and challenges related to the conservation and use of wild North American *Lactuca*.

PHYLOGENY AND TAXONOMY OF NORTH AMERICAN *LACTUCA*

In the most recent comprehensive treatment of the Asteraceae, the genus *Lactuca* was included in the subtribe Lactucinae of the tribe Cichorieae (Kilian et al., 2009). The Lactucinae host ~200 species, with the greatest diversity located in southwest Asia and the Sino-Himalayan region (Kilian et al., 2009, 2017; Wang et al., 2013). This subtribe most likely originated in the Early Miocene about 18 to 19 million yr ago (Kilian et al., 2017). Diversification of the core Lactucinae began about 12.2 to 12.7 million yr ago within the southwest Asian-European region (Kilian et al., 2017).

The recent, rapid, and reticulated history of this subtribe (or *Lactuca* alliance sensu Kilian et al., 2017) hampers unambiguous generic classification. Various taxonomic approaches have been used to deal with the richness of this group, which range from a broad definition of genera to much narrower concepts (reviewed in Lebeda et al., 2019). Recently, molecular studies (Kilian et al., 2017; Wang et al., 2013; Wei et al., 2016, 2017) have provided new data calling for a reassessment of generic relationships. These studies have demonstrated that the various classification systems in current use are not natural, often treating morphologically similar but evolutionarily only distantly related taxa as genera, now shown to be polyphyletic. In particular, the genus *Lactuca*, as currently treated, has been shown to be polyphyletic (Kilian et al., 2017; Wei et al., 2016, 2017). From the >100 species recognized within the genus *Lactuca*, ∼40 have been shown to represent a clearly monophyletic group, the "*Lactuca* lineage" (Clade I, 9.8 million yr ago) by Kilian et al. (2017), including all examined North American species.

The *Lactuca* species occurring in North America belong to three different groups: two native and one non-native. The two native clades differ with respect to chromosome numbers. The larger clade includes allotetraploids with chromosome number $2n = 34$. Allotetraploids are unique within the *Lactuca* lineage (Clade I), as all other members are diploids with two basic chromosome numbers, $x = 9$ or $x = 8$. These allotetraploids (the *L. canadensis* clade sensu Jones et al., 2018) harbor seven biennial species (*L. biennis*, *L. canadensis*, *L. floridana*, *L. graminifolia*, *L. hirsuta*, *L. ludoviciana*, and *L. brachyrrhyncha*) and one perennial species endemic to the Azores, Portugal (*L. watsoniana* Trel). The second native clade is represented by *L. tatarica* subsp. *pulchella*, a common diploid species with $2n = 18$. The third group of wild lettuce species in North America is represented by "the core *Lactuca* clade" (sensu Kilian et al., 2017), which includes three introduced species with a European–Mediterranean native distribution: *L. saligna*, *L. serriola*, and *L. virosa*.

The two native North American clades are thought to represent the relatively recent products of lineage diversification and two independent colonization events. The *L. tatarica* clade is of recent middle Pliocene origin (3.4–5.1 million yr ago) with the divergence of the two sister taxa, *L. sibirica* (L.) Benth. ex Maxim. and *L. tatarica* subsp. *pulchella*, estimated ∼3.2 million yr ago. The Beringian land bridge has been suggested as the most probable migration route to North America, and subsequent geographic isolation then resulted in divergence (Jones et al., 2018).

In contrast, the *L. canadensis* clade most likely represents a sister group to *L. plumieri* (L.) Gren. & Godr., a diploid ($x = 8$) native to the European Alpine-Pyrenean mountain range, originating about 3.8 to 4.5 million yr ago (Jones et al., 2018; Kilian et al., 2017). Thus, it seems probable that this allotetraploid clade originated via hybridization of *L. plumieri* (or its ancestor) and some now extinct ($x = 9$) species, possibly an ancestral species from the clade of *L. tatarica* and *L. quercina* L. (both $x = 9$) or even of *L. indica* L. (also $x = 9$). Such a hybridization event is further supported by morphological similarities of members of the *L. canadensis* clade with *L. plumieri* (Jones et al., 2018).

The *L. canadensis* clade originated ∼4.8 million yr ago during the late Miocene and diverged from *L. plumieri* ∼2.7 million yr ago during the late Pliocene to early Pleistocene (Jones et al., 2018). Two competing hypotheses for the origin of this hybridization event were put forward by Jones et al. (2018). According to the first hypothesis, ancestors of $x = 8$ lineage (represented most probably by *L. plumieri* or its ancestor) migrated via the North Atlantic Land Bridge to North America, whereas the ancestor of the $x = 9$ lineage (most probably ancestor of the *L. tatarica* group or *L. indica*) migrated across the Beringian Land Bridge. After these taxa met in the newly colonized area, they hybridized, giving rise to the allotetraploid *L. canadensis* clade, and the diploid progenitors subsequently went extinct. The second hypothesis favors hybridization in the area of the North Atlantic Land Bridge, where an ancestor of *L. sibirica* (member of the *L. tatarica* clade) and *L. plumieri* met and produced a new allotetraploid lineage. After the deterioration of climatic conditions and the physical collapse of the North Atlantic Land Bridge, ancestral diploid taxa became extinct, while the allotetraploid lineage migrated west to North America, where it further diversified.

The three weedy, Old World *Lactuca* species in North America all belong to one well-resolved subclade, along with *L. sativa* (Subclade I3d; Kilian et al., 2017), sister to the *L. viminea* subclade (Subclade I3b; Kilian et al. 2017), assignments supported by Wang et al. (2013) and also via whole-plastome phylogenetic inference (Wei et al. 2016), with some incongruences between internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) phylogenetic inference, observed with respect to *L. sativa* and *L. viminea* as sister clades. Kilian et al. (2017) estimated the divergence of the *L. sativa*–*quercina*–*viminea*–*tatarica* + *L. indica* clade as occurring ∼6.1 million yr ago.

HERBARIUM COLLECTIONS OF WILD NORTH AMERICAN *LACTUCA*

Dried, pressed plant specimens held in the world's herbaria have long served to document the patterns of morphological diversity that serve as the basis for classical taxonomy and the description of species. Today, as DNA extraction and sequencing technologies continue to improve, herbarium specimens are becoming increasingly valuable as historical sources of DNA samples in genetic research (Shepherd, 2017; Staats et al., 2011).

In the field of germplasm conservation, there are many examples demonstrating how data collected from herbarium specimens and their associated documentation have been used to direct germplasm exploration and improve collection use. von Bothmer and Seberg (1995) and Marek (2018) both published general surveys on the use of herbarium vouchers to target exploration efforts. Notable case studies have been presented by Edmonds (1990), who identified African regions of high morphological variability within *Corchorus* L. from herbarium specimens and used this information to maximize diversity in field collections, and Bompard (1995), who was guided by herbarium records to survey *Mangifera* L. in the tropical rainforests of Southeast Asia. Specimen labels can also be quite helpful as sources of ethnobotanical information, which can inform germplasm searches for bioactive compounds and other specialized uses (Guarino, 1995).

Depending on the quality of geographic data on specimen labels, georeferenced collection sites can be used to generate detailed distribution maps and shape ecogeographic surveys (Maxted et al., 2008), including those developed for the ranges of North American *Lactuca* presented by Lebeda et al. (2019) and the gap analyses presented later in this review. Even fairly coarse, geographic label data, when coupled with collection dates, can be used to document the dynamics of invasion and range expansion, as was done for the spread of *Chaenorrhinum minus* (L.) Lange across North America (Widrlechner, 1983), and which could be attempted to elucidate the history of North American introduction and range expansion for the Old World weeds, *L. saligna* and *L. serriola*.

In April and May 2019, we conducted a review of online resources to identify the largest holdings of wild and weedy *Lactuca* specimens from Canada, Mexico, and the United States among North American herbaria. We attempted to survey every North American herbarium with large holdings (in our case, at least 640,000 specimens) (Thiers, 2019a), beginning with a comprehensive query run on the specimen records accessible via the online portal of the Consortium of Midwest Herbaria (2019), and then proceeding to identify and query 14 other online sources for those herbaria not or inadequately covered in our initial search. In cases where few or no North American *Lactuca* specimens were found online for these largest herbaria, we contacted curatorial staff at those institutions directly, except for the Ada Hayden Herbarium at Iowa State University (herbarium codes follow Thiers, 2019b), where we conducted an actual count.

The results of this survey are clearly incomplete, as many herbaria are still in the process of making their collection data accessible online; thus, the results presented in Table 1 can only be viewed as provisional, minimal estimates. Still, the 28 largest herbarium collections that we identified (treating three herbaria housed at Harvard University [Arnold Arboretum, Gray Herbarium, and the New England Botanical Club] and Botanical Research Institute of Texas and Vanderbilt University housed at the Botanical Research Institute of Texas as single entities) have preserved >18,000 specimens of wild and weedy *Lactuca*, primarily from the United States. Only the Agriculture and Agri-Food Canada National Collection of Vascular Plants holds a large number (800+) of Canadian specimens. The next largest collection of Canadian *Lactuca* that we found is at the University of British Columbia, with 185 specimens. The size of Mexican collections is considerably smaller. The largest holder of Mexican collections is Herbario Nacional, Universidad Nacional Autónoma de México, with 102 specimens. Collectively, these specimens should be of great value in expanding our understanding of morphological diversity, native and introduced ranges, and ecological adaptation and can serve as a key resource for guiding future exploration and seed collection.

BOTANICAL GARDEN AND GENEBANK COLLECTIONS FOR NORTH AMERICAN *LACTUCA*

North American botanical gardens have focused increasing attention toward plant biodiversity conservation, organized broadly under the umbrella of the "North American Botanic Garden Strategy for Plant Conservation 2016–2020" (BGCI, 2016). To estimate the extent of their efforts directed to the ex situ conservation of wild and weedy *Lactuca*, we recently queried PlantSearch, a taxonomically organized database of botanical garden holdings, maintained by BGCI (2019). The results were striking. Only 21 North American gardens were noted as maintaining any collections of *Lactuca* species other than cultivated lettuce (A. Meyer & M. Jones, personal communication, 2019), either as living collections or as seed. In total, there were only 50 taxon records (which include one or possibly more accessions per taxon) representing 13 species, including two Old World species (*L. intricata* Boiss. and *L. plumieri*) not known to naturalize in North America.

Similarly, the Plant Collections Network of the American Public Gardens Association (2019a, 2019b) coordinates a continent-wide approach to plant germplasm preservation at botanical gardens by evaluating and recognizing the most important collections and promoting high standards of collection management and curation. There are no recognized collections of *Lactuca* within the Plant Collections Network. Taken together with the PlantSearch results, these findings are not particularly surprising, given the limited use of wild *Lactuca* in ornamental horticulture and the challenges of long-term garden cultivation of plants with a predominantly biennial life cycle.

With regard to genebanks in North America, there are five important lettuce and wild *Lactuca* seed collections (Lebeda et al., 2019), accounting for ~3.4% of total number of conserved vegetable accessions in the United States (~87,000) (USDA-ARS NPGS, 2019a). The primary national repository and distribution center for lettuce genetic resources within the US National Plant Germplasm System (NPGS) is the Western Regional Plant Introduction Station (WRPIS) in Pullman, WA. The USDA *Lactuca* Germplasm Collection in Salinas, CA (LGCS), is considered to be the largest and most diverse collection of defined and characterized lettuce accessions in the world (Leafy Vegetable Crop Germplasm Committee, 2015; Lebeda and Astley, 1999; Lebeda et al., 2004a, 2019; McGuire et al., 1993). The *Lactuca* Genetic Resources Collection, University of California, Davis (LGRCD), is a primary holder of lettuce cultivars, mapping populations, and other molecular genetic materials developed in California. Seeds for Success, a federal interagency plant material development program, coordinates the collection of wildland native seeds throughout the United States to supply the needs of research and development, conservation, and ecosystem restoration (Bureau of Land Management, 2019). To date, this program has supported the collection of 17 populations of five wild *Lactuca* species, with nearly all these collections made by the Chicago Botanic Garden in Glencoe, IL (L. Prescott, personal communication, 2019). The US National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, CO, also preserves lettuce germplasm (Leafy Vegetable Crop Germplasm Committee, 2015; McGuire et al., 1993), primarily serving as a long-term, back-up repository for working collections, such as those listed above.

Passport information, as well as observations on various morphological and horticultural traits for those *Lactuca* germplasm accessions conserved by the NPGS, is freely accessible through the Germplasm Resources Information Network (GRIN)-Global database (https://npgsweb.ars-grin.gov/gringlobal/search.aspx). As of May 2019, GRIN-Global included 2958 *Lactuca* accessions, with 779 of them being of wild *Lactuca* taxa.

Table 1. Major herbarium holdings of wild and weedy *Lactuca* species from North America in rank order, categorized by the minimum estimated total number of specimens. Herbarium codes follow Thiers (2019a).

Estimated size	Code†	Source of information
≥1250	US	https://collections.nmnh.si.edu/search/botany/
	PH	Consortium of Midwest Herbaria (2019)
≥1000	NCU	Consortium of Midwest Herbaria (2019)
≥750	ILLS	Consortium of Midwest Herbaria (2019)
	DAO	Curatorial staff
	A, GH, NEBC	https://kiki.huh.harvard.edu/databases/specimen_index.html
	ISC	Physical count
	CM	Consortium of Midwest Herbaria (2019)
	F	Curatorial staff
	MU	Curatorial staff
	WIS	Consortium of Midwest Herbaria (2019)
	MO	Consortium of Midwest Herbaria (2019)
	RM	Consortium of Midwest Herbaria (2019)
≥500	KANU	https://biodiversity.ku.edu/botany/collections/specify
	OS	http://164.107.176.186/bol/herbarium/Search
	NY	http://sweetgum.nybg.org/science/vh/
≥250	IND	Consortium of Midwest Herbaria (2019)
	MISS	Consortium of Midwest Herbaria (2019)
	BRIT, VDB	Consortium of Midwest Herbaria (2019)
	BH	Curatorial staff
	MIN	http://bellatlas.umn.edu/collections/harvestparams.php
	BRY	Curatorial staff
	CAS	http://researcharchive.calacademy.org/research/botany/coll_db/index.asp
	TENN	Consortium of Midwest Herbaria (2019)
	WILLI	Consortium of Midwest Herbaria (2019)
	RSA	Consortium of Midwest Herbaria (2019)
	COLO	Consortium of Midwest Herbaria (2019)
	DUKE	Curatorial staff

† A, Arnold Arboretum, Harvard University; BH, LH Bailey Hortorium Herbarium, Cornell University; BRIT, Botanical Research Institute of Texas; BRY, Stanley L Welsh Herbarium, Brigham Young University; CAS, California Academy of Sciences; CM, Carnegie Museum of Natural History; COLO, University of Colorado Museum; DAO, Agriculture and Agri-Food Canada, National Collection of Vascular Plants; DUKE, Duke University; F, Field Museum; GH, Gray Herbarium, Harvard University; ILLS, Illinois Natural History Survey; IND, Indiana University; ISC, Ada Hayden Herbarium, Iowa State University; KANU, McGregor Herbarium, University of Kansas; MEXU, Herbario Nacional, Universidad Nacional Autónoma de México; MIN, Bell Museum, University of Minnesota; MISS, Pullen Herbarium, University of Mississippi; MO, Missouri Botanical Garden; MU, WS Turrell Herbarium, Miami University; NCU, University of North Carolina Chapel Hill; NEBC, New England Botanical Club; NY, William and Lynda Steere Herbarium, The New York Botanical Garden; OS, Museum of Biological Diversity, Ohio State University; PH, Academy of Natural Sciences; RM, Rocky Mountain Herbarium, University of Wyoming; RSA, Rancho Santa Ana Botanic Garden; TENN, University of Tennessee; UBC, University of British Columbia; US, US National Herbarium, Smithsonian Institution; VDB, Vanderbilt University; WILLI, The College of William and Mary; WIS, University of Wisconsin.

Agriculture and Agri-Food Canada's Plant Gene Resources of Canada (PGRC) is an integral part of the Saskatoon Research Centre located at the University of Saskatchewan. The PGRC seed genebank is its main repository. The Canadian database, GRIN-CA, has adopted the information system used in the United States (http://pgrc3.agr.gc.ca/search_grinca-recherche_rirgc_e.html). Currently, however, there are no accessions of wild *Lactuca* species conserved by the PGRC.

Currently only 27 (20%) of known species are available in the world's *Lactuca* collections (mostly of European origin) (Lebeda et al., 2004a, 2007). A summary of all the wild *Lactuca* taxa reported in the NPGS GRIN-Global database was summarized by Lebeda et al. (2019). Passport data for 20 wild *Lactuca* species representing 779 accessions are entered, with 65 accessions (8%) not yet classified to species. In comparison, Europe's largest genebanks, Centre for Genetic Resources, the Netherlands (CGN), and Leibniz Institute of Plant Genetics and Crop Plant Research, Germany (IPK), maintain 994 (CGN) and 112 (IPK) accessions of wild *Lactuca* species, respectively, but for the native North American taxa, as of April 2019, only CGN conserves any (6 accessions), which is not surprising given the distance. Regarding the availability for users, a 2016 survey of the GRIN-Global database (NPGS) showed that 24% (185 accessions) were available to users at that time (Lebeda et al., 2019), whereas only 12.6% (98 accessions) are available today.

Accessions of the native North American species listed in the GRIN-Global database comprise only 3% (26 accessions) of the wild *Lactuca* species conserved within the NPGS. Regarding the availability of accessions originating from North America, it is very striking that only two accessions are available for distribution. A substantial part of this collection (78%, 606 accessions) is represented by three species: *L. serriola* (54%), *L. saligna* (12%), and *L. virosa* (12%). The remaining species are represented by very few accessions (from 1 to 18) (Lebeda et al., 2019).

A review of passport data for wild *Lactuca* represented in the GRIN-Global database, organized by continent, showed that 96% of accessions originated from Eurasia, 3% originated from North America, and only three accessions are available from Africa. However, Lebeda et al. (2019) identified some misleading information as to the primary origin of a few accessions of *L. aculeata* Boiss. & Ky., *L. altaica* Fisch & C.A. Mey., *L. indica*, and *L. inermis* Forssk., where passport records have confused the location of the original habitat with the donor site. For example, "United States, California" is listed as the place of origin for four unavailable accessions of *L. indica*. Such errors are clear in samples of species with ranges restricted to other continents.

Regarding the representation of species held by the NPGS in relation to the primary genepool of *L. sativa* (according to Koopman et al., 1998, 2001; Koopman, 1999), 59% of the collection is constituted by accessions of *L. aculeata*, *L. altaica*, *L. dregeana*, *L. georgica*, and *L. serriola* from the primary genepool, 4% by *L. saligna* and *L. virosa* from the secondary genepool, and 3% by *L. quercina*, *L. tatarica*, and *L. viminea* accessions from the tertiary genepool. This emphasis on members of the primary genepool is probably due to the fact that only recently have species of the secondary and tertiary genepools been regarded as significant donors of important traits (Lebeda et al., 2004a, 2007, 2009).

The WRPIS optimally uses current technology and information systems to meet germplasm conservation goals. Acquisition technology has been enhanced recently by use of GIS and GPS devices when planning and conducting collecting trips. Since 2014, 67 accessions of wild *Lactuca* species have been added to the WRPIS collection, collected in Armenia, Kyrgyzstan, Uzbekistan, and the Republic of Georgia (Beharav et al., 2018; Hellier, 2014). Seed storage facilities are in place to allow relatively long-term storage of original and regenerated seed lots, by using sealed packets and a temperature of −18°C. Accessions are regenerated according to protocols that respect the biology and life cycle of each species (i.e., autogamy, allogamy, biennial, and perennial species) (Lebeda et al., 2019).

A substantial part of the WRPIS lettuce collection was tested for viability, and accessions with a back-up sample viability <80% have been prioritized for regeneration (Hellier, 2013, 2014, 2016). The GRIN-Global database provides only a basic descriptor list for the characterization and evaluation of lettuce accessions, following the format approved by the Leafy Vegetable Crop Germplasm Committee. This lettuce descriptor list has five categories (disease, morphology, phenology, subset, and other), and 14 characters complemented by pictures. Descriptor data are collected on accessions during the regeneration process including photo documentation (B. Hellier, personal communication, 2016). Currently, there is no descriptor list in the NPGS specifically for wild *Lactuca* species. The LGCS has been partially described and pertinent data computerized. The LGCS also collects information on reactions to *Lettuce mosaic virus*, downy mildew (*Bremia lactucae* Regel), corky root (*Rhizorhapis suberifaciens* et al.), and big vein (*Mirafiori lettuce big-vein virus*) (McGuire et al., 1993). The LGRCD has been evaluated with emphasis on resistance to downy mildew, anthracnose [*Microdochium panattonianum* (Berl.) Sutton, Galea & Price], corky root, Verticillium wilt (*Verticillium dahliae* Kleb.), and other diseases (Lebeda et al., 2014; McGuire et al., 1993). In addition, >450 wild *Lactuca* accessions collected in the United States and Canada between 2002 and 2014 (Lebeda et al., 2011, 2012) were characterized for 25 descriptors (Doležalová et al., 2002) in the greenhouse in the Czech Republic.

CONSERVATION GAP ANALYSIS FOR NORTH AMERICAN *LACTUCA*

This section provides a new analysis of the comprehensiveness of ex situ and in situ conservation systems for North American wild *Lactuca*. We gathered reference occurrence data for all records listed as *Lactuca* from the Global Biodiversity Information Facility (GBIF, 2017), the Global Crop Wild Relative Occurrence Database (Global Crop Diversity Trust, 2017), the Consortium of Midwest Herbaria database (Consortium of Midwest Herbaria, 2019), the Biodiversity Information Serving Our Nation (BISON) database (USGS, 2017), and from the authors' own botanical explorations. We compiled genebank conservation occurrence data from the Genesys plant genetic resources portal (Global Crop Diversity Trust, 2019), the USDA National Plant Germplasm System (GRIN-Global) (USDA-ARS NPGS, 2019a), the FAO World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture (WIEWS) (FAO, 2019), and the PlantSearch database (BGCI, 2019). Duplicates were removed, and taxonomic names were standardized against USDA GRIN-Global Taxonomy (USDA-ARS NPGS, 2019b). Cultivated taxa, records listed in sample-status fields as other than wild, weedy, or null (e.g., landrace, improved, breeding material, cultivated, etc.), and fossil specimens (GBIF dataset) were removed. In preparation for analysis, we classified each record by whether it would be used only as an input for distribution modeling (labeled "H," as most of these records are from herbaria), or whether it would also be considered a "site where collected" location of an existing plant genebank or botanic garden accession (labeled "G," as most records are from genebanks). For GBIF, this classification was performed by filtering the "Basis of Record" field, assigning "living specimen" records as G, with the other categories (observation, literature, preserved specimen, human observation, machine observation, material sample, and unknown) assigned as H. All records in GRIN-Global (all occurrences were listed as "active" status), Genesys, WIEWS, and PlantSearch were assigned G, and records from the Global Crop Wild Relative Database had already been categorized accordingly.

For records without coordinates but with narrative information on localities of occurrence, georeferencing was performed via GeoLocate (Tulane University, 2017). All coordinates in the database were then mapped and evaluated by the authors, correcting or removing occurrences occurring in bodies of water or in clearly incorrect locations. Records were further constrained to the study area (North America, defined here as Canada, Mexico, and the United States). The final occurrence dataset is available in Supplemental Table S1.

We used the maximum entropy algorithm (Phillips et al., 2006, 2017) to model potential species distributions, based on all reference (H) points with coordinates, as outlined in Lebeda et al. (2019). We compiled a total of 26 ecogeographic predictors (Supplemental Table S2). These included 19 bioclimatic variables derived from WorldClim 1 (Hijmans et al., 2005), and seven major edaphic drivers of species distributions with consistent data coverage throughout North America, obtained from International Soil Reference and Information Centre (ISRIC)-World Soil Information (Hengl et al., 2014). All ecogeographic predictors were processed at a spatial resolution of 2.5 arcminutes (~5 km^2 at the equator). Ecogeographic variables were subselected per taxon by using a nonlinear, iterative partial least squares algorithm to perform principal component analyses. Those variables with the greatest contributions (greater than 0.7 or less than −0.7) to the first two principal components were identified, and a variance inflation factor was then used to select only those variables with a low degree of collinearity (Khoury et al., 2015).

Modeling was performed at a resolution of 2.5 arcminutes, using 10,000 pseudo-absence background points within North America for model training, and clipped by measuring the shortest distance between the receiver operating characteristic curve (ROC curve) and the top left corner of the plot (Liu et al., 2005). The final model was chosen among three variations: the mean and median of model replicate probabilities ($k = 10$), and the sum of thresholded areas of all model replicates, with the final choice determined by the area under the curve (Khoury et al., 2015), true skill statistic (Allouche et al., 2006; Georgopoulou et al., 2016), sensibility, and specificity values. Distribution models were clipped to a range boundary of native states as given by USDA GRIN Taxonomy for Plants (USDA-ARS NPGS, 2019b). As a final step, models were adjusted to exclude highly urban areas, water bodies, and permanent snow and ice regions (ESA, 2005).

We assessed the degree of representation of each species in conservation systems, both ex situ and in situ, following methods outlined by Khoury et al. (2019b). In short, for ex situ, three scores were calculated. The sampling representativeness score (SRS) provides a general indication of the completeness of genebank and botanic garden collections for each taxon, comparing the total count of germplasm accessions (G) against the total count of reference (H) records, with an ideal ratio of 1:1. Unique among the conservation metrics, this score makes use of all compiled reference and germplasm records, regardless of whether they possess geographical coordinates. In this and all other metrics, SRS was bound between 0 and 100, with 0 representing an extremely poor state of conservation, and 100 representing comprehensive (complete) conservation.

The geographical representativeness score ex situ (GRSex) is a geographic measure of the proportion of the range of the species that can be considered to be conserved ex situ. Buffers (CA50) of 0.5° (~50-km radius) were created around each G occurrence to estimate geographic areas already collected within the potential distribution models. Comprehensive conservation under this metric was considered to have been accomplished when the buffered areas covered the entire distribution model.

The ecological representativeness score ex situ (ERSex) is an ecological measure of the proportion of the range of the species that can be considered to be conserved in ex situ repositories. The ERSex compares the ecoregional diversity encompassed in repositories to the diversity throughout the potential distribution model, considering comprehensive conservation to have been accomplished only when every ecoregion potentially inhabited by the species was included at least once within the set of CA50-buffered areas. The layer used for estimating ERSex contained 153 distinct terrestrial ecoregions in North America (Olson et al., 2001). A final conservation score ex situ (FCSex) was derived by calculating the mean of SRS, GRSex, and ERSex.

For the analysis of the state of in situ conservation, assessed only for the native species, two metrics were calculated based on the extent of representation of the range of each species within officially recognized protected areas. We used the World Database of Protected Areas (IUCN, 2019), selecting terrestrial and coastal reserves marked as designated, inscribed, or established. The geographical representativeness score in situ (GRSin) is a geographic measure of the proportion of the range of a species that can be considered to be conserved in protected areas. The calculation compares the area (in km^2) of the potential distribution model located within protected areas vs. the total area of the distribution model, considering comprehensive conservation to have been accomplished only when the entire distribution occurs within protected areas.

The ecological representativeness score in situ (ERSin) is an ecological measure of the proportion of the range of a species that can be considered to be conserved in protected areas. The ERSin compares the ecological variation encompassed within the range located inside protected areas with the ecological variation encompassed within the total area of the distribution model, considering comprehensive conservation to have been accomplished only when every ecoregion potentially inhabited by the species is included within that species' distribution located within a protected area. A final conservation score in situ (FCSin) was derived by calculating the mean of GRSin and ERSin.

A final combined conservation score (FCSc-mean) was calculated for each native species by averaging its FCSex and FCSin values, and for each non-native species by using the FCSex. Taxa were finally categorized based on their FCSc-means, with high priority for further conservation action for species where FCS-mean < 25; medium priority where $25 \leq$ FCS-mean < 50; low priority where $50 \leq$ FCS-mean < 75; and sufficiently conserved for taxa whose FCS-mean ±75. Modeling code implemented in our analysis is available at https://github.com/ccsosa/CWR_NorthAmerica, with conservation gap analysis code available at https://github.com/dcarver1/cwrSDM. Ecogeographic variables, ecoregions, and protected areas datasets are available through Khoury et al. (2019a).

Distributions of North American *Lactuca*

A total of 18,851 records for the 10 North American *Lactuca* species were compiled and processed, of which 18,584 with coordinates were used for distribution modeling (Table 2). The number of records per species ranged from 143 (140 for modeling) (*L. hirsuta*) to 7735 (7657 for modeling) (*L. serriola*). Potential distribution models were produced for all species. The potential distributions of the species as a whole occur from northern Canada to southern Mexico (Fig. 1). Predicted species richness was highest in the northeastern, central, gulf, and west coast areas of the United States, with up to seven species potentially overlapping in the same ~5-km^2 areas.

Conservation status of North American *Lactuca*

We compiled 267 genebank and botanic garden germplasm (G) records. One species *(L. graminifolia*) had no G records at all, whereas the maximum (78) was found for *L. serriola*. Unfortunately, the vast majority of these records had no coordinate information, nor sufficient locality data to create high-confidence points through georeferencing. Thus, species conservation determinations ranged from no representation at all in North America (*L. graminifolia*) to a low level of representation in genebanks and botanic gardens (*L. virosa,* with an FCSex of 20.33) (Fig. 2, Table 2). The mean FCSex across all taxa was 2.98 on the scale of 0 to 100. With regard solely to the state of ex situ conservation, all taxa were thus considered high priority for further collecting, and this collecting will be required across the ranges of the species to sample sufficient geographic and ecological variation.

With regard to in situ conservation in officially recognized protected areas, native taxa were determined to be moderately well conserved, with the lowest score for *L. canadensis* (40.65) and the highest for *L. hirsuta* (52.55) as an average across geographic and ecological coverage (mean score across the seven species of 47.20) (Fig. 2, Table 2). Sufficiency with regard to ecoregions covered in protected areas was higher than geographic coverage, with a low of 77.78 (*L. floridana*) and a high of 100 (all ecoregions represented) (*L. hirsuta*). In total, with regard solely to the state of in situ conservation, four species (*L. canadensis*, *L. floridana*, *L. ludoviciana*, and *L. tatarica*) could be considered medium priority for further in situ conservation, and three (*L. biennis*, *L. graminifolia*, and *L. hirsuta*) could be considered low priority.

With regard to combined conservation status (both ex situ and in situ) of the native taxa, all scores were within the range between 21.69 (*L. canadensis*) to 26.63 (*L. hirsuta*), on the cusp between high and medium priority for further conservation action (Fig. 2, Table 2). The non-native species, assessed only with regard to ex situ conservation, were determined to be of high priority for further collecting.

Ecogeographic Variation in North American *Lactuca* and Its Representation Ex Situ

Ecogeographic predictor information, at a resolution of 30 arc-seconds (~1 km^2 at the equator) for 23 pertinent variables from WorldClim 2 (Fick and Hijmans, 2017) and CGIAR-Consortium for Spatial Information NASA Shuttle Radar Topography Mission datasets (Jarvis et al., 2008), were extracted for all occurrence data with coordinates, for all species (Supplemental Table S3). These data were used to characterize species with regard to their ecogeographic niches for each variable. We also assessed the representation of these niches in ex situ conservation by comparing the distributions of G points for each taxon within the full spread of its occurrences.

Substantial variation was found across species, although most species occupied fairly broad niches, especially *L. serriola*, *L. canadensis*, and *L. tatarica*, among others, for temperature variables, and *L. biennis*, *L. serriola*, and *L. graminifolia*, among others, for precipitation variables (Fig. 3, Supplemental Fig. S1). Perhaps surprisingly, the native species displayed equivalently large breadths of ecogeographic niches for most variables as those shown by the non-native taxa. The analysis of ex situ representation of ecogeographic variation in these populations demonstrated that ex situ collections currently very poorly represent this ecogeographic variation (Supplemental Fig. S2).

PHENOTYPIC AND PHENOLOGICAL VARIABILITY IN NORTH AMERICAN *LACTUCA*

Basic descriptions of the wild *Lactuca* species in North America, both native and non-native, together with their general distributions, are presented in the floras of Strother (2006) and McGregor et al. (1986). The extent of phenotypic and phenological variation differs in each plant species (Richards et al., 2006; Sultan, 2000). They are influenced by reproduction system, ecological requirements and species distribution in various habitats, population density, and species sympatry, as well as by the history of introduction for non-native taxa (Alexander and Levine, 2019).

However, this group lacks detailed data on infraspecific phenotypic and phenological variation. To our knowledge, the first more broadly aimed studies and field

Table 2. Conservation gap analysis results per species. Results of the conservation assessments within each strategy (sampling representativeness score [SRS], geographic representativeness score ex situ [GRSex], and ecological representativeness score ex situ [ERSex] for ex situ; and geographic representativeness score in situ [GRSin] and ecological representativeness score in situ [ERSin] for in situ) are displayed. The combined final conservation score (FCS-mean) is the average of the final ex situ (FCSex) and in situ (FCSin) scores. The FCS-mean is used to categorize taxa for further conservation action: high priority (HP), medium priority (MP), low priority (LP), or sufficiently conserved (SC).

Lactuca species	Total Records	Total conservation (G) records	Total conservation (G) records with coordinates	Total reference records (H) with coordinates	SRS	GRS ex	ERS ex	FCS ex	GRS in	ERS in	FCS in	FCSc_ min	FCSc_ max	FCSc_ mean	Priority category
Native															
L. biennis	1505	16	0	1489	1.07	0	0	0.36	10.94	90.77	50.85	0.36	50.85	25.61	MP
L. canadensis	2877	24	2	2853	0.84	0.57	6.78	2.73	5.03	76.27	40.65	2.73	40.65	21.69	HP
L. floridana	1545	15	4	1530	0.98	0.96	7.41	3.11	4.83	77.78	41.31	3.11	41.31	22.21	HP
L. graminifolia	724	0	0	724	0	0	0	0	11.18	91.3	51.24	0	51.24	25.62	MP
L. hirsuta	143	3	0	140	2.14	0	0	0.71	5.09	100	52.55	0.71	52.55	26.63	MP
L. ludoviciana	999	4	1	995	0.4	0.32	1.59	0.77	3.66	90.48	47.07	0.77	47.07	23.92	HP
L. tatarica	1905	3	0	1902	0.16	0	0	0.05	8.36	85.07	46.72	0.05	46.72	23.39	HP
Non-native															
L. saligna	1220	49	0	1171	4.18	0	0	1.39	NA†	NA	NA	1.39	1.39	1.39	HP
L. serriola	7735	78	0	7657	1.02	0	0	0.34	NA	NA	NA	0.34	0.34	0.34	HP
L. virosa	198	75	1	123	60.98	0	0	20.33	NA	NA	NA	20.33	20.33	20.33	HP

† NA, not applicable.

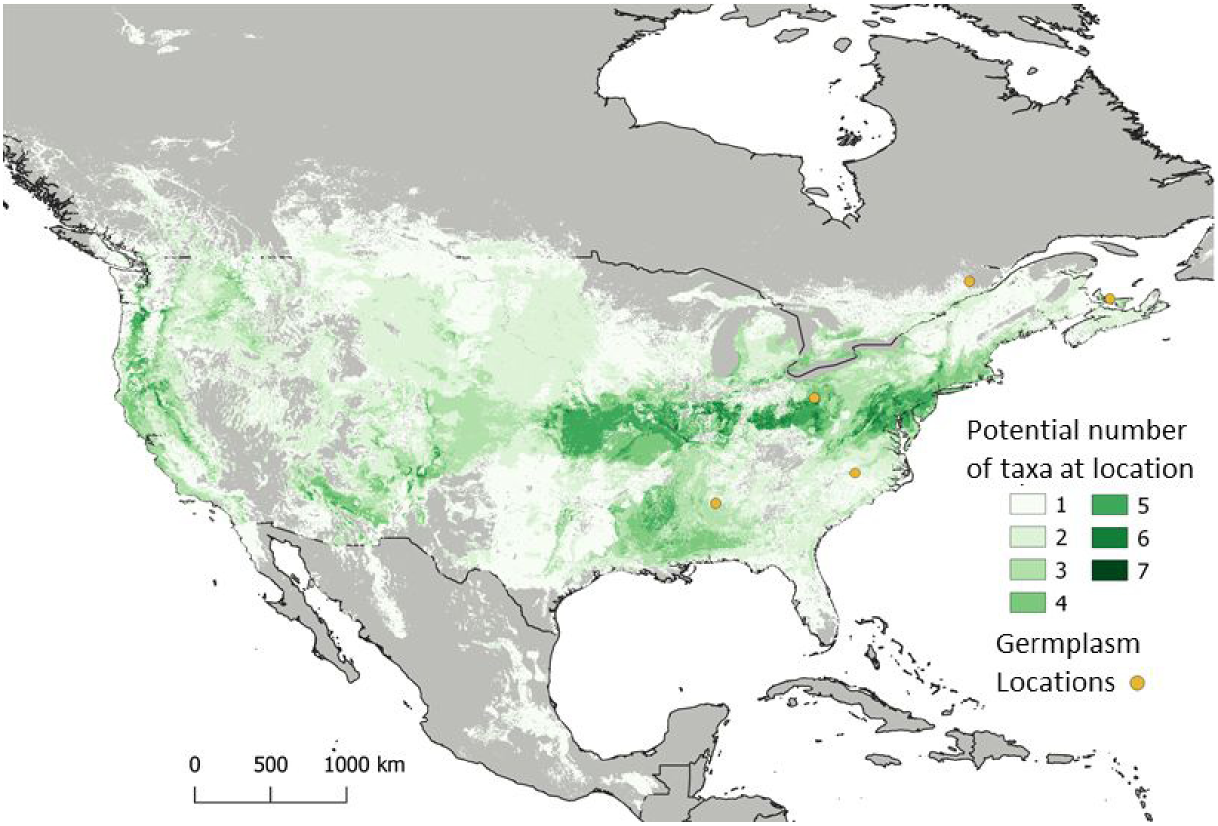

Fig. 1. Predicted species richness map for assessed lettuce wild relative potential distribution models in North America. Darker colors indicate greater numbers of species potentially occurring in the same geographic areas.

observations of wild *Lactuca* species in North America were conducted by Lebeda and coworkers only since 2000 (Lebeda et al., 2012, 2019). During these missions, wild *Lactuca* species were observed at ∼250 locations, resulting in the acquisition of 350 seed samples representing nine species. Not all species listed by Strother (2006) and McGregor et al. (1986) were found in natural habitats during the course of these studies; the spectrum of species observed included the native taxa *L. biennis*, *L. floridana*, *L. ludoviciana*, and *L. canadensis*, along with the Old World weeds *L. saligna*, *L serriola*, and *L. virosa*.

Infraspecific variation in phenology and morphological traits of wild *Lactuca* species was recorded during field trips to natural habitats across North America (Lebeda et al., 2012, 2019), and these phenomena were further studied in detail under greenhouse conditions, following the descriptor list of Doležalová et al. (2002) to describe various morphological traits on rosette and stem leaves, stems, flowers and inflorescences, fruits, and patterns of plant development.

With two exceptions, the morphological traits of these samples corresponded to their descriptions by Strother (2006) and McGregor et al. (1986). The phenotype of the plants from one sample, collected originally as *L. serriola* f. *integrifolia* in California in 2004, suggested a possible relationship to cultivated *L. sativa* (Fig. 4). The possible origin of these plants (i.e., natural mutation or spontaneous interspecific hybridization) was discussed by Lebeda et al. (2019).

To date, the limited number of samples of *L. biennis*, *L. floridana*, *L. ludoviciana*, *L. saligna*, and *L. virosa* that have been evaluated do not allow for an adequate assessment of the extent of infraspecific variation. Within a set of 44 samples of *L. canadensis* collected in Iowa and North Carolina (Lebeda et al., 2012), variation in the shape of the rosette and stem leaves, the distribution of leaf anthocyanins, and the inflorescence form were recorded. Significant variation in the color of floral ligules (bright yellow vs. pink with yellow base), of stigmata, and the distribution of anthocyanin pigmentation in involucral bracts was observed (Lebeda et al., 2019). Plants with potential hybrid phenotypes involving *L. canadensis* and *L. ludoviciana* or *L. biennis*, respectively, were observed in Iowa during field trips in 2008. The segregation of some morphological traits on plants was observed in one of these samples during greenhouse regeneration (Lebeda et al., 2019). Molecular analyses should clarify the hybrid origin of these plants and the possible involvement of *L. biennis*, which can hybridize with *L. canadensis* (Fernald, 1950). Examples of floral ligules and the composition of heads of *L. biennis*, *L. ludoviciana*, and *L. canadensis* are presented in Fig. 5.

Lactuca serriola (prickly lettuce) is now the most frequently occurring *Lactuca* species in North America,

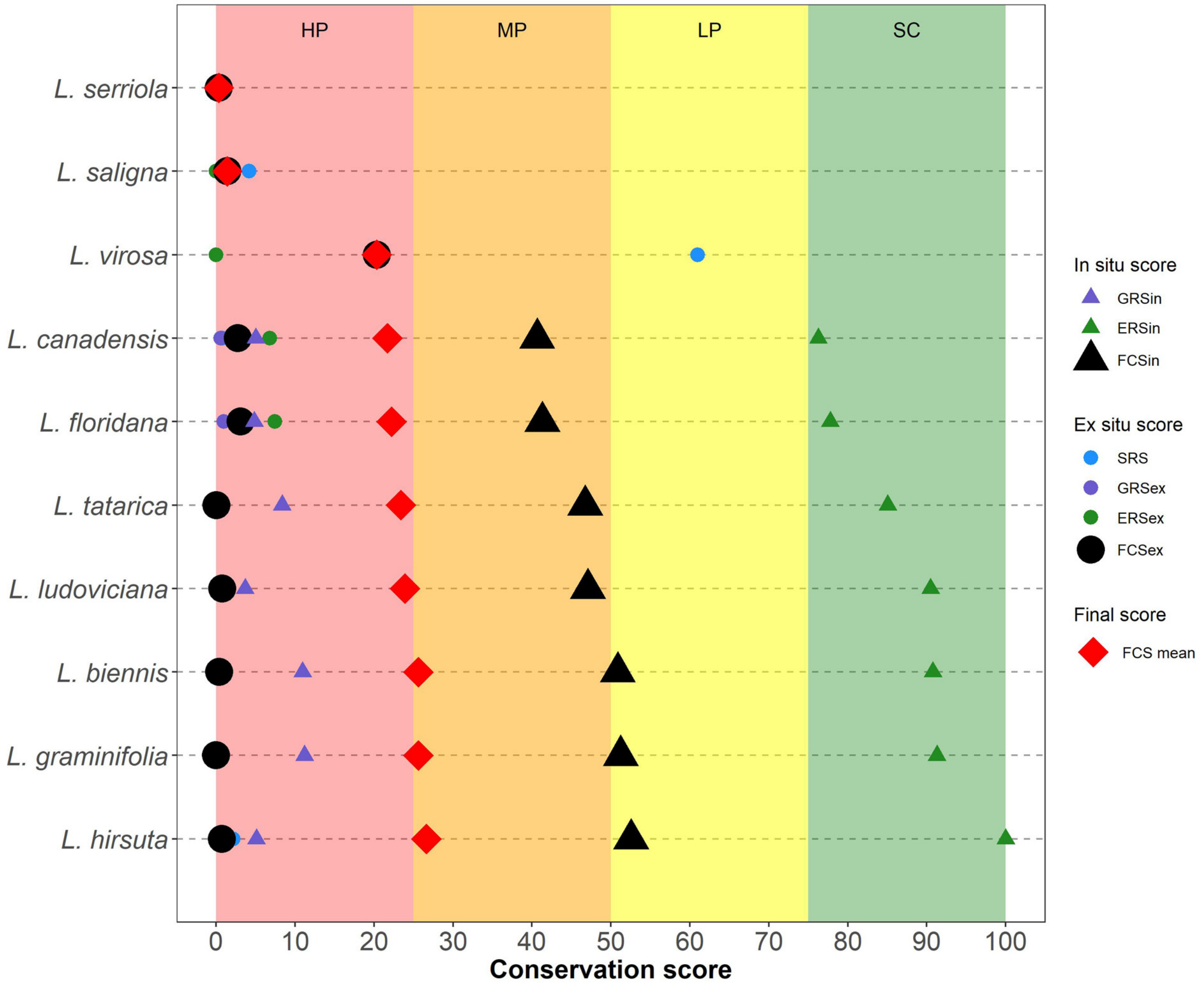

Fig. 2. Conservation gap analysis results per species. Wild relatives are listed by descending priority for further conservation action by priority categories (high priority [HP, red], medium priority [MP, orange], low priority [LP, yellow], and sufficiently conserved [SC, green]). The red diamond represents the combined final conservation score (FCS-mean) for the taxon, which is the average of the final ex situ (FCSex) (black circle) and in situ (FCSin) (black triangle) scores. Results of the conservation assessments within each strategy (sampling representativeness score [SRS], geographic representativeness score ex situ [GRSex], and ecological representativeness score ex situ [ERSex] for ex situ and geographic representativeness score in situ [GRSin] and ecological representativeness score in situ [ERSin] for in situ) are also displayed. Non-native species were not assessed with regard to in situ conservation.

within the last two centuries having spread to various and extreme habitats. It expressed considerable phenotypic variation in both human-disturbed and natural habitats (Lebeda et al., 2012), as well as during greenhouse cultivation (Lebeda et al., 2019).

For example, an assessment of morphological traits and phenological characters of prickly lettuce from Canada (44 samples), the midwestern United States (22 samples), and the western United States (165 samples) under greenhouse conditions at Palacký University (Olomouc, Czech Republic) revealed broad variation in expression of multiple traits and developmental stages. The specific expression of morphological traits (absence or presence of leaf rosette, shape of rosette and stem leaves, shape of leaf apices, distribution and quality of trichomes on leaves and stems, inflorescence type, and achene size and shape) were clearly associated with the ecological and climatic conditions of the samples' origins. The more arid or otherwise more extreme climatic conditions in the western United States and partly also in Canada have led to the development of plants with deeply divided rosette and stem leaves (Lebeda et al., 2019). Because leaf lobing reduces the distance across the lamina, the rate of heat transfer is predicted to be greater in a lobed leaf than in an unlobed leaf with equivalent area (Parkhurst et al., 1968). Deeply lobed leaves may also reflect direct selection for increased hydraulic efficiency. However, leaf shape is only one of many factors influencing leaf thermal regulation. Other factors may include water content, leaf thickness, spectral reflectance, orientation, and plant architecture (Nicotra et al., 2010, 2011), and these factors warrant further focus. Dense pubescence on both sides of stem leaves and on the upper parts of inflorescence branches on plants from one location in California (*L. serriola* var. *coriacea* Schulz-Bip.) appears to be genetically fixed, persisting during cultivation under adequate moisture conditions in the greenhouse

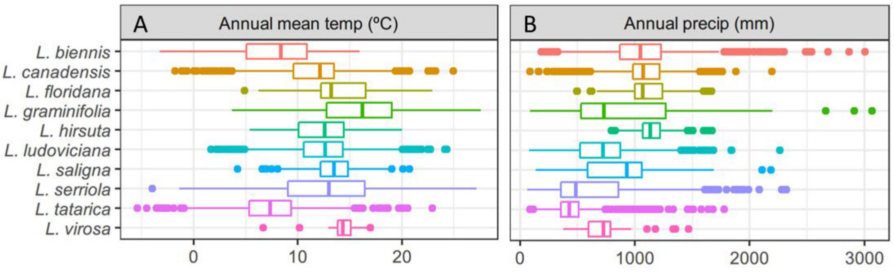

Fig. 3. Ecogeographic niche box plots of lettuce wild relative populations for annual mean temperature (temp) and annual precipitation (precip). For niches per all ecogeographic variable per species, see Supplemental Fig. S1 and S2.

(Lebeda et al., 2019). This trait may also have evolved as an adaptation to dry, hot climatic conditions.

Broad variation in developmental stages (the initiation of bolting, flowering, and fruit maturity) reflects the ecological adaptations of plants to their local climatic conditions. Data collected in the greenhouse from 231 samples of *L. serriola* from Canada, the midwestern United States, and the western United States showed that, although nearly 20% of samples *L. serriola* from the Midwest and 20% of samples from Canada entered the bolting stage at 103 d after sowing, half the samples *L. serriola* from the western United States reached the stage of fruit maturity just 1 wk later (at 110 d) (Lebeda et al., 2019). Similarly, half the samples *L. serriola* from the west were early in fruit set, whereas the samples from the Midwest were evenly divided between medium and late in their fruit set (Fig. 6). Hot, dry climates in the west, typically with winter rains and summer drought (Peel et al., 2007), select for strategies that force some plants to survive summer stresses by accelerating seed maturity via early bolting, flowering, and maturation. Milder midwestern climates (Peel et al., 2007) allow for delays in all three developmental stages. These features appear to be fixed genetically, as they were also consistently expressed under optimal greenhouse conditions (Lebeda et al., 2019). Observed genetically fixed traits in limited populations of *L. serriola* and *L. virosa* surviving under extreme conditions (sulfur evaporation, dry or almost desert sites) represent potential sources of tolerance to abiotic stresses in lettuce breeding.

GENETIC VARIATION IN NORTH AMERICAN *LACTUCA*

Species of *Lactuca* are predominantly considered to be autogamous (Lebeda et al., 2007; Davey and Anthony, 2011), which is typical for early-successional taxa that generally allocate more variation among populations than within them (Nybom et al., 2014). Selfing may be less important in the North American species than in the Old World weedy ones; however, this remains to be studied.

Most of the genetic studies involving North American *Lactuca* populations have focused on the genetic diversity of *L. sativa* cultivars, where accessions of *L. serriola*, *L. saligna*, and *L. virosa* collected in North America were used

Fig. 4. Plants originally collected as *Lactuca serriola* f. *integrifolia* (Sample no. 87/04) and regenerated in the greenhouse: (a, b) detail of stem leaves at time of bolting, and (c) plants at the beginning of flowering.

Fig. 5. Flowers (heads) of (a) *Lactuca biennis* (Sample no. 65/04), (b) *L. ludoviciana* (Sample no. 63/08), and *L. canadensis* ([c] Sample no. 58/08 and [d] Sample no. 60/08).

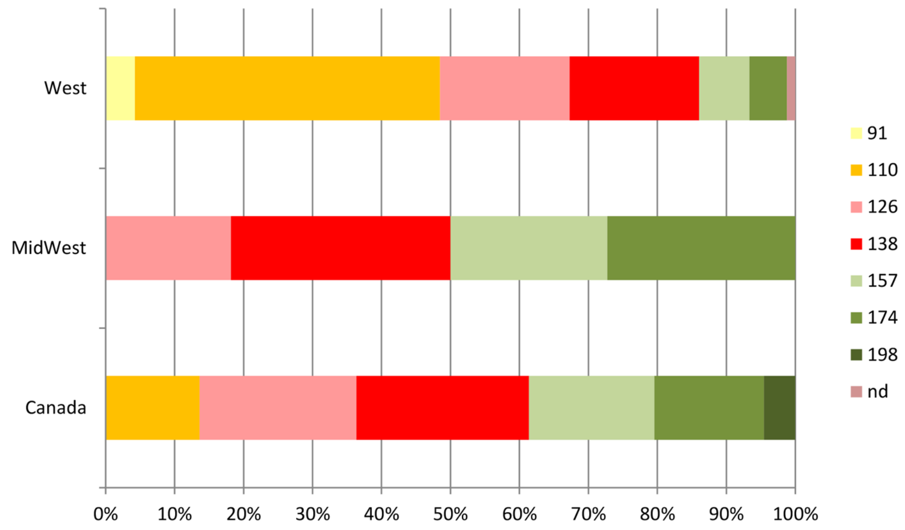

Fig. 6. Beginning of seed maturity in days after sowing (DAS) of 231 samples *Lactuca serriola* L. from the western United States (165 samples), midwestern United States (22 samples), and Canada (44 samples) cultivated in the greenhouse. Yellow tints = early seed maturity; red tints = medium maturity; green tints = late maturity. Frequency of occurrence (%) of each category (DAS) of beginning of seed maturity is expressed for each geographical area.

as reference or outgroup samples (Simko, 2009; Simko and Hu, 2008; see below), as well as in taxonomic studies (Table 3).

However, there are three studies (Table 3) assessing genetic variation in natural populations of *L. serriola* from North America, where larger numbers of individual plants (13–32) per population were analyzed (Alexander et al., 2009; Alexander, 2013; Kuang et al., 2008). In studies by Alexander et al. (2009) and Alexander (2013), the authors used seven simple sequence repeat (SSR) markers designed for *L. sativa* by van de Wiel et al. (1999). Alexander et al. (2009) compared six native populations of *L. serriola* sampled from Valais, Switzerland, with six populations sampled from part of its introduced range in the Wallowa Mountains of Oregon, USA. In the introduced area, populations of *L. serriola* were significantly more variable, with weaker population genetic structure, than was observed within its native distribution.

Table 3. Molecular marker studies analyzing native and non-native *Lactuca* species collected in the USA.

***Lactuca* species**	**Phylogenetic/ phylogeographic study**	**Population-genetic study**	**Molecular marker development**	**Other**
Native species				
Lactuca biennis	Kilian et al., 2017; Jones et al., 2018; Dias et al., 2018			
Lactuca canadensis	Kilian et al., 2017; Jones et al., 2018; Dias et al., 2018			
Lactuca floridana	Kilian et al., 2017; Jones et al., 2018; Dias et al., 2018			
Lactuca graminifolia	Kilian et al., 2017; Jones et al., 2018; Dias et al., 2018			
Lactuca hirsuta	Kilian et al., 2017; Jones et al., 2018; Dias et al., 2018			
Lactuca ludoviciana	Kilian et al., 2017; Jones et al., 2018; Dias et al., 2018			
Lactuca tatarica subsp. *pulchella* (*L. oblongifolia*)	Jones et al., 2018			
Non-native species				
Lactuca saligna			Simko, 2009; Rauscher and Simko, 2013	Simko and Hu, 2008
Lactuca sativa			Simko, 2009; Rauscher and Simko, 2013	Simko and Hu, 2008
Lactuca serriola		Alexander et al., 2009; Alexander, 2013; Kuang et al., 2008; Lebeda et al., 2011; Riar et al., 2011	Simko, 2009; Rauscher and Simko, 2013	Simko and Hu, 2008
Lactuca virosa		Beharav et al., 2018	Simko, 2009; Rauscher and Simko, 2013	

The SSR data generated by Alexander et al. (2009) were used in a subsequent study (Alexander, 2013) for detailed analyses of 75 *L. serriola* populations (730 plants) from native and non-native regions where the species has been introduced (North America, South America, South Africa, and Australia). The resulting genetic data were combined with observations from a common-garden experiment and climatic-niche modeling to study the rapid evolution of phenology associated with climate across native and introduced ranges.

Four *L. serriola* populations from California were analyzed by amplified fragment length polymorphism (AFLP) and microsatellite markers and compared with populations from Europe, Armenia, Israel, and Turkey in a study published by Kuang et al. (2008). The authors found that Turkish populations were more related to the populations from California than to those from Israel. The molecular data suggest that the California populations, recent colonizers, might be derived from the Turkish or surrounding populations. These populations were probably introduced to California by settlers. Two additional studies (Table 3) analyzed genetic variation in *L. serriola* pseudopopulations composed of individual plants collected over large geographical areas. Riar et al. (2011) used a set of 45 newly designed SSR markers to study genetic diversity among a collection of 22 *L. serriola* biotypes collected in Washington State, whereas Lebeda et al. (2011) analyzed a set of 92 *L. serriola* accessions covering a broad transect of territory in North America between the southwest (California, USA) and northeast (Quebec, Canada) with AFLP markers.

No detailed studies evaluating the genetic diversity in natural populations of other non-native *Lactuca* (*L. saligna* and *L. virosa*) or any of the native taxa have been published to date (Table 3) (Lebeda et al., 2019). Only in one recent paper were *L. virosa* samples collected in the United States (Lebeda et al. 2012) used for comparative studies (with *L. georgica* Grossh.; Beharav et al., 2018).

DISEASES AND PESTS IN NORTH AMERICAN *LACTUCA*

Interactions between many uncultivated plants and their pathogens and pests remain poorly understood (Burdon and Laine, 2019; Burdon and Thrall, 2008). This is true for interactions between the pathosystems of cultivated lettuce and its sympatric wild and weedy relatives (Lebeda et al., 2008, 2014). Variation in host-plant resistance is frequently mirrored in the diversity of pathogens and pests. The genetic diversity of pathogen populations is mostly generated by the processes of spontaneous mutation, sexual recombination, and somatic hybridization (Milgroom, 2017). Variation in pathogen–population structure can also occur through migration or a range of cytological and molecular changes (Burdon, 1993). These processes are dynamic in both time and space and can create highly unstable systems, which are often reflected in the short duration of durable resistance in cultivated crops (McDonald and Linde, 2002).

Large plant populations are important in assessing disease resistance and the identification of germplasm most relevant for incorporation into breeding programs (Davey and Anthony, 2011). Wild relatives may serve as suitable sources of resistance against biotic (pathogens and pests) and abiotic (edaphic and climatic factors) stressors (Burdon and Jarosz, 1989). Knowledge of the genetic diversity (including diversity in resistance against biotic and abiotic factors) of wild *Lactuca* species is crucial for future progress in lettuce improvement (Lebeda et al., 2007, 2009, 2014, 2019). The breeding of lettuce resistant to biotic and abiotic factors is currently most often achieved by combining desirable resistance alleles from sexually compatible wild *Lactuca* species (mostly members of the primary and secondary genepools) through introgressive hybridization (Lebeda et al., 2007, 2014, 2019; Simko, 2013; Giesbers et al., 2018). Wild *Lactuca* species as donors (sources) of important resistance genes and existing gaps in this area have been described in detail by Lebeda et al. (2007, 2009, 2014) and Simko (2013).

Many pathogens, pests, and various physiological disorders have been reported to damage lettuce (Capinera, 2001; Lot et al., 2003; Subbarao et al., 2017). In contrast, knowledge of diseases, pests, and disorders on naturally growing wild *Lactuca* taxa is very limited (Lebeda et al., 2008, 2014; Lebeda and Mieslerová, 2011), especially as related to taxa occurring in North America (Lebeda et al., 2012, 2019). Some lettuce diseases and pests have global distributions and significant economic impacts; others are damaging only on a local scale (Barrière et al., 2014). Economically important diseases and insect pests affecting yield of lettuce in North America were summarized by Simko et al. (2014b) and Lebeda et al. (2019). A relatively broad survey of wild *Lactuca* across North America (Lebeda et al., 2012) reported only a limited number of records for diseases and pests, with only occasional occurrences of downy mildew and powdery mildew [*Golovinomyces cichoracearum* (DC) Heluta, recently *G. bolayi* sp. nov.] (Braun et al., 2019). Most of these plant disease findings were made in Canada (Lebeda et al., 2012). In part because of a paucity of pertinent germplasm in genebank collections, knowledge about resistance variation in weedy and wild North American *Lactuca* against pathogens and pests is lacking (Lebeda et al., 2019). This information gap also applies to pathogenicity variation of the most important pathogens in natural *Lactuca* populations.

In summary, only a very limited amount of potential diversity in North American *Lactuca* has been described or used (Lebeda et al., 2011, 2012, 2019). Nevertheless, recent preliminary studies have shown that *L. serriola* germplasm from North America displays substantial phenotypic variation (Lebeda et al., 2019) and also broad variation in pathogen (e.g., *B. lactucae*) resistance (Lebeda, unpublished data, 2019). Examination of the current state of *Dm* genes confirmed the existence of 51 resistance genes and factors and 15 quantitative trait loci in lettuce and its wild relatives to *B. lactucae* (Parra et al., 2016). However, Parra et al. (2016) did not identify a single gene derived from wild *Lactuca* originating in North America, suggesting that there is untapped potential for the use of genetic variation in North American populations (Lebeda et al., 2019). This was confirmed by studies of Kuang et al. (2006, 2008), who determined the frequency and variation of disease resistance alleles in natural plant populations of *L. serriola*, specifically of *Dm3* in 1033 samples representing 49 populations, including 326 samples from eight California populations. We thus suspect that North American *L. serriola* germplasm could be effectively exploited in lettuce breeding for resistance to *B. lactucae* because of the existence of diverse types of resistance with many new race-specific alleles (Parra et al., 2016).

DISCUSSION OF GAPS AND CHALLENGES FOR NORTH AMERICAN *LACTUCA*

Phylogeny and Taxonomy

Although evolutionary relationships within the Lactucinae are becoming clearer, there are still many questions to be resolved. Among them, one of the most challenging is how to interpret recent findings into the creation of a workable and sustainable taxonomy for the *Lactuca* alliance. Fortunately, all the North American lettuce taxa, whether native or not, belong to the main *Lactuca* lineage (sensu Kilian et al. 2017) with well-resolved relationships and clearly fit within the genus *Lactuca*.

Regarding the allotetraploid taxa, another challenge is emerging, which is to verify their allopolyploid origin and putative parentage through appropriate methods. One of the most suitable approaches would be based on in situ visualization of different genomes via genomic in situ hybridization (GISH) or fluorescent in situ hybridization (FISH). Because the proposed ancestors of the *L. canadensis* clade seem to be well diverged, the successful application of GISH could be of high value, as it may show chromosomal changes/ and rearrangements within a defined timeframe after the two different genomes combined.

Herbaria

The primary gap relates to the degree of completeness of online herbarium records, which is gradually improving. More records need to be digitized, and the digitized information then needs to be made openly accessible. In addition, many online records do not yet include specimen images or detailed information useful for georeferencing, which would increase the ease of verifying identity and the capture of useful morphological, phenological, ecological, and geographic data (Nelson and Ellis, 2018).

Botanic Gardens and Genebank Collections

Currently, botanical gardens are only minor contributors to the ex situ conservation of North American *Lactuca*, but there is growing interest in the public garden community in wild relatives, as evidenced by the recent symposium organized by the American Public Gardens Association, "Celebrating Crop Diversity: Connecting Agriculture, Public Gardens, and Science," which may catalyze future efforts (APGA, 2019b).

In genebanks, numerous further steps can be identified. Revisions of passport data are needed, with emphases on the taxonomic determination of accessions of unknown identity and with regard to the place of origin. Genebanks will need to prioritize regeneration to make more high-quality samples available for distribution and research. Descriptor lists for wild *Lactuca* accessions held in North American genebanks should be developed, possibly based on existing descriptors developed in Europe (Doležalová et al., 2002). Wild *Lactuca* genetic resources should be characterized and evaluated for traits of importance for their management and their efficient utilization in research and lettuce breeding. Evaluations of disease and insect resistance, bolting activity, and reaction to environmental stresses are of paramount importance. Efforts should be made to acquire additional wild and weedy North American *Lactuca* and other *Lactuca* taxa from the secondary and tertiary genepools.

Ex Situ and In Situ Conservation of North American *Lactuca*

With all of the CWR of lettuce in North America assessed as high priority for further collecting for ex situ conservation and 57% of the native species as medium priority for further protection in situ, it is clear that further conservation action is needed to safeguard these potentially valuable genetic resources. Based on the available information, ex situ conservation of these species is currently quite insufficient. This said, if coordinate information on existing germplasm accessions were to be further generated and made available, it is possible that the ex situ conservation status for at least three of the native taxa (*L. biennis*, *L. canadensis*, and *L. floridana*), and all of the non-native species, could be revised in a positive direction.

Although in situ conservation of native lettuce wild relatives was determined to be substantially more advanced than the degree of protection in genebanks and botanic gardens, it is important to note that the protected areas listed in the WDPA afford only collateral protection to these species because of their overall land conservation practices. Long-term protection of native lettuces in these areas would require the formation of active species- and population-specific management plans.

Phenotypic and Phenological Variability

Morphological and phenological variation is currently poorly understood for both the native and non-native species. Patterns of variation for morphological traits and phenological characteristics within populations are not adequately treated and explained. Morphological studies are primarily aimed at the aboveground parts of the plant. Studies of the underground system (i.e., root development, architecture, and function under various conditions [including water stress, high salinity, etc.] deserve attention). Anatomical studies of trichomes, stomata, and leaves (including their waxes) have been neglected; recent technologies allow relatively fast and accurate analyses. Plant physiology and physiological modeling are essential to understand the mechanisms of plant response to various environmental factors expressed not only in morphological plant phenotypes.

Genetic Variation

Levels of genetic variation and population genetic structure in natural populations of both native and non-native North American *Lactuca* species have not been assessed. Information on the history and area(s) from which the North America has been colonized by non-native, weedy species is limited. There is a need for germplasm accessions that could be considered as representatives of individual populations (i.e., plants sampled at a minimal spacing of 2 m, with a minimum of five plants representing a population) and scattered uniformly across the species' North American ranges. The overall genetic variation in natural populations is expected (and partly verified) to be rather low due to prevailing selfing, so the arsenal of available molecular markers is limited, making the selection of appropriate marker technologies challenging. There is no population genetic study of *Lactuca* based on Sanger sequencing of partial genomic loci (e.g., ITS, trnL-trnF), most likely due to the degree of variation needed to address broader phylogenetic questions (Koopman et al., 1998; Wang et al., 2013; Kilian et al., 2017), rather than fine interpopulation differences. The use of codominant microsatellite markers seems to be an appropriate tool, especially when genotype profiles of individual plants are published, so one can directly compare results of multiple studies. However, one of the main drawbacks of SSR markers is their availability (i.e., details on nucleotide sequences of primers). This complicates their use in practice, and a panel of the most polymorphic SSR markers with primer sequences freely available to the community would be of great potential value. Amplified fragment length polymorphism markers are frequently used in cases when low level of variation is expected. Nevertheless, AFLPs suffer from their dominant character, and the inability to compare AFLP profiles generated by different studies. Future genetic and phylogeographic studies should take advantage of high-throughput markers based on next-generation technologies, as their availability and cost are becoming increasingly reasonable.

Diseases and Pests

Currently, much of the basic plant pathology and entomology information on wild *Lactuca* species in North America is lacking. Only fragmented data on distributions of some plant pathogens and pests on weedy growing *Lactuca* spp., both native and non-native, are available (Lebeda et al., 2012). Completely missing is information about resistance, as well as the genetics of resistance of all these species against biotic and abiotic stresses and their historical and current use in lettuce breeding. Until now, only a few accessions of wild *Lactuca* species (*L. serriola*, *L. virosa*, and *L. saligna*), originating mostly from Europe and Asia, have been used in lettuce resistance breeding in the United States. Further sampling, multiplication, and characterization of wild *Lactuca* accessions, especially the native species, will be required for future developments and practical applications in lettuce resistance breeding (Lebeda et al., 2014, 2019).

Conflict of Interest

The authors declare no conflict of interest.

Supplemental Material

Supplemental material is included with this manuscript. This includes ecogeographic characterization of the species (Supplemental Fig. S1), including reference to germplasm coverage (Supplemental Fig. S2); the full occurrence dataset (Supplemental Table S1); and a list of ecogeographic variables used in the potential distribution modeling (Supplemental Table S2) and ecogeographic characterization (Supplemental Table S3).

Author Contributions

A. Lebeda, E, Křístková, M. Kitner, Ľ. Majeský, I. Doležalová, C.K. Khoury, M.P. Widrlechner, J. Hu, D. Carver, H.A. Achicanoy, and C.C. Sosa compiled information and performed analyses. A. Lebeda, C.K. Khoury, M.P. Widrlechner, and J. Hu wrote the manuscript. A. Lebeda, M. Kitner, Ľ. Majeský, I. Doležalová C.K. Khoury, and M.P. Widrlechner revised the manuscript.

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