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ORGANIZACIÓN GENÉTICA DEL COMPLEJO DE SUBESPECIES *Prunus serotina* EHRH. EN NORTEAMÉRICA

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Tesis realizada por **FÉLIX ALBERTO GUZMÁN DÍAZ** bajo la supervisión del Comité Asesor indicado, aprobada por el mismo y aceptada como requisito parcial para obtener el grado de:

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DEDICATORIA

En memoria de mi padre

A mi madre

A la Morocha

Porque con su amor le dan vida a mi vida

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DATOS BIOGRÁFICOS

Félix Alberto Guzmán Díaz es un biólogo colombiano, nacido en El Cerrito (Valle del Cauca), que en 2000 obtuvo su título de pregrado en la Universidad del Valle (Cali, Colombia). En esta misma institución obtuvo el título de Magíster en Ciencias Biológicas (2007) con orientación en la Conservación de Recursos Fitogenéticos. En enero de 2014 inició estudios doctorales en el Instituto de Horticultura (Departamento de Fitotecnia) de la Universidad Autónoma Chapingo, los cuales terminó en diciembre de 2017. Durante su proceso de formación académica ha pertenecido a grupos de investigación de Colombia, Estados Unidos y México, que se dedican a la promoción de la conservación y el uso sostenible de los recursos fitogenéticos, por medio del desarrollo y aplicación de herramientas moleculares. En ejercicio de su profesión, ha integrado grupos multidisciplinarios y multiculturales cuya misión es mejorar el entendimiento de eventos evolutivos relevantes en la agricultura internacional, particularmente en Latinoamérica, en Colombia (*Bioversity International*; Centro Internacional de Agricultura Tropical – CIAT; Centro Nacional de Investigaciones de Café – Cenicafé) y los Estados Unidos (*Cornell University*; *The University of Utah; University of California* – Davis).

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RESUMEN GENERAL

ORGANIZACIÓN GENÉTICA DEL COMPLEJO DE SUBESPECIES *Prunus serotina* EHRH. EN NORTEAMÉRICA

El capulín (*Prunus serotina* Ehrh.) es un árbol frutal multipropósito nativo de Norteamérica, casi todas sus partes tienen algún uso. Esta especie conforma un complejo de cinco subespecies con diferencias morfológicas y hábitats distintivos. Sin embargo, varios aspectos biológicos de la especie son tema de debate o permanecen poco estudiados, como por ejemplo, la identidad taxonómica de sus cinco subespecies y la variabilidad molecular intraespecífica. En este estudio se analizaron combinadamente tres tipos de información (climática, morfológica y molecular) para describir la variación intraespecífica del capulín, y evaluar implícitamente las opciones de conservación y uso sustentable de sus recursos genéticos. La altitud y siete parámetros bioclimáticos desempeñaron un papel no despreciable en la diferenciación de las cinco subespecies. La variabilidad climática indicó que las subespecies *eximia*, *hirsuta* y *serotina* prosperan en ambientes más húmedos y fríos, mientras que ssp. *virens* lo hace en ambientes más secos y cálidos. La subespecie *capuli* presentó la mayor heterogeneidad ambiental. Las diferencias morfológicas incluyeron hojas más largas y más anchas, y pedicelo de fruta más grueso en la ssp. *capuli*; y hojas más cortas y más pequeñas en ssp. *virens*. Aunque la variación morfológica asociada con el clima fue relativamente baja, el estudio mostró posibles efectos de la variabilidad climática sobre la morfología y distribución del complejo, y proporcionó información de referencia útil para una descripción integral de sus recursos genéticos. Se usaron 16 marcadores microsatélite para evaluar la estructura genética de 18 poblaciones naturales, pertenecientes a las subspecies *capuli*, *eximia*, *serotina* y *virens*. Esta evaluación sugiere la existencia de seis grupos genéticos principales que no correspondieron con las subespecies estudiadas. Asimismo, detectó flujo de genes entre poblaciones geográficamente cercanas. En conjunto, los resultados permiten concluir que las subespecies de capulín deberían manejarse como una sola entidad o unidad biológica de conservación.

Palabras clave: capulín, variación morfológica asociada al clima, estructura genética, conservación de recursos fitogenéticos, maderable.

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GENERAL ABSTRACT

GENETIC ORGANIZATION OF THE SUBSPECIES COMPLEX *Prunus serotina* EHRH. IN NORTH AMERICA

Black cherry (*Prunus serotina* Ehrh.) is a multipurpose fruit tree native to North America, almost all parts of which have some use. This species is a complex of five subspecies with morphological differences and distinctive habitats. However, several biological aspects of the species are currently being debated or remain poorly studied, such as the taxonomic identity of its five subspecies and its intraspecific molecular variability. In the present study, three types of information (climatic, morphological and molecular) were subjected to a combined analysis to describe the variation of the species; these analyses implicitly evaluate the options for the conservation and sustainable use of the genetic resources of the species. Altitude and seven climatic parameters played a considerable role in the differentiation of subspecies. Climate variability indicated that ssp. *eximia*, *hirsuta* and *serotina* thrive in more humid and cold environments, while ssp. *virens* prefers drier and warmer environments. Subspecies *capuli* exhibited the greatest environmental heterogeneity. Morphological differences included longer and wider leaves, thicker fruit pedicel in ssp. *capuli* and shorter, smaller leaves in ssp. *virens*. Even though morphological variation associated with climate was relatively low, the study did show possible effects of climatic variability on the morphology and distribution of the *P. serotina* complex and provided reference information that may be useful for an integrated description of its genetic resources. The genetic structure of 18 natural populations of black cherry was evaluated with 16 microsatellite markers, representing the subspecies *capuli, eximia, serotina* and *virens*. This molecular evaluation detected six main clusters that do not clearly correspond to the four putative subspecies studied. Gene flow among geographically nearby populations was also revealed. As a whole, the results allow us to conclude that black cherry subspecies should be managed as a single unit or biological conservation entity.

Keywords: black cherry, climate-associated morphological variation, genetic structure, plant genetic resources conservation, timber.

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GENERAL INTRODUCTION

Black cherry (*Prunus serotina* Ehrh.) is a North American fruit tree valued since pre-Hispanic times for its timber, leaves, fruits and seed. This species makes up a botanic complex of five subspecies (*capuli*, *eximia*, *hirsuta*, *serotina* and *virens*). McVaugh (1951) establishes the basis for taxonomic identification of the complex and states that the five subspecies are geographical races with distinctive morphological characters and habitats, but that recognizing them visually in the field would be impossible if they were not geographically segregated since they exhibit continuity in their variation and a certain degree of overlapping in almost all characters used to distinguish them.

Rzedowski and Calderón de Rzedowski (2005) described the overall characteristics of the three subspecies present in Mexico (*serotina*, *capuli* and *virens*), and highlighted that these are difficult to separate. These authors coincide with Avendaño-Gómez *et al.* (2015) in stating that black cherries are still undergoing domestication. According to all of them, ssp. *capuli* is the only one really domesticated, is native to Mexico and its distribution range has spread due to human influence as a consequence of its more popular uses: forestry and consumption of its fresh fruits.

Fresnedo-Ramírez, Segura and Muratalla-Lúa (2011) analyzed the morphological variability of the three subspecies present in the central and western parts of Mexico, but only discriminated two large groups that clearly did not correspond with the subspecies evaluated. Rohrer (2014) considers that this botanic complex is composed of four varieties (*alabamensis*, *capuli*, *rufula* and *serotina*), implying that morphological differences are subtler than classically proposed by McVaugh (1951). In any case, determining the degree of similarity or heterogeneity of black

cherry's infraspecific taxa is important for the conservation and sustainable use of its genetic resources.

In México, there are some selections of *P. serotina* intended for horticultural research (Segura-Ledesma, Zavala-Robles, Equihua-Cervantes, Andrés-Agustín & Yepez-Torres, 2009); however, at the present time, there is a lack of formal *ex situ* collections of its germplasm. The Germplasm Resources Information Network (GRIN) in its public website (GRIN-Global; GRIN, 2015), catalogs 41 accessions of *P. serotina* in the United States (4), México (16), Ecuador (1), Poland (6), and the United Kingdom (14). Moreover, it indexes 37 accessions belonging to three conspecific taxa. But, all these accessions are referenced as "Historical record only", thus they are not available. These observations emphasize that *ex situ* conservation is urgently needed and strongly recommended for the natural populations of black cherry.

Molecular markers are an important tool for guiding decision making in conservation actions by providing genetic information necessary for managing plant germplasm; in addition, the use of molecular markers promotes deployment of the diversity of genetic resources (Vicente, Guzmán, Engels & Ramanatha Rao, 2006). To date, no studies are available on the genetic structure of black cherries throughout their natural geographic distribution range, even though individuals of the species have been included in microsatellite evaluations conducted in the United States, Ecuador and several European countries (Beck, Ferguson, Mayfield & Shaw, 2014; Downey & Iezzoni, 2000; Guadalupe *et al*., 2015; Pairon, Jonard & Jacquemart, 2006; Pairon *et al*., 2010; Petitpierre *et al*., 2009).

This introductory context emphasizes that in spite of black cherry's importance as a multipurpose fruit species: 1) infraspecific variation still has relevant aspects that need to be evaluated, 2) morphological variability studies conducted to date have not taken into account the influence of environmental factors on this variability, and 3) few molecular studies are available that include populations of the natural geographic distribution range and only a limited number of genotypes have been evaluated.

The present study proposes refining the description and quantification of intraspecific variation of black cherries. To carry out the study, the first combined analysis was conducted of bioclimatic variables present in the environment where the five subspecies are present and of a group of morphological descriptors. In addition, the study did the first molecular evaluation of differences in the genetic structure amongst and within four of its subspecies. As a whole, these data will improve the understanding of the geographic and taxonomic distribution of genetic diversity within the species, and will help define strategies for the conservation and sustainable use of black cherry's genetic resources.

OBJECTIVES

OVERALL

Contribute to develop strategies for the conservation and sustainable use of the *Prunus serotina* botanic complex in North America, and broaden the benefits derived from this species.

SPECIFICS

Describe and analyze the current state-of-the-art of the multipurpose nature of black cherry (Chapter 1).

Associate morphological variation of the five subspecies and climatic conditions in its natural geographic distribution range in Mexico and the United States (Chapter 2).

Establish the genetic structure and molecular variability of natural black cherry populations using microsatellite markers (Chapter 3).

Discuss ecoclimatic, morphologic and molecular results in the general context of conservation of genetic resources and in the identification of gene pools that could potentially be used for broadening the benefits derived from the species and in plant breeding programs (Chapter 4).

HYPOTHESIS

A thorough description of the genetic resources of the *Prunus serotina* botanical complex will facilitate proposing strategies for the conservation and sustainable use of this species.

OUTLINE OF THE STUDY

The present study integrates three types of information (climatic, morphologic and molecular) to describe variation in natural populations of black cherries, and implicitly evaluates their importance as an option for the conservation and sustainable use of the species' genetic resources.

Chapter 1 is the review of literature which summarizes six relevant biological aspects of the species.

Chapter 2 presents the association between climatic conditions in the areas of the species' natural distribution and morphologic variation. A multivariate analysis was conducted to determine the discriminatory power of climatic variables, and an additional analysis to correlate with variation in a set of morphologic descriptors. Additionally, two atmospheric circulation models were used to estimate the potential effect of climate change on the future distribution of the species, suggesting possible genetic erosion.

Chapter 3 describes molecular evaluation (16 microsatellites) of 18 natural black cherry populations representing four of the five subspecies classically recognized. Molecular diversity and cluster analyses suggested that–in spite of having detected molecular variability and genetic structure–for conservation purposes, the four taxa evaluated should be managed as one biological entity.

Chapter 4 integrates results of evaluations presented in the previous chapters to illustrate and support the overall discussion of the thesis.

LITERATURE CITED

Avendaño-Gómez, A., Lira-Saade, R., Madrigal-Calle, B., García-Moya, E., Soto-Hernández, M., & Romo de Vivar-Romo, A. (2015). Management and domestication syndromes of capulin (*Prunus serotina* Ehrh. ssp. *capuli* (Cav.) Mc Vaugh) in communities of the state of Tlaxcala. *Agrociencia*, 49, 189-204.

Beck, J.B., Ferguson, C.J., Mayfield, M.H., & Shaw, J. (2014). Reduced genetic variation in populations of Black Cherry (*Prunus serotina* subsp. *serotina*, Rosaceae) at its western range limit in Kansas. *Northeastern Naturalist*, 21(3), 472-478.

Downey, S.L., & Iezzoni, A.F. (2000). Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. *Journal of the American Society for Horticultural Science*, 125(1), 76-80.

Fresnedo-Ramírez, J., Segura, S., & Muratalla-Lúa, A. (2011). Morphovariability of capulín (*Prunus serotina* Ehrh.) in the central-western region of Mexico from a plant genetic resources perspective. *Genetic Resources and Crop Evolution*, 58(4), 481-495, doi:10.1007/s10722-010-9592-2.

Germplasm Resources Information Network – GRIN (2015). *Prunus serotina*. Consulted on-line https://npgsweb.ars-grin.gov/gringlobal/view2.aspx?dv=web_taxonomyspecies_view_accessionlist&params=:taxonomyid=30099.

Guadalupe, J.J., Gutiérrez, B., Intriago-Baldeón, D.P., Arahana, V., Tobar, J., Torres A.F., & Torres, M.L. (2015). Genetic diversity and distribution patterns of Ecuadorian capuli (*Prunus serotina*). *Biochemical Systematics and Ecology*, 60, 67-73, doi:10.1016/j.bse.2015.04.001.

McVaugh, R. (1951). A revision of the North American black cherries (*Prunus serotina* Ehrh., and relatives). *Brittonia*, 7(4), 279-315, doi:10.2307/2804698.

Pairon, M., Jonard M., & Jacquemart, A.L. (2006). Modeling seed dispersal of black cherry, an invasive forest tree: how microsatellites may help? *Canadian Journal of Forest Research*, 36(6), 1385-1394.

Pairon, M., Petitpierre, B., Campbell, M., Guisan, A., Broennimann, O., Baret, P.V., Jacquemart, A.L., & Besnard, G. (2010). Multiple introductions boosted genetic diversity in the invasive range of black cherry (*Prunus serotina*; Rosaceae). *Annals of Botany*, 105(6), 881-890, doi:10.1093/aob/mcq065.

Petitpierre, B., Pairon, M., Broennimann, O., Jacquemart, A.L., Guisan, A., & Besnard. G. (2009). Plastid DNA variation in *Prunus serotina* var. *serotina* (Rosaceae), a North American tree invading Europe. *European Journal of Forest Research*, 128(5), 431-436.

Rohrer, J.R. (2014). *Prunus* (Rosaceae). In Flora of North America Editorial Committee (Eds.), *Flora of North America North of Mexico* (pp. 363-365). New York, Oxford 20+ vols. and. Vol 9, 1993+.

Rzedowski, J., & Calderón de Rzedowski, G. (2005). *Prunus serotina* Ehrh. In J. Rzedowski and G. Calderón de Rzedowski (Eds.), *Flora del Bajío y Regiones Adyacentes*. Instituto de Ecología A. C. Centro Regional del Bajío, Pátzcuaro, Michoacán, México. pp. 101-111.

Segura-Ledesma, S., Zavala-Robles, D., Equihua-Cervantes, C. Andrés-Agustín, J., & Yepez-Torres, E. (2009). Genetic resources of fruit trees in Michoacan. *Revista Chapingo Serie Horticultura*, 15, 207-305, doi:10.5154/r.rchsh.2009.15.041.

Vicente de, M.C., Guzmán, F.A., Engels, J., & Ramanatha Rao, V. (2006). Genetic characterization and its use in decision making for the conservation of crop germplasm. In International workshop: The Role of Biotechnology for the Characterization and Conservation of Crop, Forestry, Animal and Fishery Genetic Resources. 5-7 March 2005, Turin, Italy, FAO, FOBIOTECH, ECOGENE, SIGA. pp.121-128.

CAPÍTULO I. SINOPSIS DE LA ESPECIE FRUTAL MULTIPROPÓSITO *Prunus serotina* EHRH.

CHAPTER I. SYPNOSIS OF THE MULTIPURPOSE FRUIT TREE *Prunus serotina* EHRH.

1.1. RESUMEN

El capulín (*Prunus serotina* Ehrh.) es un árbol frutal nativo de Norteamérica, y casi todas sus partes tienen algún uso. Sin embargo, varios aspectos biológicos de la especie son tema de debate o permanecen pobremente estudiados, como la identidad taxonómica de sus cinco subespecies y su variabilidad molecular intraespecífica. Estos vacíos de conocimiento obstaculizan la planeación acertada de estrategias de conservación y de utilización agronómica. Ampliar el conocimiento sobre la diversidad de los recursos genéticos del capulín ofrecerá bases sólidas, entre otros, para tomar decisiones con fines de conservación a partir de su estructuración genética, caracterizar agro-morfológicamente capulines que sean utilizables en la producción frutal, o identificar individuos con valor potencial para el mejoramiento de la especie o de especies emparentadas. Esta revisión tiene como objetivo resumir seis aspectos biológicos del capulín (Clasificación taxonómica, distribución geográfica, fenología, enfermedades y plagas, relevancia etnobotánica y ecológica, y variabilidad molecular), que en conjunto resaltan su importancia biológica y comercial, y la necesidad de incluirlo en programas formales de conservación y uso sostenible. La información revisada muestra que el capulín es un recurso genético multipropósito con importancia, real y potencial, en áreas tan diversas como la explotación maderera, el fitomejoramiento de especies *Prunus* con importancia comercial o como fuente primaria de compuestos de valor farmacológico, por mencionar algunos.

Palabras clave: *Prunus serotina*, alotetrapolide, valor etnobotánico, especie invasora, complejo de subespecies.

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1.2. ABSTRACT

Black cherry (*Prunus serotina* Ehrh.) is a fruit tree native to North America, almost all parts of which have some use. However, several biological aspects of the species are currently debated or remain poorly studied, such as the taxonomic identity of its five subspecies and its intraspecific molecular variability. These gaps in knowledge hamper the successful planning of conservation and agronomic utilization strategies. Increasing knowledge on the diversity of genetic resources of black cherry will provide a solid foundation, among others, to make conservation decisions based on their genetic structure, characterize agro-morphologically black cherries that can be used in fruit production, or identify individuals with potential value for the improvement of the species or related species. This review aims to summarize six biological aspects of black cherry (Taxonomic delimitation, geographic distribution, phenology, pests and diseases, ethnobotanic and ecological relevance, and molecular variability), which together emphasize its biological and commercial importance, and the need to include it in formal programs of conservation and sustainable use. The revised information shows that black cherry is a multipurpose genetic resource with actual and potential importance in areas as diverse as logging, breeding of economically important *Prunus* species or as a primary source of compounds of pharmacological value, just to mention a few.

Keywords: *Prunus serotina*, allotetraploid, ethnobotanical value, invasive species, subspecies complex.

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1.3. INTRODUCCIÓN

El capulín (*Prunus serotina* Ehrh.) es un frutal nativo de Norteamérica conocido desde épocas prehispánicas por el consumo de su fruto y semilla, y por el uso de sus hojas y madera. Tradicionalmente el fruto se come en fresco y se usa como ingrediente de otras preparaciones (e.g., tamales); pero la pluralidad de usos va desde consumir su semilla después de tostar el hueso (Raya-Pérez, Aguirre-Mancilla, Tapia-Aparicio, Ramírez-Pimentel & Covarrubias, 2012), pasando por emplear sus hojas con fines medicinales (Mendoza-Castelán & Lugo-Pérez, 2010), hasta utilizar su tronco con fines maderables (Rohrer, 2014). Asimismo, por su follaje se ha usado como ornamental y barrera rompe vientos. Ecológicamente también tiene relevancia porque su capacidad para regenerar en ambientes perturbados la ha convertido en una especie invasora que amenaza la diversidad de los bosques de varios países europeos (Starfinger, Kowarik, Rode & Schepker, 2003). Sin embargo, a pesar del gran potencial en el mercado, tanto por su apetecido fruto como por sus propiedades farmacológicas y madera, el capulín sigue siendo una especie poco estudiada.

Esta especie conforma un complejo botánico de cinco subespecies, cuyas bases de identificación taxonómica y distribución geográfica fueron propuestos por McVaugh (1951): *Prunus serotina* ssp. *capuli* (Cav.) McVaugh (sur de México a Guatemala), *Prunus serotina* ssp. *eximia* (Small) Little (Meseta Edwards de Texas), *Prunus serotina* ssp. *hirsuta* (Elliot) McVaugh (Georgia y Alabama), *Prunus serotina* ssp. *serotin*a (Ehrh.) McVaugh (oriente de los Estados Unidos y Canadá, reapareciendo en el oriente mexicano), y *Prunus serotina* ssp. *virens* (Wooton et Standl.) McVaugh (occidente de Texas hasta Arizona y el norte de México). Sin embargo, sus identidades botánicas son ambiguas y su clasificación es imprecisa porque se establecieron con base en pocas diferencias morfológicas, conduciendo a la subutilización de su germoplasma (Fresnedo-Ramírez, Segura & Muratalla-Lúa*,* 2011).

Más recientemente, en México se han hecho algunas revisiones parciales del complejo botánico, diferenciando morfológicamente con dificultad tres

subespecies (*capuli*, *serotina* y *virens*) (Fresnedo-Ramírez *et al*., 2011; Rzedowski & Calderón de Rzedowski, 2005). Por otro lado, Rohrer (2014) delimitó cuatro variedades botánicas (var. *alabamensis* = ssp *hirsuta*, var. *capuli* = ssp *capuli*, var. *rufula* = ssp. *virens*, and var. *serotina* = ssp. *serotina* + ssp. *eximia*), y sugirió que las diferencias taxonómicas entre los grupos infra específicos son más sutiles que lo propuesto clásicamente por McVaugh (1951).

El capulín es una especie alotetraploide (2*n* = 4*x* = 32) de la cual se desconocen sus progenitores (Pairon & Jacquemart, 2005). No obstante, aunque los estudios citológicos son escasos, se han reportado individuos dipolides (Forbes, 1969), y se han referenciado individuos pentaploides y hexaploides (Dickson, Arumuganathan, Kresovich & Doyle, 1992) Los estudios moleculares o genómicos de la especie también son escasos (Wang & Pijut, 2014). El primer estudio molecular se realizó para demostrar la utilidad de las técnicas moleculares en la evaluación de la diversidad del capulín (Downey & Iezzoni, 2000). Posteriormente, los trabajos moleculares han tenido diversos objetivos, tales como: mejorar el entendimiento de su comportamiento invasivo en los bosques europeos y establecer estrategias eficientes de control (Pairon, Jonard, Jacquemart & 2006; Pairon *et al*., 2010; Petitpierre *et al*., 2009), evaluar su variabilidad molecular en Ecuador (Gordillo, Tobar, Arahana & Torres, 2015; Guadalupe *et al*., 2015), y usar el capulín como especie modelo para probar la Hipótesis Centro-Abundante (Beck, Ferguson, Mayfield & Shaw, 2014). En el contexto del uso maderable, en los EE.UU. se ha incluido en proyectos de mejoramiento genético que obtuvieron capulines transgénicos más resistentes al ataque de insectos, para reducir la ocurrencia de gomosis y mejorar las ganancias económicas que ofrece el uso de su madera (Wang & Pijut, 2014). Sin embargo, aún no se conocen trabajos de estructuración genética en su rango de distribución natural, y la información genómica que se ha generado aún no está disponible públicamente (Staton *et al*., 2015).

Esta revisión tiene como objetivo resumir la clasificación taxonómica del capulín, su distribución geográfica, fenología, enfermedades y plagas, relevancia

etnobotánica y ecológica, y su variabilidad molecular; ya que en conjunto resaltan su importancia biológica y comercial, y la necesidad de incluirlo en programas formales de conservación y uso sostenible.

1.4. ASPECTOS TAXONÓMICOS

1.4.1. El género *Prunus*

El género *Prunus* L. (Rosaceae) comprende más de 200 especies, incluyendo todos los cultivos económicamente importantes conocidos como frutos de hueso [almendro (*Prunus dulcis* (Miller) D.A. Webb), durazno (*Prunus persica* L.), cerezo dulce (*Prunus avium* L.), cerezo ácido (*Prunus cerasus* L.), ciruelo europeo (*Prunus domestica* L.), ciruelo japonés (*Prunus salicina* L.) y chabacano (*Prunus armeniaca* L.)], muchas especies ornamentales, otras usadas con fines forestales y medicinales, y especies silvestres que no son usadas por los pueblos (Bortiri, Vanden Heuvel & Potter, 2006; Chin, Shaw, Haberle, Wen & Potter, 2014; Potter, 2011).

Prunus ha sido incluido dentro de la Subfamilia Spiraeoideae C. Agardh (Tribu Amigdaleae Juss.), que se distingue de otras rosáceas porque agrupa principalmente árboles y arbustos perennifolios o caducifolios con una cantidad significativa de sorbitol, presencia generalizada de glicósidos cianogénicos, hojas simples y alternas de margen entero o serrado (provistas de estípulas), flores comúnmente hermafroditas (rara vez unisexuales), 1 a 5 pistilos, hipantio generalmente libre de ovario(s), ovarios habitualmente separados (Potter *et al*., 2007; Rzedowski & Calderón de Rzedowski, 2005), por mencionar algunos caracteres. A su vez, los caracteres morfológicos distintivos de la tribu Amigdaleae son la presencia de estípulas deciduas, el pistilo solitario, los frutos drupáceos y el número cromosómico básico X = 8 (Potter *et al*., 2007; Raven, 1975).

El género está ampliamente distribuido tanto en la zona templada del hemisferio norte (Rehder, 1940), como en los bosques tropicales de Asia, África, Suramérica

y Australia (Kalkman, 1965). Aún hay muchas especies tropicales poco conocidas o sin descripción taxonómica (Pérez-Zabala, 2007). Varios estudios filogenéticos moleculares claramente demuestran la monofilia del género (Bortiri *et al*., 2001, Bortiri *et al*., 2006; Chin, Lutz, Wen & Potter, 2013; Chin *et al*., 2014; Lee & Wen, 2001; Potter *et al*., 2007; Shi, Li, Sun, Yu & Zhou, 2013; Wen *et al*., 2008). Sin embargo, la delimitación genérica de *Prunus* ha sido controversial porque se ha realizado usando pocas muestras y por la falta de sustento monofilético de sus grupos infra genéricos (Potter*,* 2011). La clasificación infra genérica de Rehder (1940) es la más ampliamente aceptada; esta plantea cinco subgéneros: *Amygdalus* (L.) Focke, *Cerasus* Focke, *Laurocerasus* (Tourn. Ex Duham) Rehder, *Padus* (Moench) Focke y *Prunophora* (Necker) Focke; pero, su monofilia no ha sido determinada por los trabajos que sustentan la monofilia del género. Por otro lado, *P. serotina* está incluida en el subgénero *Padus* (Rehder, 1940) y ha sido considerada representativa del mismo, junto a especies tales como: *Prunus maackii* Rupr., *Prunus napaulensis* (Ser.) Steud, *Prunus padus* L. y *Prunus virginiana* L. (Potter, 2011).

La identificación de acervos génicos dentro de *Prunus* es clave para conservar y usar su germoplasma eficientemente. Watkins (1976) propuso que *Prunus* tiene dos acervos génicos desde el punto de vista del fitomejoramiento; uno integrado por los subgéneros *Amygdalus* (durazno y almendra) y *Prunus* [sección *Armeniaca*: chabacano y sección *Prunophora*: ciruelo japonés (diploide) y cerezo europeo (hexaploide)], y otro conformado por el subgénero *Cerasus* [cerezo dulce (diploide) y cerezo tarto (tetraploide)]. Por su parte, Aradhya, Weeks y Simon (2004) usaron Polimorfismos en la Longitud de los Fragmentos Amplificados (AFLP) para analizar la diferenciación y variabilidad genética (intraespecífica e interespecífica) de siete especies cultivadas y siete silvestres de *Prunus*, y propusieron cuatro acervos génicos diferentes que corresponden a las cuatro secciones del género (*Amygdalus*, *Armeniaca*, *Cerasus* y *Prunophora*). Sin embargo, Wen *et al*. (2008) usando secuencias de cloroplasto (*ndhF*) y nucleares (ITS), obtenidas de 53 taxones representativos de los cinco subgéneros de *Prunus* y de 13 grupos externos, encontraron inconsistencias

entre ambas filogenias subgenéricas. Estos autores concluyeron que se necesitan más marcadores de cloroplasto y nucleares de copia única, o de pocas copias, para evaluar todos los esquemas de clasificación existentes, y que la diversificación del género pudo involucrar evolución reticulada, poliploidía y otros procesos moleculares.

En cuanto al origen geográfico de *Prunus*, Chin *et al*. (2014) usaron cuatro loci de plastidio y la región nuclear ribosomal ITS para evaluar una muestra que incluyó especies originarias de regiones tropicales del sudeste de Asia (su centro de diversidad de cultivos) y las Américas, que no habían estado bien representadas en estudios previos. En sus conclusiones estos autores indicaron que el género se originó en el oriente de Asia y que alcanzó su distribución actual por medio de complejos eventos geológicos y oscilaciones climáticas, los cuales favorecieron o impidieron su migración dentro y entre el Viejo Mundo y el Nuevo.

1.4.2. Aspectos taxonómicos

McVaugh (1951) sentó las bases taxonómicas de la identificación de esta especie y sus cinco subespecies (*capuli*, *hirsuta*, *eximia*, *serotina* y *virens*). Además, en la subespecie *virens* distinguió dos variedades*: virens* y *rufula*. Este autor referenció que las cinco subespecies son razas geográficas con caracteres morfológicos y hábitats distintivos; sin embargo, también manifestó que la diferenciación de sus poblaciones naturales sería imposible si no fuera porque están geográficamente segregadas.

Esencialmente, las diferencias entre las subespecies están en los grados de pubescencia, el número de dientes en el margen de las segundas hojas basales del racimo floral, el tamaño de la inflorescencia y en algunas estructuras florales (McVaugh, 1951). Las subespecies mexicanas *serotina* y *capuli* se diferencian en que la primera incluye individuos silvestres con racimos cortos, flores y frutos pequeños, y hojas elípticas, mientras que *capuli* está conformada por individuos con hojas lanceoladas, frutos grandes y racimos largos, y no se encuentra en estado silvestre (McVaugh, 1951).

Rzedowski y Calderón de Rzedowski (2005) describieron al capulín como un "árbol o a veces arbusto caducifolio hasta de 30 m de alto; ramas nigrescentes, ramillas café-rojizas, glabras o pubérulas, con lenticelas; estípulas lineares, de 4 a 10(20) mm de largo, escariosas, por lo general pubérulas, deciduas, peciolos de 4 a 30 mm de largo, láminas foliares elípticas a lanceoladas, ovadas u obovadas, de (3)5 a 12(18) cm de largo, de (1)2 a 6(8) cm de ancho, agudas a acuminadas en el ápice, cuneadas a redondeadas en la base, margen más bien finamente serrado desde el ápice hasta la base, el par de dientes cercanos a la base a menudo apareciendo como 2 glándulas ubicadas a veces en la parte superior del peciolo, verdes oscuras en el haz, más pálidas en el envés, de textura membranácea, con la costa prominente en el envés, brillantes en ambas caras, muchas veces totalmente glabras, pero en otras ocasiones con pelos rojizos o amarillentos, principalmente a lo largo de la costa en el envés; racimos ubicados en posición terminal de ramillas que llevan en la base 1 a 4(10) hojas de tamaño algo reducido, los racimos de (3)5 a 15(18) cm de largo, por lo general plurifloros, sus ejes glabros a densamente pubérulos, brácteas lineares a ovadas o espatuladas, de 1 a 5(8) mm de largo, pedicelos delgados, hasta de 7(10) mm de largo; cáliz con el tubo anchamente campanulado a acopado, de 1.5 a 2 mm de largo, por lo general glabro por fuera y por dentro, sus lóbulos anchamente triangulares, de 0.8 a 2.5 mm de largo, valvados en el botón; pétalos blancos, obovados a suborbiculares, de 2.5 a 3.5 mm de largo, glabros; estambres 20, exsertos, anteras oblongas, de 0.4 a 1 mm de largo; estilo de 1 a 2.5 mm de largo, estigma disciforme; fruto globoso u oblato, de 0.6 a 2.5 cm de diámetro, casi negro y brillante en la madurez, mesocarpio jugoso y comestible, hueso globoso o biconvexo, de 0.3 a 0.6 cm de diámetro, liso."

Según Rzedowski y Calderón de Rzedowski (2005), las subespecies *serotina*, *capuli* y *virens* son de separación difícil en El Bajío mexicano y las regiones adyacentes, principalmente porque el hombre ha sometido árboles a frecuente cultivo en función de su fruto comestible o ha protegido individuos con características deseables.

Fresnedo-Ramírez *et al*. (2011) describieron la variabilidad morfológica de las subespecies *serotina*, *capuli* y *virens* en la región centro-occidental de México, y diferenciaron dos grandes grupos que no correspondieron a las subespecies estudiadas: uno formado por individuos de ssp. *capuli* y ssp. *serotina* recolectados en los estados de Tlaxcala, Querétaro y México; y el otro grupo incluyó individuos de ssp. *serotina* y ssp. *virens* obtenidos en Michoacán.

Recientemente Rohrer (2014) delimitó cuatro variedades botánicas (var. *alabamensis* = ssp. *hirsuta*, var. *capuli* = ssp. *capuli*, var. *rufula* = ssp. *virens*, y var. *serotina* = ssp. *serotina* + ssp. *eximia*), planteando que las diferencias taxonómicas entre los grupos infra específicos son más sutiles que lo propuesto clásicamente por McVaugh (1951).

1.5. ASPECTOS DE DISTRIBUCIÓN

1.5.1. Medio ecológico

Popenoe y Pachano (1922) indicaron que *P. serotina* se adapta bien a climas templados-frío (Cw_0, Cw_1, Cw_2 y Cs) y subtropical [tipos A(c) y (A)c], y que en regiones subtropicales se adapta bien al clima seco, pero no en cercanía a las costas.

El capulín crece óptimamente en suelos arenosos y de marga aluvial, terrenos altamente arcillosos, laderas secas y rocosas, y áreas sueltas de arenas volcánicas (Popenoe & Pachano, 1922). Se desarrolla preferentemente en suelos de tipo regosol éutrico, cambisol pedregoso, acrisol órtico y toba; además, se desarrolla bien en suelos tepetatosos, suelos ácidos o ricos en materia orgánica. Por su rusticidad tolera bajas temperaturas (superiores a -5 ºC en el periodo invernal) y periodos de sequía prolongados, ya que se establece sobre suelos profundos en función de su vigorosa raíz pivotante, que alcanza una longitud hasta de 10 m (Muratalla, 1984). Regularmente esta especie se identifica con la zona templada subhúmeda, siendo una especie secundaria acompañada de *Pinus maximinoi* H.E. Moore, *Abies religiosa* Kunth Schltdl. et Cham. y *Oreopanax xalapensis* (Kunth) Decne. et Planch. Esta especie se desarrolla

mejor en claros porque es intolerante a la sombra; los individuos se convierten en entes dominantes durante la sucesión secundaria y tienen éxito en terrenos donde recientemente se han producido alteraciones o desastres naturales (Vázquez-Yanes, Batis Muñoz, Alcocer Silva, Gual Díaz & Sánchez Dirzo,1999).

1.5.2. Distribución geográfica

Prunus serotina es un árbol de rápido crecimiento, sobre todo en zonas templadas. La especie es nativa de Norteamérica (Marquis, 1990; Maynard, Kavanagh, Fuernkranz & Drew, 1991; Niembro Rocas, Vázquez Torres & Sánchez Sánchez, 2010) y se distribuye naturalmente desde Canadá hasta Guatemala (McVaugh, 1951; Niembro *et al*., 2010; Popenoe & Pachano, 1922; Rzedowski & Calderón de Rzedowski, 2005). La Figura 1 presenta la distribución geográfica natural de cada subespecie.

Las poblaciones más occidentales del rango natural de *P. serotina* se distribuyen hacia el sur desde Iowa, el extremo oriental de Nebraska y el norte de Arizona en los EE.UU.; y las más orientales, desde el suroriente de Canadá (Nueva Escocia, Nuevo Brunswick, Quebec y el oriente de Ontario) (Marquis, 1990).

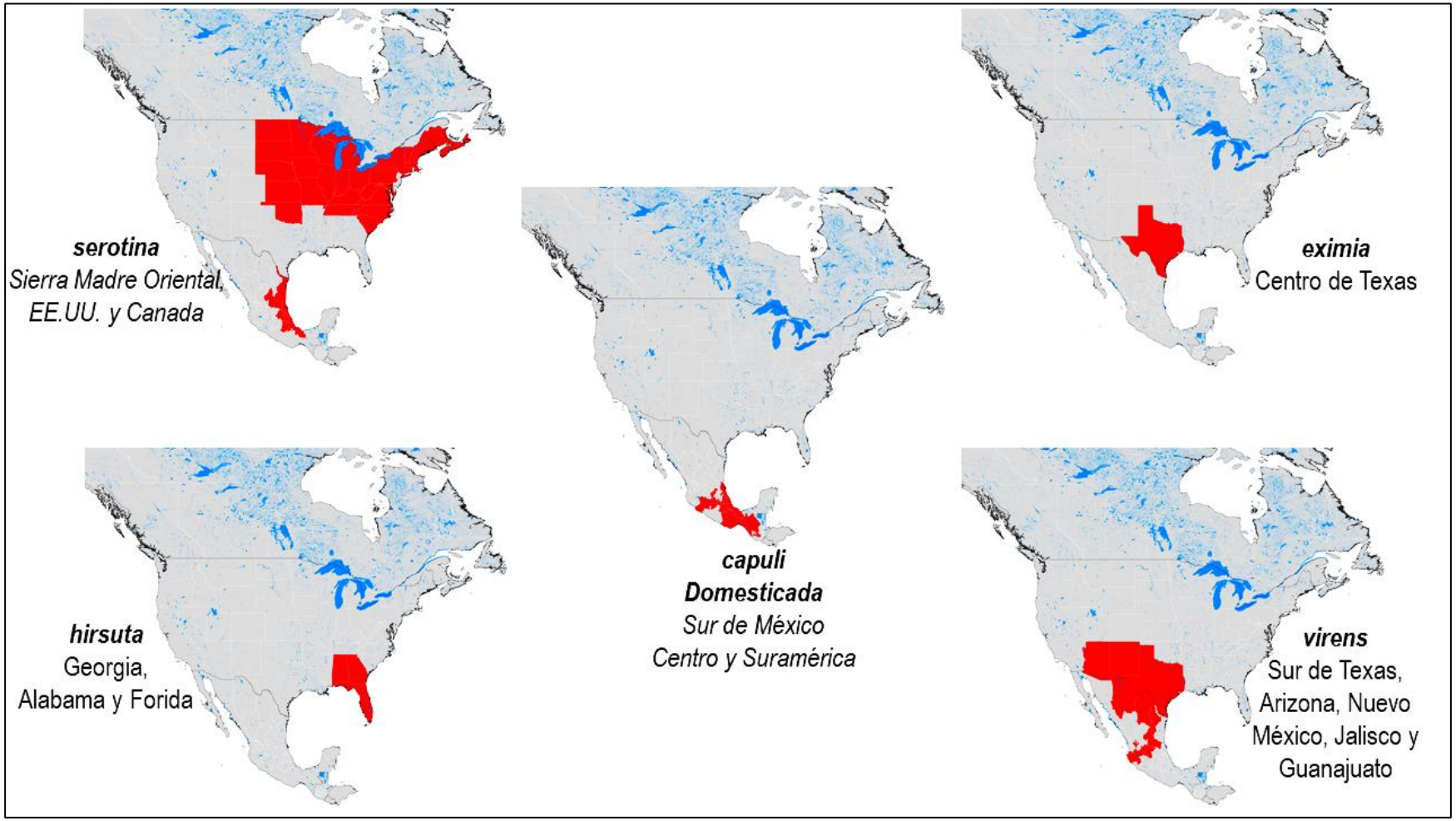

Figura 1. Localización geográfica de las cinco subespecies del complejo *P. serotina* en Norteamérica. Adaptado de McVaugh (1951) y Marquis (1990).

En los EE.UU. se han registrado poblaciones naturales en Dakota del Norte, oriente de Minnesota y Nebraska, Iowa, Kansas, Oklahoma, Texas, Arizona, Nuevo México, Carolina del Norte, Carolina del Sur, Georgia, noroccidente de Florida y el nororiente de Alabama. En México el capulín ha sido reportado en Chihuahua, Coahuila, Nuevo León, Querétaro, Durango, San Luis Potosí, Hidalgo, Tlaxcala, México, Veracruz, Guanajuato, Michoacán, Jalisco, Morelos, Chiapas y Oaxaca (McVaugh, 1951; Marquis, 1990; Rzedowski & Calderón de Rzedowski, 2005; Vázquez-Yanes *et al*., 1999). En Guatemala, el capulín ocurre naturalmente en Chimaltenango, Guatemala, Huehuetenango, Quetzaltenango, Quiché, Sacatepéquez y Sololá (McVaugh, 1951).

Por otro lado, el capulín fue introducido a Europa en el siglo XVII con fines ornamentales y forestales, y actualmente se considera una especie invasiva en los bosques de varios países de ese continente, como Francia, Alemania, Holanda, Bélgica, norte de Italia, entre otros (Starfinger *et al*., 2003); además, ha sido considerada una especie naturalizada en Colombia, Ecuador, Perú y Bolivia (Popenoe & Pachano, 1922).

1.6. COMPORTAMIENTO FENOLÓGICO

En altitudes superiores a 2000 m, el capulín pierde su follaje durante el invierno, generalmente por un poco más de un mes en enero y febrero. La brotación de yemas se presenta en marzo, lo que hace suponer que la diferenciación de yemas vegetativas a florales ocurre durante su corto letargo, el cual puede reducirse si la cantidad de horas frío del sitio es superior a 300, con base en la fórmula del método de Da Mota (Almaguer Vargas, 1998). En el Valle de México, hay poblaciones de tipo perennifolio y caducifolio; y en condiciones de humedad constante ambas inician el proceso de diferenciación floral en el verano.

La floración ocurre habitualmente desde marzo a abril, aunque en altitudes superiores a 3000 m algunos individuos pueden retrasarla hasta mayo, tal como ocurre en zonas adyacentes al volcán Cofre de Perote entre Puebla y Veracruz (México). Esto ocurre debido a la acumulación de calor dada en mayor cantidad

de tiempo. La viabilidad de la flor está alrededor de las 24 horas, y su polinización es entomófila. Cabe destacar que es difícil encontrar sincronía fenológica entre distintos individuos en un mismo lugar.

La fructificación para la zona centro de México se presenta entre los meses de mayo, junio y al principio del mes de julio (Calderón de Rzedowski & Rzedowski, 2001); sin embargo, en zonas altas como la Sierra Madre Oriental o la Sierra Nevada y zonas más altas del Eje Volcánico Transversal se presenta hasta entrado el mes de agosto. El tiempo que tarda la maduración del fruto es de 70 a 120 días, teniendo un desarrollo y crecimiento de tipo doble sigmoidal, común entre las drupas de los *Prunus* (Almaguer Vargas, 1998), la cual tiene dos pausas, una para la formación del endocarpio lignificado y otra una vez alcanzada la madurez y en la acumulación de azúcares. El crecimiento del fruto está fuertemente influenciado por la precipitación pluvial, ya que al coincidir con la etapa de crecimiento acelerado ocasionan que el fruto se reviente. Este fenómeno denominado *Craking fruit* se considera en otros frutales del género como la respuesta a un conjunto de factores ambientales, fisiológicos y de manejo (Webster & Lonney, 1996).

En algunas ocasiones, durante los meses de noviembre y octubre se presenta la diferenciación floral en individuos del Valle de México y algunas regiones de Michoacán, por lo que hay una mínima fructificación entre la primera quincena del mes de noviembre y la primera de diciembre, la cual antecede a la caída del follaje. Esto se relaciona con condiciones ambientales como la prolongación de la canícula durante el verano, lo que genera un corto período de letargo que permite la diferenciación de yemas vegetativas a florales (Almaguer Vargas, 1998). Cierta proporción de estos frutos presentan deformaciones, como fusiones de pericarpio o frutos dobles unidos por la cáscara y pulpa.

1.7. ASPECTOS FITOPATOLÓGICOS Y ENTOMOLÓGICOS

Según Avendaño-Gómez (2000) las principales enfermedades que afectan al capulín son de tipo fungoso, destacándose: 1) la mancha foliar provocada por *Alternaria* sp. y *Cephalosporium* sp., que se caracteriza por ser una lesión necrótica de color pardo a negruzca, bien definida y delimitada; y 2) la pudrición radical causada por *Phymatotrichum* sp., que provoca el amarillamiento y bronceado de las hojas, estas posteriormente se marchitan, adquieren color castaño, se secan y permanecen adheridas a las plantas, por su parte las raíces mueren desde sus ápices tornándose oscuras. La principal enfermedad de los frutos es la mancha negra, causada por *Enthomosporium* sp., que no es identificable fácilmente debido a la coloración del fruto en su etapa de madurez (Avendaño-Gómez, 2000).

Por otro lado, el daño causado por insectos minadores del cambium da origen a la gomosis, que afecta principalmente el tallo con exudaciones gomosas inicialmente cristalinas y después amarillentas (Avendaño-Gómez, 2000; Wang & Pijut, 2014). Estas secreciones afectan la calidad de la madera del capulín y disminuyen su valor comercial hasta en 75 % (Cassens, 2004). Algunos insectos que ocasionan gomosis son el escarabajo de corteza de durazno (*Phloeotribus liminaris* Harris), la polilla del melocotonero (*Synanthedon pictipes* Grote & Robinson), el gusano perforador del duraznero *(Synanthedon exitiosa* Say), y el agromícido *Phytobia pruni* (Gross.) que se alimenta del cambium del tallo (Avendaño-Gómez, 2000; Barnd & Ginzel, 2008; Wang & Pijut, 2014).

Avendaño-Gómez (2000) propuso que las principales plagas que afectan al capulín son : 1) la escama globosa (*Cerococus koebelei* Hopk.) que ataca tronco, ramas, frutos y brotes tiernos; 2) el barrenador del tronco (*Megacyllene erythropa* Gahan.), cuyas hembras abren pequeñas escotaduras en la corteza para ovopositar en la zona del cambium; 3) la mosca del capulín (*Rhagoletis sp.*) que penetra los frutos para ovopositar, por lo que el fruto maduro podría presentar hasta tres larvas que consumen la pulpa y ocasionan el rechazo del consumidor al producto; y 4) Neumogen (*Malacosoma invircum* var. *aztecum*), que consume

las hojas dañándolas de tal forma que reduce el crecimiento y causa la mortalidad de ramas o individuos completos en los casos de infestaciones severas. En Michoacán, Estado de México y Ciudad de México se tienen problemas severos con plantas parásitas del género *Phoradendrum*, las cuales pueden llegar a matar al árbol (Avendaño-Gómez, 2000).

1.8. RELEVANCIA ETNOBOTÁNICA Y ECOLÓGICA

1.8.1. Domesticación y valor etnobotánico

Actualmente se conocen tres trabajos cuyos enfoques estiman el efecto del proceso de domesticación en *P. serotina*: uno evaluó la variación morfológica de poblaciones del centro y occidente mexicano (Fresnedo-Ramírez *et al*., 2011), otro documentó el manejo tradicional en una comunidad de Michoacán (Rodríguez & Farfán, 2014), y el más reciente se centró en aspectos de manejo y síndromes de domesticación en cuatro comunidades del estado de Tlaxcala (Avendaño-Gómez *et al*., 2015).

Fresnedo-Ramírez *et al*. (2011) usaron 39 descriptores morfológicos (22 cuantitativos y 17 cualitativos) de diferentes órganos de la planta para describir la variabilidad morfológica de las subespecies *serotina*, *capuli* y *virens* en la región centro-occidental de México. Los análisis permitieron identificar dos grandes ecorregiones: una formada por individuos de ssp. *capuli* y ssp. *serotina* recolectados en los estados de Tlaxcala, Querétaro y México (grupo de la región central de México); y la otra incluyó individuos de ssp. *serotina* y ssp. *virens* recolectados en Michoacán (grupo del occidente). Para estos autores, las similitudes morfológicas encontradas en las poblaciones del centro posiblemente son una consecuencia de la selección humana, ya que en esta zona el capulín es usado para producir frutos y semillas para consumo humano. De otra parte, argumentaron que las poblaciones del occidente, a diferencia de las del centro, han estado menos sujetas al interés antropocéntrico por el fruto y más al servicio agroecológico en barreras rompevientos de parcelas de cultivo (i.e., los árboles

con fruto se usan como distractor de aves que se alimentan del maíz), y en consecuencia parecen estar sometidas a un proceso de domesticación incipiente.

Los sistemas de agricultura tradicional son el escenario natural donde continuamente se desarrolla el proceso de fitodomesticación. En consecuencia, el enfoque etnobotánico es fundamental para entender este fenómeno y a sus elementos, tales como: la selección, uso, manejo y conservación del germoplasma. Rodríguez y Farfán (2014) realizaron 60 encuestas para documentar el conocimiento del ciclo de vida del capulín, de las características de sus variedades, de las formas de uso del fruto y el árbol, y de las actividades del manejo tradicional de los árboles en zonas agrícolas, ruderales y forestales. El análisis de las respuestas permitió documentar que: 1) la gente conoce detalladamente las tres etapas de vida del capulín; 2) diferencia tres variedades morfológicas de frutos, distinguiéndolos con base en el color (negro, blanco y colorado) y subdividiéndolos por tamaño (grande, mediano y pequeño); 3) la intensidad de las prácticas *in situ* de manejo silvícola han establecido tres tipos de poblaciones [silvestre, manejada *in situ* (o tolerada) y cultivada] que se diferencian en gradiente de manejo, intensidad de selección, presencia de variedades y abundancia de árboles. En las poblaciones silvestres se hace recolección selectiva de los frutos con mayor tamaño y mejor sabor; mientras que en las toleradas el aprovechamiento del capulín es más intenso porque se realiza junto a otros frutales dentro del huerto y se usa en cercos vivos. Los frutos de las poblaciones cultivadas presentan las características más deseadas por la gente de la zona, por esto son los preferidos para consumo doméstico y comercialización.

La apropiación de los recursos vegetales es un aspecto fundamental en la relación del hombre con el medio ecológico. Esta relación se ha originado en la recolección, e implica que el humano debe modificar las poblaciones vegetales, y hasta el ecosistema, para obtener el producto de su interés. Por lo tanto, el primer paso que debe dar el hombre es acumular conocimiento sobre la planta de interés, el medio ecológico y sus componentes, y su respectivo orden causal.

En consecuencia, la domesticación debe entenderse como un proceso, no como un evento puntual. En este sentido, Avendaño-Gómez *et al*. (2015) buscaron evidencia etnobotánica, morfológica y fitoquímica que pudiera relacionarse con el proceso de domesticación del capulín en el estado de Tlaxcala (México). El trabajo se realizó durante cuatro años en cuatro comunidades para documentar el consumo y comercio de la semilla de capulín, y la presencia de la especie en los sistemas agrícolas (i.e., las formas de manejo en la zona de estudio). Se midieron 32 caracteres morfológicos (13 de hoja, nueve de flor y 10 de semilla), y se determinó el contenido de glucósidos cianogénicos de la semilla para establecer si la selección artificial había disminuido estos compuestos. Según estos autores, el uso del fruto es secundario en las comunidades documentadas, por esto no lo estudiaron profundamente.

Los resultados de este trabajo mostraron que en el área de estudio: 1) a pesar de que hay dos subespecies (*serotina* y *capuli*), los caracteres morfológicos de los individuos evaluados correspondieron a la subespecie *capuli*; 2) hay tres grados de manejo (tolerado, fomentado y cultivado) cuyas diferencias en el contenido de glucósidos cianogénicos son estadísticamente no significativas; 3) los individuos tolerados son usados como fuente de sombra en parcelas de temporal, los fomentados tanto por su semilla como en metepantles, y los cultivados para aprovechar su semilla; y 4) la selección artificial produjo variedades que se diferencian por la semilla (tamaño, forma y textura) y que las comunidades se organizan para aprovecharlas. En sus conclusiones, los autores destacaron que la domesticación del capulín en el área de estudio se advierte en el reconocimiento de la variación de la especie, y en la influencia de la selección en las formas y tamaños de semilla de individuos con diferentes grados de manejo; además, en la organización social para aprovechar la especie, lo que destaca la integración, pertenencia y el valor del capulín en la región.

1.8.2. Usos tradicionales y modernos

El capulín tradicionalmente se ha utilizado como productor de fruta, madera, y planta ornamental (Niembro *et al*., 2010). En el ámbito alimenticio, el capulín

forma parte de la dieta mexicana y popularmente sus frutos se consumen frescos, secos o como ingredientes de otras preparaciones (*e.g.*, jaleas, tamales o licores) (Niembro *et al*., 2010, Raya-Pérez *et al*., 2012). Su madera se utiliza como fuente de energía (leña) o como materia prima para realizar diversos trabajos de ebanistería (Adriano-Morán & McClung de Tapia 2008; Niembro *et al*., 2010), y en los EE.UU. hay plantaciones comerciales con fines forestales (Barnd & Ginzel, 2008; Wang & Pijut, 2014). Además, por su follaje ha sido usada como cerco vivo, barrera rompevientos y ornamental (Niembro *et al*., 2010).

Adriano-Morán y McClung de Tapia (2008) usaron vestigios de carbón de leña, recuperados de 15 excavaciones arqueológicas de México, para reconstruir los patrones de uso y explotación de la madera desde el período Preclásico hasta el Postclásico (c.a. 400 a.C. – 1500 d.C.) por parte de los habitantes del Valle de Teotihuacán. Sus análisis identificaron 16 taxones de árboles y arbustos que fueron usados como leña. El capulín fue identificado en siete excavaciones, conformando un grupo secundario de especies usadas como combustible por los habitantes prehispánicos de la zona estudiada.

A la especie se le han atribuido propiedades medicinales como expectorante, sedante y antiespasmódica; por esto sus frutos y hojas se usan en infusiones para combatir la tos (Mendoza-Castelán & Lugo-Pérez, 2010). También se ha demostrado que sus inflorescencias y hojas son excelente fuente de antioxidantes (Olszewska, 2007).

Además, están en aumento los estudios bioquímicos que buscan implementar su uso innovador en el desarrollo de nuevos productos en las industrias de alimentos funcionales, cosmética, terapéutica y nutracéutica. El consumo de la decocción de sus hojas tiene efectos benéficos en el tratamiento de la hipertensión (Ibarra-Alvarado *et al*., 2009; Ibarra-Alvarado *et al*., 2010). La evaluación del valor nutracéutico y propiedades antihipertensivas de sus frutos permitió determinar que el extracto podría usarse en la prevención de la hipertensión y como coadyuvante en su tratamiento (Luna-Vázquez *et al*., 2013). Posteriormente se

estableció que el ácido ursólico y el uvaol son los principales compuestos vasodilatadores no-polares en los frutos de capulín (Luna-Vázquez *et al*., 2016).

Asimismo, se ha establecido que su contenido de fenoles totales es superior al de cultivos comercialmente importantes, como la ciruela y la fresa (Vasco, Riihinen, Ruales & Kamal-Eldin, 2009). Del mismo modo, se ha encontrado que el extracto etanólico del fruto de capulín tiene alta actividad antioxidante, y mayor actividad antimicrobiana contra cuatro bacterias gram negativas y una gram positiva; lo cual resalta su potencial como aditivo en alimentos (Jiménez, Castillo, Azuara & Beristain, 2011).

La almendra del capulín es consumida en la Ciudad de México y en los estados de México, Hidalgo y Tlaxcala; en estos lugares pueden encontrarse sitios de comercio donde se vende el hueso de capulín crudo o tostado (Raya-Pérez *et al*., 2012). El perfil electroforético de las proteínas de reserva de la almendra indicó que la fracción mayoritaria son las albúminas, y que la solubilidad de las proteínas se incrementa a pH alcalino; además, se estableció que la actividad inhibitoria contra enzimas tipo tripsina prácticamente desaparece al tostar la semilla (Raya-Pérez *et al*., 2012). Por lo tanto, el potencial de aprovechamiento de la almendra del capulín aumentaría si se tiene en cuenta que puede ser fuente valiosa de proteína, minerales y aceite.

Por otro lado, los análisis del aceite de la almendra sugieren que es fuente de aceite para usos especiales (Aguerrebere, Rojas Molina, Oomah & Drover, 2011). Por ejemplo, el ácido α-eleosteárico ha sido efectivo para suprimir el crecimiento de células cancerígenas y se ha propuesto como agente quimoterapéutico contra el cáncer de seno (Aguerrebere *et al*., 2011). En conclusión, hay un conjunto de hallazgos recientes que aumentan significativamente el potencial terapéutico del capulín como alimento funcional, ingrediente nutracéutico y suplemento alimenticio.

1.8.3. Especie invasora en Europa

El capulín es una especie nativa de Norteamérica; pero, por distintos motivos fue introducida a Europa hace cientos de años, especialmente a Alemania, Bélgica y Países Bajos. En el siglo XVII fue inicialmente introducida como ornamental, y a finales del siglo XVIII era ampliamente sembrada como forestal. Sin embargo, cuando su uso comercial como maderable fue cuestionado comenzó a usarse con fines no maderables, tales como la mejora de suelos debido a la baja proporción C/N en sus hojas (Starfinger *et al*., 2003). A mediados del siglo XX la especie se había naturalizado en el occidente de Europa, y a finales de ese siglo fue considerada una plaga forestal invasiva (Figura 2) (Closset-Kopp, Chabrerie, Valentin, Delachapelle & Decocq, 2007; Pairon *et al*., 2006a; Starfinger *et al*., 2003).

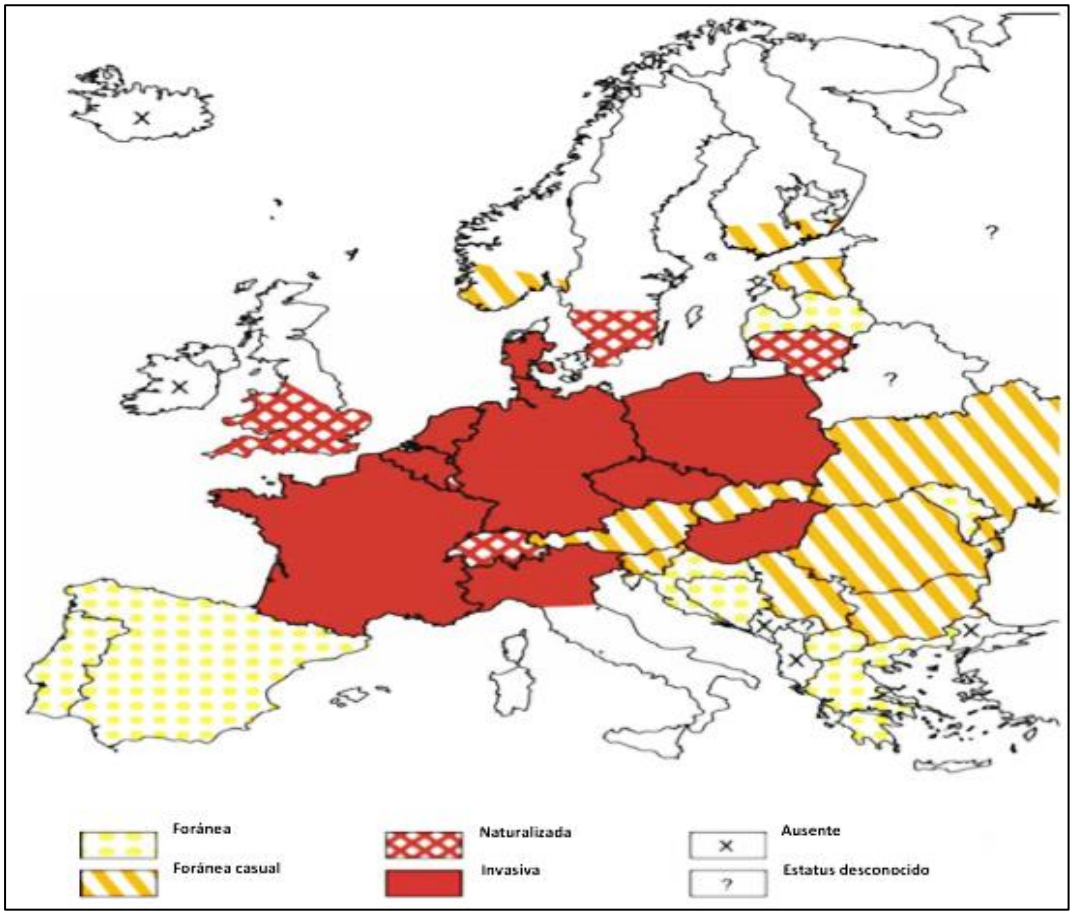

Figura 2. Invasión de *P. serotina* a los bosques europeos. Tomado de Vanhellemont (2009).

Varios trabajos han sido planteados para investigar y caracterizar los factores que contribuyen a la invasividad del capulín en los bosques europeos. En estas investigaciones se ha evaluado la capacidad de *P. serotina* para invadir bosques considerando los efectos de la estructura del paisaje (Deckers, Verheyen, Hermy & Muys, 2005), las variables ecológicas (Godefroid, Phartyal, Weyembergh & Koedam, 2005; Verheyen, Vanhellemont, Stock & Hermy, 2007), sus características reproductivas (Pairon *et al*., 2006a), la historia de la perturbación (Chabrerie, Verheyen, Saguez & Decocq, 2008), y la presión de propágulos (Vanhellemont, Verheyen, De Keersmaeker, Vandekerkhove & Hermy, 2009).

Deckers *et al*. (2005) examinaron los efectos de la estructura del paisaje sobre las dinámicas espacial y temporal de la expansión de una población de capulín dentro de un paisaje rural en Flanders (Bélgica), caracterizado por poseer una densa red de setos interconectados. Se estudiaron 2962 árboles de capulín encontrados en 251 ha, que poblacionalmente se caracterizaban por tener una distribución exponencial negativa de la edad, alta tasa de crecimiento, y una reproducción temprana y continua a lo largo del ciclo de vida. Además, la tasa de expansión de la especie a lo largo de los setos había incrementado progresivamente en el tiempo, especialmente durante la década previa al estudio. El análisis del patrón de puntos espaciales reveló que los árboles presentaban una distribución significativamente agregada, especialmente alrededor de las fuentes de semilla, intersecciones de los setos y árboles de percha. Por otro lado, una regresión logística confirmó los efectos de la estructura del paisaje sobre la ocurrencia de *P. serotina*, sugiriendo dispersión direccional a larga distancia por acción de las aves y resultando en una presión diferencial de semilla a lo largo de la red de setos, ya que las las aves dispersoras preferían percharse cerca de las intersecciones de setos estructuralmente ricos con árboles grandes. Por lo tanto, la distribución del capulín en este paisaje agrícola estuvo fuertemente mediado por los procesos de dispersión de semilla. Adicionalmente, la disminución de la agregación a lo largo del ciclo de vida (plántulas y árboles jóvenes muy agregados, pero los adultos con tendencia aleatoria) se consideró

un indicativo de mortalidad dependiente de la densidad, probablemente causada por la competencia intraespecífica.

Por otro lado, Godefroid *et al*. (2005) estudiaron los factores ecológicos que afectan la abundancia de *P. serotina* en los bosques de Bélgica, y observaron una correlación negativa entre la riqueza de especies en la capa herbácea y la abundancia de *P. serotina* en la capa arbustiva. Además, encontraron que la pendiente y la abundancia de luz fueron los únicos factores abióticos medidos que explicaron porciones significativas de la variación en la abundancia del capulín. Las mediciones de la intensidad de luz sugirieron que la respuesta a los cambios de intensidad es diferente cuando el individuo de capulín madura: las plántulas mostraron respuesta positiva entre 58 % y 80 % de intensidad de luz total, y respuesta negativa a intensidades de luz más bajas; de otra parte, la tendencia se invertía en los árboles juveniles. Según Closset-Kopp *et al*. (2007), nuevamente se requieren altas intensidades de luz en la etapa del crecimiento de los individuos juveniles hasta su madurez y producción de semilla; en este sentido, los árboles jóvenes pueden adoptar una estrategia de "sentarse-y-engordar" (*sit-and-weight strategy*), formando bancos de plántulas, hasta que un hueco o abertura en el dosel (claro de luz) permita el paso de suficiente luz. En consecuencia, el establecimiento y la persistencia de *P. serotina* dependen de la apertura del dosel.

Pairon *et al*. (2006a) para mejorar el entendimiento de la capacidad invasiva del capulín, analizaron su dinámica de regeneración sexual durante dos años consecutivos en una plantación belga de pinos (*Pinus sylvestris* L. y *Pinus nigra* Arn.). Los resultados mostraron que la producción de frutos es alta (aún en el sotobosque de una plantación de pinos) debido a la gran cantidad de flores producidas por árbol, y a pesar de la baja proporción fruto/flor. Por su parte, las semillas se agruparon en dos clases, permitiendo la cobertura completa del área de estudio: la mayoría eran grandes y se dispersaban por acción de la gravedad, y las más pequeñas por las aves. La alta densidad de semillas permitía que cada año el suelo del bosque estuviera cubierto por plántulas de capulín, aunque las

tasas de germinación y de supervivencia de plántulas menores de cuatro años fueran bajas; además, la alta tasa de supervivencia de los individuos juveniles asegura el mantenimiento de la población. Para Potter (2011) las características ecológicas y reproductivas de *P. serotina* parecen haberla predispuesto a ser una especie invasora altamente exitosa en los bosques europeos.

Chabrerie *et al*. (2008) compararon comunidades vegetales, propiedades del suelo e historia de la perturbación entre dos sitios (uno invadido por *P. serotina* y el otro no) de un bosque con cierto grado de manejo. Ellos se plantearon determinar si estas características eran diferentes entre los sitios debido a la presencia del capulín, y si la magnitud de las diferencias estaba asociada con la densidad de árboles de *P. serotina* en el dosel del bosque. Para hacer esto usaron un análisis de redundancias con partición de la variación, que permitió encontrar que los dos sitios: 1) diferían en varias características del suelo; pero, que estas eran componentes de la invasibilidad más que efectos de la invasión; 2) presentaban similar historia de perturbación; excepto por la cantidad de árboles caídos debido a tormentas, que se correlacionó con la densidad de la invasora; 3) las áreas perturbadas por jabalíes salvajes fueron más importantes debajo del dosel del capulín, sugiriendo un efecto positivo sobre su establecimiento; y 4) las condiciones del hábitat y las perturbaciones explicaron la mayoría de la variación, tanto en diversidad vegetal como en densidad de *P. serotina* (estas dos últimos factores presentaron débil asociación directa). Basándose en estos resultados los autores concluyeron que en el corto plazo en los ecosistemas forestales manejados, donde las comunidades vegetales están sujetas principalmente a factores no-interactivos y a procesos de migración, las especies no-nativas se naturalizan sin la influencia directa de características medibles de la comunidad vegetal del ambiente receptor.

De otra parte, Vanhellemont *et al*. (2009) notaron que la mayoría de los estudios europeos sobre *P. serotina* se realizaban en áreas fuertemente invadidas donde la especie había sido introducida intencionalmente, por esto estudiaron su comportamiento invasivo en un ambiente en el que la especie no hubiera sido

favorecida por la introducción deliberadamente antropocéntrica y con baja presión de propágulos. Analizando los inventarios de tres años distintos (1986, 1996 y 2006) de una reserva forestal de Bélgica con las características deseadas, encontraron que la propagación de *P. serotina* en esta reserva había disminuido desde su primera introducción en 1970. Estos autores asumieron que los principales impulsores de la presencia del capulín eran la fuente de semilla y la disponibilidad de luz, ya que los eventos de dispersión a larga distancia y la perturbación parecían impulsar la colonización. De otra parte, no encontraron evidencias de que *P. serotina* hubiera inhibido la regeneración de las especies nativas de este sotobosque. Finalmente concluyeron que el capulín no podía ser considerado una invasora agresiva en el área de estudio; sin embargo, resaltaron que futuras perturbaciones del dosel podrían acelerar su propagación y hacerla invasiva.

Por varias décadas se han buscado estrategias de erradicación intensa para controlar esta especie invasiva (Starfinger *et al*., 2003). Sin embargo, estas no se han popularizado porque son costosas, toman mucho tiempo y su éxito es variable. Curiosamente, parece que el mejor método para controlarla implica asumir que *P. serotina* seguirá haciendo parte de los bosques europeos, y que su impacto y dominancia pueden reducirse controlando las perturbaciones en el dosel, incluso en aquellos sin especies tolerantes a la sombra que le compitan (Starfinger *et al*., 2003; Vanhellemont *et al*., 2009; Vanhellemont, Baeten, Verbeeck, Hermy & Verheyen, 2011).

1.9. ASPECTOS GENÉTICOS

1.9.1. Relaciones filogenéticas con otras especies de *Prunus*

El capulín está incluido en el subgénero *Padus* (Rehder, 1940) y se ha usado en algunos estudios filogenéticos que evalúan la clasificación infra genérica de *Prunus*; estos han permitido identificar sus parientes cercanos. Sin embargo, discrepancias en los resultados de algunos trabajos, sugerirían que la

identificación incorrecta de las accesiones usadas condujo a errores de interpretación de las especies más emparentadas con el capulín.

Lee y Wen (2001) evaluaron la clasificación infra genérica analizando secuencias ITS nucleares de 35 especies (39 taxones), y reportaron que *P. serotina* era especie hermana de *P. virginiana*. Posteriormente, Bortiri *et al*. (2001) reconstruyeron la filogenia del género usando 48 especies, y analizando secuencias nucleares ITS y del espaciador de cloroplasto *trnL-trnF*. La accesión de *P. serotina* usada por estos autores fue diferente a la de Lee y Wen (2001), y sirvió para mostrar que la especie sería hermana de *Prunus mahaleb* L. (subgénero *Cerasus*).

Shaw y Small (2004) usaron la secuencia de siete regiones no-codificantes de cloroplasto (cuatro intrones y tres espaciadores intergénicos) para evaluar la monofilia de la sección *Prunocerasus* del subgénero *Prunus*. Sus resultados indicaron que *P. serotina* sería cercana a un clado conformado por *P. virginiana* y *Prunus laurocerasus* L., y que *Prunus caroliniana* Aiton está emparentada con estas tres; pero, no apoyaron la relación estrecha entre *P. serotina* y *P. mahaleb* planteada en el trabajo de Bortiri *et al*. (2001).

Debido a estos contrastes, Bortiri *et al*. (2006) utilizaron una accesión de *P. serotina* distinta a la usada por Lee y Wen (2001) y Bortiri *et al*. (2001), para incluirla en su estudio filogenético del género. La identidad de esta accesión fue confirmada morfológicamente, antes de incluirla en los análisis. En este trabajo se utilizaron 25 caracteres morfológicos de 38 especies, y datos de secuencia de una región ITS nuclear y de tres de cloroplasto (dos espaciadores intergénicos y un intrón). Según los resultados, con esta nueva accesión, *P. serotina* es especie hermana de *P. caroliniana*. A su vez, el clado integrado por estas dos especies es muy cercano a *Punus ilicifolia* (Nutt. ex Hook. & Arn.) D. Dietr., y el clado más cercano a estas tres quedó conformado por *P. virginiana* y *P. padus* (*European bird cherry*). En conclusión, este trabajo y el de Shaw y Small (2004), no encontraron evidencia de que el capulín se relacione estrechamente con *P.*

virginiana (Lee & Wen, 2001) o con *P. mahaleb* (Bortiri *et al*., 2001); pero, contrastan en la cercanía de *P. serotina* con *P. laurocerasus* y *P. caroliniana*.

Para Bortiri *et al*. (2006) la explicación más admisible de las diferencias entre los resultados de estos trabajos es que la accesión usada por Lee y Wen (2001) realmente era *P. virginiana*, y estaba erróneamente identificada como *P. serotina*. Según estos autores, las dos especies son similares morfológicamente; pero, difieren principalmente en que *P. serotina* tiene hipantio persistente, y esta característica solo es observable cuando el espécimen tiene frutos. Del mismo modo, sugirieron que la accesión usada por Bortiri *et al*. (2001) probablemente también fue mal identificada y en realidad sería *P. mahaleb*. En resumen, los estudios filogenéticos de *Prunus* basados en caracteres morfológicos, secuencias de ITS de genes ribosomales y secuencias espaciadoras de cloroplasto indicaban que las especies del género más emparentadas con el capulín son *P. laurocerasus*, *P. virginiana*, *P. caroliniana*, *P. ilicifolia* y *P. padus* (European bird cherry).

Complementariamente, Chin *et al*. (2014) realizaron una amplia revisión del género, que no solo incluyó especies cultivadas y a sus parientes cercanos, sino especies de las regiones tropicales de América y del sureste de Asia. Para hacerlo utilizaron cuatro marcadores de plastidio y una región nuclear ITS. Los resultados mostraron un grupo al que se denominó Templado-racemoso, y en el que se resolvieron tres subclados: 1) *P. laurocerasus – P. wallichii*, 2) *P. serotina – P. hypoleuca* y 3) *P. virginiana – P. padus*, *P. grayana*. Sin embargo, las relaciones entre estos clados no se resolvieron, sino que se unieron en una politomía.

Hace poco se secuenció el genoma de cloroplasto del capulín (Luan, Gao, He, Bi & He, 2017), y su análisis filogenético bayesiano reveló una estrecha relación con la especie frutal silvestre y multipropósito originaria de Asia localmente conocida como *Sohiong* (*P. napaulensis*). Esta secuencia tendrá gran utilidad en estudios genómicos poblacionales para obtener información valiosa con fines de uso y conservación *in situ* y *ex situ* de la especie.

1.9.2. Nivel de ploidía

Los niveles de ploidía y las relaciones filogenéticas entre las subespecies de *P. serotina* no han sido descritos conjuntamente; hasta ahora solo se conoce que hay autoincompatibilidad e incompatibilidad cruzada entre algunos individuos (Forbes, 1969). Establecer con certeza la ploidía de las subespecies de *P. serotina* permitiría usarlas más efectivamente.

Para determinar el nivel de ploidía generalmente se usan dos métodos: i) el conteo de cromosomas y ii) la citometría de flujo. Esta última es una técnica de cuantificación del genoma que se desarrolló para investigación biomédica, y que posteriormente fue adaptada para analizar genomas vegetales (Galbraith *et al*., 1983).

Determinar la variación inter e intraespecífica del contenido de ADN es importante para anticipar el éxito de eventos de hibridación en los programas de mejoramiento y manipulación genética (Dolezel & Bartos, 2005; Dolezel, Dolezelová & Novák, 1994). Segura *et al*. (2007) usaron la citometría de flujo para estimar con precisión el nivel de ploidía de 23 especies de *Opuntia*, y concluyeron que no había correlación entre los niveles de ploidía que ellos determinaron y el origen geográfico de las especies evaluadas, aunque resaltaron que sus resultados representan una oportunidad para evaluar la utilidad del tamaño del genoma de *Opuntia* como predictor de respuestas ambientales y evolutivas.

Algunos integrantes de la familia *Rosaceae* presentan alteraciones en el número cromosómico, dándoles características particulares a los individuos que las portan. Por ejemplo, en el cerezo estas alteraciones controlan la auto-incompatibilidad gametofítica, presente en la mayoría de los individuos tetraploides, que tiene consecuencias en el interés productivo y de investigación de esta especie.

Algunos trabajos sugieren que el capulín no sería la excepción a estas alteraciones cromosómicas. Se ha reportado que el número cromosómico básico del género *Prunus* es $x = 8$ (Ewert, 1922) y que *P. serotina* es tetraploide ($2n =$

4*x* = 32) (Pairon & Jacquemart, 2005). Forbes (1969) realizó cariotipos a partir de semilla y observó 32 cromosomas en individuos tetraploides de Tennessee, mientras que en diploides aseguró haber observado claramente de 22 a 25 cromosomas y segmentos. No obstante, también se han referenciado niveles de ploidía 5*x* y 6*x* (Dickson *et al*., 1992). Por lo tanto, en el capulín las variaciones de ploidía irían desde diploides hasta hexaploides, aunque no se han referenciado individuos haploides y triploides. Sin embargo, los estudios citológicos de la especie son realmente escasos. El valor-C (peso y número de bases del genoma de una especie) reportado para *P. serotina* en la base de datos del Jardín Botánico del Kew, indica tetraploidía (4*x*) y que el valor-C es no-cambiante (Bennett & Leitch, 2012).

Actualmente *P. serotina* es considerada una especie de origen alopoliploide, aunque no hay certeza sobre sus especies progenitoras (Pairon & Jacquemart, 2005). Los alopoliploides se forman por un proceso de hibridación y la subsecuente duplicación cromosómica (Bennett, 2004). Además, es conocido que los alopoliploides segregan de manera disómica y que durante la meiosis hay escasa o nula recombinación entre los genomas progenitores. Pairon, Jacquemart y Potter (2008) realizaron cinco cruces controlados de capulín para evaluar 67 microsatélites desarrollados en tres especies de *Prunus* con importancia económica, cinco de estos fueron identificados como SSR genoma-específicos (i.e., los cebadores son específicos a uno de los dos genomas que inicialmente formaron la especie alopoliploide) y permitieron establecer un típico patrón de herencia disómica en los embriones resultantes, en el cual no existía evidencia de recombinación intergenómica.

1.9.3. Análisis de la variabilidad molecular

Los primeros trabajos moleculares se enfocaron principalmente en la transferabilidad de los microsatélites o Repeticiones de Secuencia Simple (SSR, sus siglas en inglés: *Simple Sequence Repeats*), e identificaron un conjunto que posteriormente ha sido muy útil para evaluar la variabilidad molecular del capulín (Cuadro 1). La transferibilidad de los SSR facilita el trabajo con especies no-

modelo que carezcan de secuencias publicadas en bases de datos porque no hay que desarrollarlos *de novo.* La tasa de transferabilidad aumenta si los taxa implicados son filogenéticamente cercanos.

Los SSR por ser codominantes, reproducibiles, abundantes y multialélicos han sido útiles en estudios de diversidad genética de *Prunus* (Dirlewanger *et al*., 2002; Downey & Iezzoni, 2000; Hormaza, 2002), patrón de dispersión de sus semillas (Godoy & Jordano, 2001; Schueler, Tusch, Schuster & Ziegenhagen, 2003), ligamiento genético (Aranzana *et al*., 2003), cartografía molecular (Decroocq, Hagen, Favé, Eyquard & Pierronnet, 2004) y certificación de cultivares (Aranzana, Garcia-Mas, Carbó & Arús, 2002; Schueler *et al*., 2003). Además, esta técnica molecular es ideal en el diseño de estrategias para la conservación y uso de recursos genéticos (Vicente, 2002). Los microsatélites son particularmente útiles para entender los patrones de la variación genética, cuando son utilizados conjuntamente con marcadores de ADN de cloroplasto (Saltonstall, 2003).

Cuadro 1. Marcadores SSR informativos usados en la evaluación de la variabilidad molecular del capulín. Los siete SSR en negrita y subrayados son genómico-específicos.

Evaluación original (*Prunus* spp.)	Nombre del locus	Cebadores (5' → 3')	Evaluación en *P. serotina*	Tamaños de alelo (pb)	Número de alelos		Temperatura de alineamiento (ºC)
					Máximo por individuo	Total	
Cipriani *et al*. (1999)	UDP96-001	F: agtttgattttctgatgcatcc	Pairon *et al*. (2008)	97 – 107	4	n/a	56
Testolin *et al*. (2000)		R: tgccataaggaccggtatgt	Guadalupe *et al*. (2015)	102 – 120	n/a	7	56.3
	UDP96-005	F: gtaacgctcgctaccacaaa	Pairon *et al*. (2008)	82 – 88	2	4	56
		R: cctgcatatcaccacccag	Beck *et al*. (2014)	91 – 110		9	53
	UDP96-019	F: ttggtcatgagctaagaaaaca	Pairon *et al*. (2008)	202 – 236	4	n/a	56
		R: tagtggcacagagcaacacc					
	UDP97-402	F: tcccataaccaaaaaaaacacc	Pairon *et al*. (2008)	123 – 145	4	n/a	56
		R: tggagaagggtgggtacttg					
Testolin *et al*. (2000)	UDP98-022	F: ctagttgtgcacactcacgc	Pairon *et al*. (2008)	112 – 138	4	n/a	56
		R: gtcgcaggaacagtaagcct					
	UDP98-025	F: gggaggttactatgccatgaag	Pairon *et al*. (2008)	109 – 127	2	9	56
		R: cgcagacatgtagtaggacctc					
Cipriani *et al*. (1999)	**UDP98-405**	F: acgtgatgaactgacaccca	Pairon *et al*. (2008)	113 – 119	2	3	56
Testolin *et al*. (2000)		R: gagtctttgctctgccatcc	Beck *et al*. (2014)	109 – 125		9	53
	UDP98-406	F: tcggaaactggtagtatgaacaga	Pairon *et al*. (2008)	77 – 101	4	n/a	56
		R: atgggtcgtatgcacagtca					
	UDP98-408	F: acaggcttgttgagcatgtg	Pairon *et al*. (2008)	65 – 406	4	n/a	56
		R: ccctcgtgggaaaatttga					
Testolin *et al*. (2000)	UDP98-410	F: aatttacctatcagcctcaaa	Pairon *et al*. (2008)	114	1	1	56
		R: tttatgcagtttacagaccg	Guadalupe *et al*. (2015)	110 – 121	n/a	4	49
	UDP98-416	F: ttttctcagcagccaaacaa	Pairon *et al*. (2008)	102 – 106	4	n/a	56
		R: atgtttcgtgcttctgctcc	Guadalupe *et al*. (2015)	98 – 111	n/a	7	

Cuadro 1. Continuación.

Evaluación original (*Prunus* spp.)	Nombre del locus	Cebadores (5' → 3')	Evaluación en *P. serotina*	Tamaños de alelo (pb)	Número de alelos		Temperatura de alineamiento (ºC)
					Máximo por individuo	Total	
Downey & Iezzoni (2000)	**pchpgms2**	F: gtcaatgagttcagtgtctacactc	Pairon & Jacquemart (2005)	130 – 154	2	6	60
		R: aatcataacatcattcagccactgc	Guadalupe *et al*. (2015)	130 – 153	n/a	5	52
	pchpgms3	F: acgctatgtccgtaccatctccatg	Pairon & Jacquemart (2005)	182 – 232	4	16	60
		R: caacctgtgattgctcctattaaac	Guadalupe *et al*. (2015)	169 – 230	n/a	8	
	PceGA34	F: gaacatgtggtgtgctggtt	Pairon & Jacquemart (2005)	136 – 162	2	9	60
		R: tccactaggaggtgcaaatg	Beck *et al*. (2014)	128 – 174	2	21	53
			Guadalupe *et al*. (2015)	140 – 168	n/a	10	60
	Ps12a02	F: gccaccaatggttcttcc	Pairon & Jacquemart (2005)	146 – 168	4	6	56
		R: agcaccagatgcacctga	Guadalupe *et al*. (2015)	150 – 160	n/a	4	62
Dirlenwager *et al*. (2002)	BBPCT-002	F: tcgacagcttgatcttgacc	Pairon *et al*. (2008)	179 – 191	4	n/a	56
		R: caatgcctacggagataaaagac					
	BBPCT-017	F: caatgcctacggagataaaagac	Pairon *et al*. (2008)	316 – 364	4	n/a	56
		R: aagcataatttagcataaccaagc	Guadalupe *et al*. (2015)	310 – 360	n/a	4	53.2
	BBPCT-024	F: gaggaatgtgcctcttctgg	Pairon *et al*. (2008)	92 – 96	4	n/a	56
		R: ctcccgtacgcgtttacc					
	BBPCT-025	F: tcctgcgtagaagaaggtagc	Pairon *et al*. (2008)	188 – 206	4	n/a	56
		R: cgacataaagtccaaatggc					
	BPPCT-007	F: tcattgctcgtcatcagc	Pairon *et al*. (2008)	149	1	1	56
		R: cagatttctgaagttagcggta					
	BPPCT-033	F: gtagccggagccgtgtat	Pairon *et al*. (2008)	144	1	1	56
		R: ctagaaccctataaacacatggc					
Yamamoto *et al*. (2002)	M12a	F: aggtgcctcatcttcttctcttg	Pairon & Jacquemart (2005)	170 – 216	4	14	56
		R: gtgtggtgaggggtgagagc					
	M4c	F: gaatttgttctctctctctc	Pairon & Jacquemart (2005)	65 – 89	4	11	60
		R: ggaagcgttcgtctgcaaat	Beck *et al*. (2014)	65 – 95		15	53

Cuadro 1. Continuación.

Evaluación original (*Prunus* spp.)	Nombre del locus	Cebadores (5' → 3')	Evaluación en *P. serotina*	Tamaños de alelo (pb)	Número de alelos		Temperatura de alineamiento (ºC)
					Máximo por individuo	Total	
Sosinski *et al*. (2003)	PS01H03	F: tgaggagcataatgacagt	Pairon *et al*. (2008)	Amplificación poco confiable			56
		R: tcaccatgtgtcatact					
	PS08E08	F: cccaatgaacaactgcat	Pairon *et al*. (2008)	135	1	1	56
		R: catatcaatcactgggatg					
	PS7A02	F: cagggaaatagataagatg	Pairon *et al*. (2008)	66 – 92	4	n/a	56
		R: tctaatggtggtgttcatt					
Struss *et al*. (2003)	UCD-CH11	F: tgctattagcttaatgcctccc	Pairon *et al*. (2008)	78 – 118	4	n/a	56
		R: atgctgatgtcataaggtgtgc					
	UCD-CH13	F: acccgcttactcagctgaac	Pairon *et al*. (2008)	120	1	1	56
		R: ttagcactaagcctttgctgc					
	UCD-CH14	F: gtacacggacccaatcctg	Pairon *et al*. (2008)	136 – 164	2	12	56
		R: tctaacatcatgttaaacatcg	Beck *et al*. (2014)	124 – 178		28	53
	UCD-CH15	F: tcactttcgtccattttccc	Pairon *et al*. (2008)	Amplificación poco confiable			56
		R: tcattttggtctttgagctcg					
	UCD-CH19	F: gtacaaccgtgttaacagcctg	Pairon *et al*. (2008)	Amplificación poco confiable			56
		R: acctgcactacata agcattgg					
Ahmad *et al*. (2004)	**UCD-CH24**	n/a	Pairon *et al*. (2008)	80 – 106	2	8	56

El primer estudio de diversidad genética del capulín fue realizado por Downey y Iezzoni (2000). Ellos estudiaron 66 accesiones de capulín originarias de los EE.UU., México y Ecuador, evaluando un marcador de ADN cloroplasto derivado de cerezo ácido (*P. cerasus*) y ocho microsatélites, que originalmente habían sido desarrollados en cerezo ácido, durazno (*P. persica*) y cerezo dulce (*P. avium*). Estos autores detectaron polimorfismo en el marcador de cloroplasto y en cuatro SSR, mostrando la utilidad de las técnicas moleculares en estudios genéticos (poblacionales y evolutivos) del capulín, sin necesidad de desarrollar nuevos cebadores.

Pairon y Jacquemart (2005) aumentaron el uso potencial de los SSR en capulín al realizar el primer estudio de patrones herencia en esta especie. Su objetivo era determinar si *P. serotina* es alotetraploide o autotetraploide, usando ocho SSR nucleares (seis de durazno, uno de cerezo dulce y otro de cerezo ácido). Cuatro de estos ya habían sido probados en capulín por Downey y Iezzoni (2000), y los otros cuatro fueron descritos en durazno por Yamamoto *et al*. (2002). La evaluación de la progenie obtenida de tres cruces controlados permitió describir la herencia disómica de los seis SSR polimórficos (dos de estos presentaron la típica herencia diploide mendeliana), ofreciendo argumentos a favor del origen alopoliploide del capulín.

Posteriormente, Pairon *et al*. (2008) realizaron cinco cruces controlados en una población invasiva de capulín en Bélgica y evaluaron 67 microsatélites que habían sido desarrollados en tres especies de *Prunus* con importancia económica, para identificar SSR genoma-específicos en *P. serotina* (i.e., los cebadores son específicos a uno de los dos genomas que inicialmente formaron la especie alopoliploide). Veintiseis SSR amplificaron exitosamente, tanto en los progenitores como en la progenie: cinco fueron monomórficos, y entre los 21 SSR restantes se identificaron cinco loci genoma-específicos. Estos últimos permitieron demostrar un típico patrón de herencia disómica en los embriones resultantes, en el que no existía evidencia de recombinación intergenómica de estos cinco SSR.

El capulín fue introducido a Europa hace casi 400 años y actualmente se considera una especie invasiva que amenaza la diversidad de los bosques europeos (Starfinger *et al*., 2003), por esto se han realizado trabajos que buscan entender su comportamiento invasivo para establecer estrategias de control eficientes. Pairon, Chabrerie, Casado y Jacquemart (2006b) usaron dos enfoques para modelizar la dispersión de la semilla de *P. serotina* producida en el dosel de un área dominada por pinos, considerando la fuerza gravedad (distancias cortas) y la mediación de las aves (distancias largas): un enfoque fue el modelo matemático tradicional o no-genético, y el genético se fundamentó en el uso de SSR polimórficos para relacionar la semilla dispersada con su progenitor materno. La respectiva genotipificación se realizó usando cuatro SSR previamente reportados por Pairon y Jacquemart (2005). En sus conclusiones los autores resaltaron que el enfoque no-genético fue preciso en la predicción del destino de las semillas dispersadas por la gravedad y poco confiable con las de larga distancia. Por su parte, los SSR presentaron información más precisa sobre los eventos individuales de dispersión de semilla mediada por aves y condujeron a un mejor entendimiento del proceso general, mostrando que un gran número de semillas caen cerca del árbol progenitor y sugiriendo que las aves permanecen perchadas mientras comen frutos y regurjitan su semilla.

Petitpierre *et al*. (2009) evaluaron polimorfismos de cloroplasto de *P. serotina* var*. serotina* muestreando cinco individuos por población en siete poblaciones del rango de distribución natural (EE.UU.), y en cuatro invasivas europeas (Italia, Francia, Reino Unido y Dinamarca); adicionalmente incluyeron dos de *P. serotina* var*. rufula* obtenidos en Arizona (EE.UU.) y dos individuos de *P. virginiana*. Su objetivo era deducir el patrón de introducción a Europa. Para hacerlo usaron Polimorfismos de la Longitud de los Fragmentos de Restricción (RFLP) de tres espaciadores intergénicos y siete loci microsatélite amplificados con cebadores universales publicados por Weising y Gardner (1999). En conjunto, el polimorfismo de tres loci SSR y dos RFLP sugirieron que no hubo reducción importante de la diversidad genética durante la invasión; además, la combinación de estos cinco loci permitió identificar seis haplotipos de cloroplasto que

sustentarían la hipótesis de múltiples introducciones de *P. serotina*. y serían particularmente útiles en estudios poblacionales. Según estos autores, el uso combinado de estos cinco loci con los SSR nucleares disponibles (*e.g.*, los de Pairon *et al*. 2008), favorecería la descripción del origen materno de las poblaciones invasivas presentes en Europa. Otro hallazgo interesante de este trabajo es que el locus SSR (ccmp5) presentó una variante rara de 125 pb en los dos individuos de var. *rufula*, que los diferenció de los var. *serotina*.

Pairon *et al*. (2010) analizaron la variabilidad genética de 23 poblaciones invasivas (442 individuos) de ocho países europeos y de 22 poblaciones norteamericanas (321 individuos de EE.UU. y Canadá), usando ocho SSR nucleares descritos por Pairon *et al*. (2008) y cinco regiones no-codificantes de cloroplasto descritas previamente (Petitpierre *et al*., 2009). Este estudio fue realizado para buscar las posibles fuentes de las poblaciones europeas de *P. serotina* y para determinar si las poblaciones invasivas tienen menos diversidad genética que las nativas. La diversidad detectada en el cloroplasto sugirió que hubo múltiples introducciones desde una sola región, el noreste de EE.UU., concretamente del este de los Montes Apalaches y principalmente de la Meseta de Allegheny. Además, los marcadores de ambos genomas permitieron estimar que en el área de invasión hubo poca reducción de la diversidad genética. Poblaciones de Dinamarca, Países Bajos, Bélgica y Alemania mostraron alta diversidad genética pero poca diferenciación entre poblaciones, sustentando la hipótesis de que varios eventos de introducción han ocurrido durante los dos siglos de plantación de *P. serotina* en Europa.

El capulín también fue introducido a algunos países de Suramérica; pero, solo en Ecuador alcanzó relevancia regional en las actividades comerciales (Popenoe & Pachano, 1922). Intriago-Baldeón, Torres, Arahana y Tobar (2013) usaron 12 SSR heterólogos probados anteriormente (Dirlewanger *et al*., 2002; Downey & Iezzoni, 2000; Pairon *et al*., 2008; Testolin *et al*., 2000) para evaluar la diversidad genética de 88 individuos de *P. serotina* recolectados en tres provincias de la Sierra ecuatoriana (Pichincha, Cañar y Azuay). Los resultados de los ocho SSR

polimórficos sugirieron una ligera difrenciación genética entre dos grupos, uno formado por los individuos obtenidos en el norte de Ecuador (Pichincha) y otro por individuos del sur (Cañar y Azuay). Sin embargo, los autores resaltaron la necesidad de aumentar la validez de sus observaciones mejorando la representatividad del muestreo con poblaciones de todas las provincias de la Sierra ecuatoriana. En este sentido, Guadalupe *et al*. (2015) usaron estos ocho SSR polimórficos para evaluar la diversidad genética de 217 individuos de *P. serotina* que representaban ocho provincias a lo largo de la Sierra ecuatoriana. Los análisis revelaron que la diversidad genética de *P. serotina* en Ecuador (He = 0,71) es equivalente a la que encontraron Pairon *et al*. (2010) en poblaciones de Norteamérica (Hs = 0,76) y Europa (Hs = 0,70); y que la riqueza alélica de las poblaciones ecuatorianas es menor frente a la reportada para poblaciones de EE.UU., Canadá (Pairon *et al*., 2010) y México (Downey & Iezzoni, 2000). Estos hallazgos se atribuirían respectivamente a la reproducción alógama del capulín y al efecto fundador inherente a su introducción en Ecuador. Nuevamente se detectó diferenciación genética sutil entre las poblaciones del norte y del sur de la Sierra, sugiriendo que en este país la diversidad genética del capulín está estructurada geográficamente. Según Guadalupe *et al*. (2015), antropocéntricamente, la tenue diferenciación geográfica se derivaría del papel activo del capulín en el intercambio comercial propio del norte y de su poca participación en la del sur; asimismo, podría explicarse con base en las diferencias de las condiciones agroclimáticas y agroecológicas entre las dos regiones. Adicionalmente, sugirieron que la escasa diferenciación poblacional se explicaría considerando la reciente introducción de *P. serotina* y, principalmente, la homogenización ocasionada por sus altas tasas de alogamia y su autoincompatibilidad.

Una característica de los frutales de las rosáceas es la incompatibilidad gametofítica, es decir que los pistilos rechazan el polen de la misma planta. Esta incompatibilidad es determinada por el locus S, que es multialélico, altamente polimórfico y contiene dos genes muy ligados físicamente. En este contexto, Gordillo *et al*. (2015) evaluaron 80 individuos de la subespecie *capuli* obtenidos

en ocho provincias de la Sierra ecuatoriana, para estimar la diversidad alélica del locus S analizando la secuencia de 15 amplicones polimórficos obtenidos con cebadores degenerados, que se diseñaron de regiones conservadas del gen S-RNasa en varias especies *Prunus*. En este trabajo, pionero para *P. serotina*, el análisis de las secuencias permitió identificar 11 alelos putativos del intrón I del gen S-RNasa, que presentaron alta identidad (> 82 %) con los de otras especies *Prunus*.

Beck *et al*. (2014) compararon la variación genética de cinco SSR en siete poblaciones de la subespecie *serotina*, tres recolectadas en el borde occidental (Kansas) de su rango de distribución natural (este de los EE.UU.) y cuatro del centro del mismo (Wisconsin, Missouri, Tennessee y Georgia). Cuatro de los SSR evaluados habían sido reportados previamente como genoma-específicos (Pairon & Jaquemart, 2005; Pairon *et al*., 2008), y los resultados de este estudio lo confirmaron (Cuadro 1). La heterocigosidad esperada no mostró diferencias entre los dos conjuntos de poblaciones; pero, la riqueza alélica fue significativamente más baja en las poblaciones del borde. Basándose en la Hipótesis Centro-Abundante (Brown, 1984), los autores resaltaron que este resultado es análogo a la pérdida de alelos raros por deriva genética esperada en poblaciones demográficamente inestables del borde.

En el contexto del uso forestal del capulín en los EE.UU., Wang y Pijut (2014) desarrollaron protocolos para su micropropagación *in vitro*, la regeneración adventicia y enraizamiento de brotes, y la transformación genética mediada por *Agrobacterium*, orientándose al desarrollo de capulín transgénico más resistente al ataque de insectos, para reducir la ocurrencia de gomosis y mejorar las ganancias económicas que ofrece el uso de su madera. Según estos autores, el entendimiento de la interacción planta-insecto y la comprensión del mecanismo molecular de floración, gracias a la previa clonación y caracterización de genes relacionados con este evento (*AG* y *TFL1,* homólogos de *Arabidopsis*), les han permitido plantear varias estrategias que podrían conocerse en el futuro cercano, y que modificarían la reproducción y la resistencia del capulín. En esencia, este

trabajo sienta las bases del mejoramiento del capulín usando ingeniería genética para favorecer su esterilidad reproductiva y resistencia a plagas.

En cuanto al estudio del genoma nuclear, se ha reportado la caracterización genómica preliminar del capulín y otras nueve especies forestales que carecen de programas de mejoramiento, usando tecnologías de secuenciación de bajo costo (Staton *et al*., 2015). Sin embargo, en este momento los resultados finales no están disponibles públicamente.

1.10. CONCLUSIONES Y PERSPECTIVAS FUTURAS

El capulín es una especie de origen americano que se aprovecha desde épocas prehispánicas en distintas zonas del continente. Es evidente que los estudios frutícolas y de sus propiedades farmacológicas o forestales son parciales en cuanto a la inclusión de los cinco taxones que componen a la especie. En México se ha estudiado a las subespecies *capuli y serotina*, y con menor frecuencia a *virens*. En los Estados Unidos la subespecie *serotina* es la más estudiada. En Europa se supone que la subespecie *serotina* es la que invade sus bosques, pero hasta ahora no se ha reportado la verificación de su identidad taxónómica. Las evaluciones parciales y los vacíos de información, dificultan la generalización de los resultados de caracterización citológica, molecular, morfológica y de las áreas de distribución del complejo botánico. Por lo tanto, se requieren estudios que incluyan a las cinco subespecies para asociar su variación morfológica con la climática. Finalmente, con la disponibilidad de marcadores moleculares y de secuencias del cloroplasto del capulín, los estudios futuros deben centrarse en el estudio de la estructura genética de poblaciones del rango natural de distribución geográfica, la clarificación de las identidades taxonómicas, y en aportar información objetiva para planear la conservación y uso sustentable de los recursos genéticos de la especie.

1.11. LITERATURA CITADA

Adriano-Morán, C.C., & McClung de Tapia, E. (2008). Trees and shrubs: the use of wood in prehispanic Teotihuacan. *Journal of Archaeological Science*, 35(11), 2927-2936.

Aguerrebere, I.A., Rojas Molina, A., Oomah, B.D., & Drover, J.C.G. (2011). Characteristics of *Prunus serotina* seed oil. *Food Chemistry*, 124, 983-990.

Ahmad, R., Potter, D., & Southwick, S.M. (2004). Identification and characterization of plum and pluot cultivars by microsatellite markers. *Journal of Horticultural Science and Biotechnology*, 79(1),164-169, doi:10.1080/14620316.2004.11511743.Almaguer Vargas, G. (1998). *Principios de Fruticultura* (3ª ed.). México: Mundi-Prensa. 370 p.

Aradhya, M.K., Weeks, C., & Simon, C.J. (2004). Molecular characterization of variability and relationships among seven cultivated and selected wild species of *Prunus* L. using amplified fragment length polymorphism. *Scientia Horticulturae*, 103, 131-144, doi:10.1016/j.scienta.2004.05.004.

Aranzana, M.J., Garcia-Mas, J., Carbó, J., & Arús, P. (2002). Development and variability analysis of microsatellite markers in peach. *Plant Breeding*, 121: 87-92, doi:10.1046/j.1439-0523.2002.00656.x.

Aranzana, M.J., Pineda, A., Cosson, P., Dirlewanger, E., Ascasibar, J., Cipriani, G., Ryder, C.D., Testolin, R., Abbott, A., King, G.J., Iezzoni, A.F., & Arús, P. (2003). A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. *Theoretical and Applied Genetics*, 106, 819-825, doi:10.1007/s00122-002-1094-y.

Avendaño-Gómez, A. (2000). *Manejo y aprovechamiento de Prunus serotina subsp. capuli Ehrh. (Rosaceae) en comunidades campesinas de Tlaxcala y el Estado de México* (Tesis de maestría). Colegio de Postgraduados, Montecillo, Texcoco, México. 125 p.

Avendaño-Gómez, A., Lira-Saade, R., Madrigal-Calle, B., García-Moya, E., Soto-Hernández, M., & Romo de Vivar-Romo, A. (2015). Management and domestication syndromes of capulin (*Prunus serotina* Ehrh. ssp. *capuli* (Cav.) Mc Vaugh) in communities of the state of Tlaxcala. *Agrociencia*, 49, 189-204.

Barnd, B.D., & Ginzel, M.D. (2008). Causes of gummosis in black cherry (*Prunus serotina*). USDA Forest Service and Purdue University. FNR-229-W. 6 p.

Beck, J.B., Ferguson, C.J., Mayfield, M.H., & Shaw, J. (2014). Reduced genetic variation in populations of Black Cherry (*Prunus serotina* subsp. *serotina*, Rosaceae) at its western range limit in Kansas. *Northeastern Naturalist*, 21(3), 472-478.

Bennett, M.D. (2004). Perspectives on polyploidy in plants – ancient and neo. *Biological Journal of the Linnean Society*, 82(4), 411-423, doi:10.1111/j.1095-8312.2004.00328.x.

Bennett, M.D., & Leitch, I.J. (2012). Angiosperm DNA C-values database (release 8.0, Dec. 2012). Consultada en http://data.kew.org/cvalues/CvalServlet?querytype=2.

Bortiri, E., Oh, S.H., Jiang, J., Baggett, S., Granger, A., Weeks, C., Buckingham, M., Potter, D., & Parfitt, D. E. (2001). Phylogeny and systematics of *Prunus* (Rosaceae) as determined by sequence analysis of ITS and the chloroplast trnL-trnF spacer DNA. *Systematic Botany*, 26(4), 797-807, doi:10.1043/0363-6445-26.4.797.

Bortiri, E., Vanden Heuvel, B., & Potter, D. (2006). Phylogenetic analysis of morphology in *Prunus* reveals extensive homoplasy. *Plant Systematics and Evolution*, 259, 53-71, doi:10.1007/s00606-006-0427-8.

Brown, J.H. (1984). On the relationship between abundance and distribution of species. *The American Naturalist*, 124(2), 255-279.

Calderón de Rzedowski, G., & Rzedowski, J. (2001). *Flora Fanerogámica del Valle de México* (2ª ed.). Instituto de Ecología, A.C. y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. Pátzcuaro, México. 1406 p.

Cassens DL (2004) Factors affecting the quality of hardwood timber and logs for face veneer. Purdue University Cooperative Extension Service. Department of Forestry and Natural Re- sources, Purdue University, West Lafayette, IN. *Forestry and Natural Resources*, 239: 1-12.

Chabrerie, O., Verheyen, K., Saguez, R., & Decocq, G. (2008). Disentangling relationships between habitat conditions, disturbance history, plant diversity, and American black cherry (*Prunus serotina* Ehrh.) invasion in a European temperate forest. *Diversity and Distributions*, 14, 204-212, doi:10.1111/j.1472-4642.2007.00453.x.

Chin, S.W., Lutz, S., Wen, J., & Potter, D. (2013). The bitter and the sweet: inference of homology and evolution of leaf glands in *Prunus* (Rosaceae) through anatomy, micromorphology, and ancestral-character state reconstruction. *International Journal of Plant Sciences*, 174, 27-46, doi:10.1086/668219.

Chin, S.W., Shaw, J., Haberle, R., Wen, J., & Potter, D. (2014). Diversification of almonds, peaches, plums and cherries – Molecular systematics and biogeographic history of *Prunus* (Rosaceae). *Molecular Phylogenetics and Evolution*, 76, 34-48, doi:10.1016/j.ympev.2014.02.024.

Cipriani, G., Lot, G., Huang, W.G., Marrazzo, M.T., Peterlunger, E., & Testolin, R. (1999). AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. *Theoretical and Applied Genetics*, 99, 65-72, doi:10.1007/s001220051209.

Closset-Kopp, D., Chabrerie, O., Valentin, B., Delachapelle, H., & Decocq, G. (2007). When Oskar meets Alice: does a lack of trade-off in r/K-strategies

make *Prunus serotina* a successful invader of European forests? *Forest Ecology and Management*, 247, 120-130, doi:10.1016/j.foreco.2007.04.023.

Deckers, B., Verheyen, K., Hermy, M., & Muys, B. (2005). Effects of landscape structure on the invasive spread of black cherry *Prunus serotina* in an agricultural landscape in Flanders, Belgium. *Ecography*, 28, 99-109, doi:10.1111/j.0906-7590.2005.04054.x.

Decroocq, V., Hagen, L.S., Favé, M.G., Eyquard, J.P., & Pierronnet, A. (2004). Microsatellite markers in the hexaploid *Prunus domestica* species and parentage lineage of three European plum cultivars using nuclear and chloroplast simple-sequence repeats. *Molecular Breeding*, 13(2), 135-142, doi:10.1023/B:MOLB.0000018761.04559.b3.

Dickson, E. E., Arumuganathan, K., Kresovich, S., & Doyle, J.J. (1992). Nuclear DNA content variation within the Rosaceae. *American Journal of Botany*, 79(9), 1081-1086.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M., Poizat, C., Zanetto, A., Arús, P., & Laigret, F. (2002). Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theoretical and Applied Genetics*, 105, 127-138, doi:10.1007/s00122-002-0867-7.

Dolezel, J., Dolezelová, M., & Novák, F.J. (1994). Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). *Biologia Plantarum*, 36, 351-357, doi:10.1007/BF02920930.

Dolezel, J., & Bartos, J. (2005). Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany*, 95, 99-110, doi:10.1093/aob/mci005.

Downey, S. L., & Iezzoni, A.F. (2000). Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. *Journal of the American Society for Horticultural Science*, 125(1), 76-80.

Ewert, R. (1922). The influence of Bee-Keeping upon the fertilization and yield of fruit plantations. *Bee World*, 4(1), 22-23, doi:10.1080/0005772X.1922.11096310.

Forbes, D. (1969). *Self incompatibility and cross-incompatibility in black cherry* (*Prunus serotina*) (Ph.D. dissertation). University of Florida, Gainesville, USA. 92 p.

Fresnedo-Ramírez, J., Segura, S., & Muratalla-Lúa, A. (2011). Morphovariability of capulín (*Prunus serotina* Ehrh.) in the central-western region of Mexico from a plant genetic resources perspective. *Genetic Resources and Crop Evolution*, 58(4), 481-495, doi:10.1007/s10722-010-9592-2.

Galbraith, D. W., Harkins, K. R., Maddox, J. M., Ayres, N. M., Sharma, D. P., & Firoozabady, E. (1983). Rapid flow cytometric analysis of the cell cycle in

intact plant tissues. *Science*, 220, 1049-1051, doi:10.1126/science.220.4601.1049.

Godefroid, S., Phartyal, S.S., Weyembergh, G., & Koedam, N. (2005). Ecological factors controlling the abundance of non-native invasive black cherry (*Prunus serotina*) in deciduous forest understory in Belgium. *Forest Ecology and Management*, 210, 91-105, doi:10.1016/j.foreco.2005.02.024.

Godoy, J.A., & Jordano, P. (2001). Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. *Molecular Ecology*, 10(9), 2275-2283.

Gordillo, M., Tobar, J., Arahana, V.S., & Torres, M.L. (2015). Identification of S alleles associated with self-incompatibility in capuli (*Prunus serotina* subsp. *capuli*) samples by amplification of the Intron I of the S-RNasa gene. *Avances en Ciencias e Ingenierías*, 7(1), B17-B23.

Guadalupe, J.J., Gutiérrez, B., Intriago-Baldeón, D.P., Arahana, V., Tobar, J., Torres A.F., & Torres, M.L. (2015). Genetic diversity and distribution patterns of Ecuadorian capuli (*Prunus serotina*). *Biochemical Systematics and Ecology*, 60, 67-73, doi:10.1016/j.bse.2015.04.001.

Hormaza, J.I. (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequences repeats. *Theoretical and Applied Genetics*. 104, 321-328, doi:10.1007/s001220100684.

Ibarra-Alvarado, C., Rojas, A., Luna, F., Rojas, J.I., Rivero-Cruz B., & Rivero-Cruz, J.F. (2009). Vasorelaxant constituents of the leaves of *Prunus serotina* "capulín". *Revista Latinoamericana de Química*, 37, 164-173.

Ibarra-Alvarado, C., Rojas, A., Mendoza, S., Bah, M., Gutiérrez, D.M., Hernández-Sandoval, L., & Martínez, M. (2010). Vasoactive and antioxidant activities of plants used in Mexican traditional medicine for the treatment of cardiovascular diseases. *Pharmaceutical Biology*, 48(7), 732-739, doi:10.3109/13880200903271280.

Intriago-Baldeón, D.P., Torres, M.L., Arahana, V., & Tobar, J. (2013). Evaluación de la variabilidad genética del capulí (*Prunus serotina* subsp. *capulí*) en tres provincias del Ecuador. *Revista Ecuatoriana de Medicina y Ciencias Biológicas*, 34, 11-24, doi:10.26807/remcb.v34i1-2.231.

Jiménez, M., Castillo, I., Azuara, E., & Beristain, C.I. (2011). Antioxidant and antimicrobial activity of capulin (*Prunus serotina* subsp. *capuli*) extracts. *Revista Mexicana de Ingeniería Química*, 10(1), 29-37.

Kalkman, C. (1965). The Old World species of *Prunus* subg. Laurocerasus including those formerly referred to Pygeum. *Blumea* 13(1), 1-115. Consultada en http://repository.naturalis.nl/document/566657.

Lee, S., & Wen, J. (2001). A phylogenetic analysis of *Prunus* and the Amygdaloideae (Rosaceae) using ITS sequences of nuclear ribosomal DNA. *American Journal of Botany,* 88(1),150-160, doi:10.2307/2657135.

Luan, A., Gao, A., He, J., Bi, G., & He, Y. (2017). Characterization of the complete chloroplast genome of black cherry (*Prunus serotina* Ehrh.). *Conservation Genetics Resources*, publicado en línea: 22-agosto-2017, doi:10.1007/s12686-017-0826-x.

Luna-Vázquez, F.J., Ibarra-Alvarado, C., Rojas-Molina, A., Rojas-Molina, J.I., Yahia, E.M., Rivera-Pastrana, D.M., Rojas-Molina, A., & Zavala-Sánchez, A.M. (2013). Nutraceutical value of black cherry *Prunus serotina* ehrh. Fruits: Antioxidant and antihypertensive properties. *Molecules*, 18, 14597-14612, doi:10.3390/molecules181214597.

Luna-Vázquez, F.J., Ibarra-Alvarado, C., Rojas-Molina, A., Romo-Mancillas, A., López-Vallejo, F.H., Solís-Gutiérrez, M., Rojas-Molina, J.I., & Rivero-Cruz, F. (2016). Role of nitric oxide and hydrogen sulfide in the vasodilator effect of ursolic acid and uvaol from black cherry *Prunus serotina* fruits. *Molecules*, 21, 78, doi:10.3390/molecules21010078.

Marquis, D.A. (1990). *Prunus serotina* Ehrh. Black Cherry. In R.M. Burns & B.H. Honkala, *Silvics of North America*. Volume 2, Hardwoods. USDA Forest Service. Agriculture Handbook 654, pp. 594-604, Washington, D.C. Consultada en https://www.srs.fs.usda.gov/pubs/misc/ag_654_vol2.pdf.

Maynard, C.A., Kavanagh, K., Fuernkranz, H., & Drew, A.P. (1991). Black Cherry (*Prunus serotina* Ehrh.). In Y. P. S. Bajaj (Ed.). Trees III: Biotechnology in Agriculture and Forestry 16. pp. 3-22. Heidelberg, Berlin: Springer.

Mendoza-Castelán, G., & Lugo-Pérez, R. (2010). *Farmacia viviente: conceptos, reflexiones y aplicaciones. Universidad Autónoma Chapingo*. Consultada en http://www.sagarpa.gob.mx/Delegaciones/hidalgo/Documents/Agricultura%20Familiar/LIBRO%20GMC_Farmacia_Viviente_2010%20DR.%20GUILLERMO%20MENDOZA%20CASTELAN.pdf.

McVaugh, R. (1951). A revision of the North American black cherries (*Prunus serotina* Ehrh., and relatives). *Brittonia*, 7(4), 279-315, doi:10.2307/2804698.

Muratalla, L.A. 1984. Factores limitantes en la producción de capulín (*Prunus serotina* ssp. *capuli*), *Boletín Técnico Colegio de Postgraduados*. Montecillo, Texcoco. México. pp. 18-27.

Niembro Rocas, A., Vázquez Torres, M., & Sánchez Sánchez, O. (2010). *Árboles de Veracruz: 100 especies para la reforestación estratégica.* México: Gobierno del Estado de Veracruz. pp. 86-87.

Olszewska, M. (2007). Quantitative HPLC analysis of flavonoids and chlorogenic acid in the leaves and inflorescences of *Prunus serotina* Ehrh. *Acta Chromatographica*, 19, 253-269.

Pairon, M.C., & Jacquemart, A.L. (2005). Disomic segregation of microsatellites in the tetraploid *Prunus serotina* Ehrh. (Rosaceae). *Journal of the American Society for Horticultural Science*, 130(5), 729-734.

Pairon, M., Jonard M., & Jacquemart, A.L. (2006a). Modeling seed dispersal of black cherry, an invasive forest tree: how microsatellites may help? *Canadian Journal of Forest Research*, 36(6), 1385-1394.

Pairon, M, Chabrerie, O., Casado, C.M., & Jacquemart, A.L. (2006b). Sexual regeneration traits linked to black cherry (*Prunus serotina* Ehrh.) invasiveness. *Acta Oecologica*, 30(2), 238-247, doi:10.1016/j.actao.2006.05.002.

Pairon, M., Jacquemart, A., & Potter, D. (2008). Detection and characterization of genome specific microsatellite markers in allotetraploid *Prunus serotina*. *Journal of the American Society for Horticultural Science*, 133(3), 390-395.

Pairon, M., Petitpierre, B., Campbell, M., Guisan, A., Broennimann, O., Baret, P.V., Jacquemart, A.L., & Besnard, G. (2010). Multiple introductions boosted genetic diversity in the invasive range of black cherry (*Prunus serotina*; Rosaceae). *Annals of Botany*, 105(6), 881-890, doi:10.1093/aob/mcq065.

Pérez-Zabala, J.A. (2007). Estudios sobre el género *Prunus* (Rosaceae) en el Geotrópico: novedades taxonómicas y nomenclaturales para Colombia. *Anales del Jardín Botánico de Madrid*, 64(2), 177-190.

Petitpierre, B., Pairon, M., Broennimann, O., Jacquemart, A.L., Guisan, A., & Besnard. G. (2009). Plastid DNA variation in *Prunus serotina* var. *serotina* (Rosaceae), a North American tree invading Europe. *European Journal of Forest Research*, 128(5), 431-436.

Popenoe, W., & Pachano, A. (1922). The Capulín Cherry: A superior form of the Northern black cherry develop in the highlands of tropical America. *Journal of Heredity, 13*(2)*,* 51-62.

Potter, D. (2011). *Prunus*. In C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources), Temperate Fruits* (pp. 129-145)*.* Heidelberg, Berlin: Springer, doi:10.1007/978-3-642-16057-8_7.

Potter, D., Eriksson, T., Evans, R.C., Oh, S., Smedmark, J.E.E., Morgan, D.R., Kerr, M., Robertson, K.R., Arsenault, M., Dickinson, T.A., & Campbell, C.S. (2007). Phylogeny and classification of Rosaceae. *Plant Systematics and Evolution*, 266, 5-43, doi:10.1007/s00606-007-0539-9.

Raven, P.H. (1975). The Bases of Angiosperm Phylogeny: Cytology. *Annals of the Missouri Botanical Garden*, 62(3): 724-764, doi:10.2307/2395272.

Raya-Pérez, J.C., Aguirre-Mancilla, C.L., Tapia-Aparicio, R., Ramírez-Pimentel, J.G., & Covarrubias-Prieto, J. (2012). Storage proteins characterization and mineral composition of black cherry seed (*Prunus serotina*). *Polibotánica*, *34*, 203-215.

Rehder, A. (1940). Manual of cultivated trees and shrubs hardy in North America exclusive of the subtropical and warmer temperate regions. New York, USA: The Macmillan Company. 996 p.

Rodríguez, L., & Farfán, B. (2014). Manejo tradicional del capulín (*Prunus serotina* spp.) en San Francisco Pichátaro, Michoacán. In G. Arias, F. Zurita & M.L. Uranga (Eds.), *Sustentabilidad e interculturabilidad paradigmas entre la relación cultura y naturaleza*. Michoacán, México. (pp. 145-152).

Rohrer, J.R. (2014). *Prunus* (Rosaceae). In Flora of North America Editorial Committee (Eds.), *Flora of North America North of Mexico*. New York, Oxford. 20+ vols. and. Vol 9, 1993+. pp. 363-365.

Rzedowski, J., & Calderón de Rzedowski, G. (2005). *Prunus serotina* Ehrh. In J. Rzedowski and G. Calderón de Rzedowski (Eds.), *Flora del Bajío y Regiones Adyacentes*. Instituto de Ecología A. C. Centro Regional del Bajío, Pátzcuaro, Michoacán, México. pp. 101-111.

Saltonstall, K. (2003). Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular Ecology*, 12(7): 1689-1702, doi:10.1046/j.1365-294X.2003.01849.x.

Schueler, S., Tusch, A., Schuster, M., & Ziegenhagen, B. (2003). Characterization of microsatellites in wild and sweet cherry (*Prunus avium* L.) - Markers for individual identification and reproductive processes. *Genome*, 46(1), 95-102, doi:10.1139/g02-107.

Segura, S., Scheinvar, L., Olalde, G., Leblanc, O., Filardo, S., Muratalla, A., Gallegos, C., & Flores, C. (2007). Genome sizes and ploidy levels in Mexican cactus pear species (*Opuntia* (Torn.) Mill. Series *Streotacanthae* Britton et Rose, *Leucotrichae* DC., *Heliabravoannae* Scheinvar and *Robustae* Britton et Rose. *Genetic Resources and Crop Evolution*, 54(5), 1033-1041, doi:10.1007/s10722-006-9196-z.

Shaw, J., & Small, R.L. (2004). Addressing the ‘‘hardest puzzle in American pomology’’: phylogeny of *Prunus* sect. *Prunocerasus* (Rosaceae) based on seven noncoding chloroplast DNA regions. *American Journal of Botany*, 91(6), 985-996, doi:10.3732/ajb.91.6.985.

Shi, S., Li, J., Sun, J., Yu, J., & Zhou, S. (2013). Phylogeny and Classification of *Prunus sensu lato* (Rosaceae). *Journal of Integrative Plant Biology*, 55(11), 1069-1079, doi:10.1111/jipb.12095.

Sosinski, B., Gannavarapu, M., Hager, L.D., Beck, L.E., King, G.J., Ryder, C.D., Rajapakse, S., Baird, W.V., Ballard, R.E., & Abbott, A.G. (2000). Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. *Theoretical and Applied Genetics*, 101(3), 421-428, doi:10.1007/s001220051499.

Starfinger, U., Kowarik, I., Rode, M., & Schepker, H. (2003). From desirable ornamental plant to pest to accepted addition to the flora? – the perception of an alien tree species through the centuries. *Biological Invasions*, 5, 323-335.

Staton, M., Best, T., Khodwekar, S., Owusu, S., Xu, T., Xu, Y., Jennings, T., Cronn, R., Arumuganathan, A.K., Coggeshall, M., Gailing, O., Liang, H., Romero-Severson, J., Schlarbaum, S., & Carlson, J.E. (2015). Preliminary genomic characterization of ten hardwood tree species from multiplexed low coverage whole genome sequencing. *PLOS ONE*, 10(12), e0145031, doi:10.1371/journal.pone.0145031.

Struss, D., Ahmad, R., Southwick, S.M., & Boritzki, M. (2003). Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. *Journal of the American Society for Horticultural Science*, 128(6), 904–909.

Testolin, R., Marrazzo, T., Cipriani, G., Quarta, R., Verde, I., Dettori, M.T., Pancaldi, M., & Sansavini, S. (2000). Microsatellite DNA in peach and its use in fingerprinting and testing the genetic origin of cultivars. *Genome*, 43(3), 512-520, doi:10.1139/g00-010.

Vanhellemont, M. (2009). *Present and future population dynamics of Prunus serotina in forests in its introduced range* (Ph.D. dissertation). Ghent University, Ghent, Belgium. 159 p.

Vanhellemont, M., Verheyen, K., De Keersmaeker, L., Vandekerkhove, K., & Hermy, M. (2009). Does *Prunus serotina* act as an aggressive invader in areas with a low propagule pressure?. *Biological Invasions*, 11(6),1451-1462, doi:10.1007/s10530-008-9353-8.

Vanhellemont, M., Baeten, L., Verbeeck, H., Hermy, M., & Verheyen, K. (2011). Long-term scenarios of the invasive black cherry in pine-oak forest: Impact of regeneration success. *Acta Oecologica*, 37(3), 203-211, doi:10.1016/j.actao.2011.02.003.

Vasco, C., Riihinen, K., Ruales, J., & Kamal-Eldin, A. (2009). Phenolic compounds in *Rosaceae* fruits from Ecuador. *Journal of Agricultural and Food Chemistry*, 57(4), 1204-1212, doi:10.1021/jf802656r.

Vázquez-Yanes, C., Batis Muñoz, A.I., Alcocer Silva, M.I., Gual Díaz, M., & Sánchez Dirzo, C. (1999). Árboles y arbustos potencialmente valiosos para la restauración ecológica y la reforestación. Reporte Técnico del Proyecto J084. CONABIO. Universidad Nacional Autónoma de México. México. pp. 227-229.

Vicente de, M.C. (2002). Molecular techniques to facilitate prioritization of plant genetic resources conservation and further research. AgBiotechNet.4 ABN 092, 1-5.

Verheyen, K., Vanhellemont, M., Stock, T., & Hermy, M. (2007). Predicting patterns of invasion by black cherry (*Prunus serotina* Ehrh.) in Flanders (Belgium) and its impact on the forest understorey community. *Diversity and Distributions*, 13(5), 487-497, doi:10.1111/j.1472-4642.2007.00334.x.

Wang, Y., & Pijut, P.M. (2014). Can *Prunus serotina* be genetically engineered for reproductive sterility and insect pest resistance?. *Springer Science Reviews*, 2, 75-93, doi:10.1007/s40362-014-0024-0.

Watkins, R. (1976). Cherry, plum, peach, apricot and almond. In N.W. Simmonds (Ed.), *Evolution of Crop Plants*. London: Longman. pp. 242-247.

Wen, J., Berggren, S.T., Lee, C.H., Ickert-Bond, S., Yi, T.S., Yoo, K.O., Xie, L., Shaw, J., & Potter, D. (2008). Phylogenetic inferences in *Prunus* (Rosaceae) using chloroplast ndhF and nuclear ribosomal ITS sequences. *Journal of Systematics and Evolution*, 46(3), 322-332, doi:10.3724/SP.J.1002.2008.08050.

Webster, A.D., & Lonney, N.E. (1996). *Cherries: Crop Physiology, Production and Uses*. CAB International Publishing. Wallingford, Oxfordshire, UK. 513 p.

Weising, K., & Gardner, R.C. (1999). A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome*, 42(1), 9-19.

Yamamoto, T., Mochida, K., Imai, T., Shi, Y.Z., Ogiwara, I., & Hayashi, T. (2002). Microsatellite markers in peach (*Prunus persica* (L.) Batsch) derived from an enriched genomic and cDNA libraries. *Molecular Ecology Resources*, 2(3), 298-301, doi:10.1046/j.1471-8286.2002.00242.x.

CHAPTER II. MORPHOLOGICAL VARIATION IN BLACK CHERRY (*Prunus serotina* EHRH.) ASSOCIATED WITH ENVIRONMENTAL CONDITIONS IN MEXICO AND THE UNITED STATES

CAPÍTULO II. VARIACIÓN MORFOLÓGICA DEL CAPULÍN (*Prunus serotina* EHRH.) ASOCIADA A LAS CONDICIONES AMBIENTALES DE MÉXICO Y LOS ESTADOS UNIDOS

2.1. ABSTRACT

Black cherries are native to North America and make up a complex of five morphologically different subspecies growing in significantly different niches. This study used 474 presence points, 19 climate parameters and altitude to: 1) describe climatic preference of subspecies, 2) associate the more discriminant bioclimatic variables with variation of 17 quantitative morphological descriptors and 3) estimate the potential effect of climate change in the distribution areas of the complex. Altitude and seven climatic parameters played a considerable role in the differentiation of subspecies. Climate variability indicated that ssp. *eximia*, *hirsuta* and *serotina* thrive in more humid and cold environments, while ssp. *virens* prefers drier and warmer environments. Subspecies *capuli* exhibited the greatest environmental heterogeneity. Morphological differences included longer and wider leaves, and thicker fruit pedicel in ssp. *capuli*; and shorter, smaller leaves in ssp. *virens*. Even though morphological variation associated with climate was relatively low, the study did show possible effects of climatic variability on the morphology and distribution of the *P. serotina* complex and provided reference information that may be useful for an integrated description of its genetic resources. Climate change could potentially cause genetic erosion due to the loss of suitable habitats. Future studies on population genetics can increase understanding of the complex and favor efficient strategic guidelines for use and conservation of these resources.

Keywords: *Prunus serotina*, capulín, genetic erosion, multipurpose fruit tree, timber.

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2.2. RESUMEN

El capulín es un frutal nativo de Norteamérica que conforma un complejo de cinco subespecies, que se diferencian morfológicamente y se desarrollan en nichos significativamente diferentes. En este estudio se usaron 474 puntos de presencia, 19 parámetros climáticos y la altitud para: 1) describir las preferencias climáticas de las subespecies, 2) asociar las variables bioclimáticas más discriminantes y la variación de 17 descriptores morfológicos cuantitativos, y 3) estimar el efecto potencial del cambio climático en las áreas de distribución del complejo. La altitud y siete parámetros climáticos desempeñaron un papel no despreciable en la diferenciación de las subespecies. La variabilidad climática indicó que las ssp. *eximia*, *hirsuta* y *serotina* prosperan en los ambientes más húmedos y fríos, mientras que ssp. *virens* lo hace en los más secos y cálidos. La ssp. *capuli* presentó la mayor heterogeneidad ambiental. La variación morfológica mostró que las hojas de ssp. *capuli* tienden a ser más largas y anchas, y el pedicelo del fruto más grueso. A su vez, las hojas de ssp. *virens* fueron las más cortas y pequeñas. Aunque la variación morfológica asociada al clima resultó relativamente baja, el estudio permite vislumbrar los posibles efectos de la variabilidad climática sobre la morfología y distribución del complejo *P. serotina*, y proporciona información de referencia que será útil en la descripción integral de sus recursos genéticos. El cambio climático potencialmente ocasionaría erosión genética debido a la pérdida de áreas aptas. Futuros estudios genéticos poblacionales aumentarán la comprensión del complejo, y favorecerán el planteamiento de estrategias eficientes de uso y conservación de sus recursos genéticos.

Palabras clave: *Prunus serotina,* black cherry, erosión genética, frutal multipropósito, maderable.

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2.3. INTRODUCTION

Black cherry (*Prunus serotina* Ehrh.), or capulín in Spanish, is a multiuse species native to North America. Its ripe fruits and seeds are consumed in Mexico (Raya-Pérez, Aguirre-Mancilla, Tapia-Aparicio, Ramírez-Pimentel & Covarrubias, 2012); along with the plant's leaves and inflorescences, these have antioxidant and antimicrobial activity and have been used traditionally to treat respiratory diseases (Olszewska, 2007). Mexican peoples have used its timber for firewood since pre-Hispanic times (Adriano-Morán & McClung de Tapia, 2008); while in the United States its timber is valued for its hardness and durability (Maynard, Kavanagh, Fuernkranz & Drew, 1991; Rohrer, 2014), the thickness of its logs and its reddish color (Barnd & Ginzel, 2008; Rohrer, 2014; Wang & Pijut, 2014).

This species grows in a broad range of biotic and abiotic conditions, its natural distribution ranging from temperate zones in Canada and the United States to Mexico and Guatemala, and from sea level to altitudes of 3000 m.a.s.l. (Marquis, 1990; McVaugh, 1951; Rzedowski & Calderón de Rzedowski, 2005). The species was introduced in the seventeenth century in Europe for ornamental and forestry purposes. Currently it is considered an invasive species in several countries (Belgium, France, Germany, Holland, northern Italy, among others) (Starfinger Kowarik, Rode & Schepker, 2003); and a naturalized species in South America (Bolivia, Colombia, Ecuador and Peru) (Popenoe & Pachano, 1922).

Taxonomic treatment of black cherries is a theme for discussion. Black cherry is a botanical complex of five subspecies natives to North America: *Prunus serotina* ssp. *capuli* (Cav.) McVaugh, *Prunus serotina* ssp. *eximia* (Small) Little, *Prunus serotina* ssp. *hirsuta* (Elliot) McVaugh, *Prunus serotina* ssp. *serotina* (Ehrh.) McVaugh, and *Prunus serotina* ssp. *virens* (Wooton *et* Standl.) McVaugh (McVaugh, 1951). According to this author, the five subspecies are geographical races with distinctive morphological characters and habitats. However, partial evaluations of the complex recently done in Mexico hardly differentiated the three Mexican subspecies (*capuli*, *serotina* and *virens*) (Fresnedo-Ramírez, Segura & Muratalla-Lúa, 2011; Rzedowski & Calderón de Rzedowski, 2005). In turn, Rohrer

(2014) proposed four botanical varieties (*alabamensis*, *capuli*, *rufula*, and *serotina*) instead of the five subspecies, thus suggesting that taxonomic differences among infraspecific groups are slighter than firstly stated by McVaugh (1951).

Research has studied its process of domestication and ethnobotanical value (Avendaño-Gómez *et al*., 2015), its physical and chemical characteristics (Aguerrebere, Rojas Molina, Oomah & Drover, 2011), and its innovative use in the therapeutic (Ibarra-Alvarado Rojas, Luna, Rojas, Rivero-Cruz & Rivero-Cruz, 2009; Ibarra-Alvarado *et al*., 2010; Palomares-Alonso *et al*., 2017), functional food (Jiménez, Castillo, Azuara & Beristain, 2011; Raya-Pérez *et al*., 2012) and nutraceutical industries (Luna-Vásquez *et al*., 2013; Luna-Vásquez *et al*., 2016). Nonetheless, little is known about its morphological variability (Fresnedo-Ramírez *et al*., 2011) and the association of this variability with climatic conditions in the areas where black cherries thrive.

Morphological variability of plants is a response to local environmental fluctuations (Ellison, Buckley, Miller & Gotelli, 2004), and it is mainly influenced by climate (Guerin, Wen & Lowe, 2012). Consequently, this variability must be evaluated in order to manage plant genetic resources in a sustainable manner. Based on the central hypothesis that the five subspecies have distinctive morphological variation and that this is related to the climatic conditions they require to thrive, this study was undertaken to: 1) identify and describe the climatic factors that explain the natural geographic distribution of the five black cherry subspecies in Mexico and the United States, 2) associate these factors with variation in the set of morphological descriptors, and 3) estimate potential effects of future climate change on the distribution of the species.

2.4. MATERIALS AND METHODS

2.4.1. Selection of presence points

A data base was constructed by refining information collected of presence sites for the five subspecies of *P. serotina* in North America, from three sources: 1) 36 samples collected in natural distribution areas in 12 Mexican states and four samples in Texas (United States); 2) 622 herbarium specimens reviewed, with their corresponding taxonomic identification; and 3) 154 occurrence data from the portal of the Global Biodiversity Information Facility (GBIF, 2017). Taxonomic identification of materials was verified, based on our knowledge of black cherries and consulting the opinion of botanists with expertise in Mexican flora. Points reported before 1980 were eliminated to avoid possible georeferencing errors.

The 622 specimens examined are deposited in four institutional herbaria in Mexico and two in the United States [*Instituto de Ecología* A.C. (IEB, XAL): 190, *Instituto Politécnico Nacional* (ENCB): 31, National Autonomous University of Mexico (MEXU): 41, University of Sonora (USON): 5, University of Texas at Austin (LL, TEX): 125, and Ohio State University (OS): 230].

Preselected presence points were treated following the procedure proposed by Scheldeman and Zonneveld (2010) to avoid inconsistencies, correct coordinates when possible and eliminate duplicate or doubtful data. As a result of taxonomic verification and refining, the 474 points making up the data base included 234 Mexican records (24 states) and 240 US records (15 states); and represented 137 presence points of ssp. *capuli*, 13 of ssp. *eximia*, seven of ssp. *hirsuta*, 113 of ssp. *serotina* and 204 of ssp. *virens* (Appendix I).

2.4.2. Description of environmental conditions

Distribution of the 474 presence points was described based on classification of ecological units known as ecoregions. This was achieved by using the master layer of spatial data of The Nature Conservancy's (TNC) Terrestrial Ecoregions of the World (http://maps.tnc.org/gis_data.html). This layer provides information

graded by ecozone, terrestrial ecoregion and main habitat type. In the United States it is based on Bailey's ecoregions of the USDA Forest Service and in the remaining countries, it is based on ecoregions identified by the World Wildlife Fund (WWF) (http://maps.tnc.org/files/metadata/TerrEcos.xml).

2.4.3. Climatic values associated with presence points

Altitude and values for the 19 derived bioclimatic variables (Busby, 1991), associated to all presence points, were taken from the WorldClim data base (Hijmans, Cameron, Parra, Jones & Jarvis, 2005), using DIVA-GIS 7.5 (Hijmans, Guarino, Cruz & Rojas, 2001) with a spatial resolution of 2.5 minutes (approximately 4.5 km at the equator).

2.4.4. Characterization of climatic variation

The 20 variables were subjected to the Discriminant Analysis (DA) to construct functions that could explain the climatic preferences of the five subspecies, even based on the small number of variables. STATISTICA 7.0 (StatSoft. Inc., 2004) was used for the analysis, employing the standardized matrix and the forward stepwise selection method for the inclusion of variables. This strategy incorporates variables, one-by-one, in the discriminant function, enabling the construction of a function using only those variables that are actually useful for classifying and evaluating individual contribution of each variable to the model. The variable that minimized the global Wilks Lambda (λ) was introduced in the model in each step, since the model's discriminant power is inversely proportional to the value of this statistic.

2.4.5. Association between morphological variation and climate

The smaller Mahalanobis distances, calculated in the DA, identify presence points that would represent an average of typical environment for each subspecies. Consequently, using the smaller distances to the centroid of each group, 39 sites (14 in Mexico and 25 in the United States) were selected to conduct morphological measurements.

Table 2 shows the 17 quantitative descriptors used, including characteristics of the tree, flowers, fruit and seeds. Their usefulness in differentiating subspecies have been previously established by Fresnedo-Ramírez *et al*. (2011). Morphological measurements in the 14 Mexican sites were done on botanized samples from our field collections, while those in the 25 USA sites were done on herbarium specimens [University of Texas at Austin (LL, TEX) and The Ohio State University (OS)]. When specimens were not available having both flowers and fruits, the corresponding characters were measured in separate specimens.

Table 2. Descriptors used for the morphological evaluation. The code was assigned following the Fresnedo-Ramírez *et al*. (2011) proposal.

Organ	Descriptor number	Morphological descriptor	Code	Unit
Stems and branches	1	Internode length in young stems	ILS	cm
	2	Thickness of young stems	TYS	cm
Leaves	3	Second basal leaf of flowering branch length	2BL	cm
	4	Second basal leaf of flowering branch thickness	2BT	cm
	5	Length/thickness quotient length of second basal leaf of flowering branch	LT2	NA
	6	Petiole length of second basal leaf of flowering branch	PL2	cm
	7	Teeth quantity in second basal leaf margin of flowering branch per 1 cm^2	TQM	Number*c m^{-2}
	8	2BL/PL2 quotient	LPQ	NA
Flower	9	Flower branch length	FBL	cm
	10	Flower pedicel length	FPL	cm
	11	Stamen length	STL	cm
	12	Flower diameter	FLD	cm
	13	Number of flowers per branch	NFB	#
Fruit	14	Fruit pedicel length	FPL	cm
	15	Thickness of fruit pedicel	TFP	cm
Seed	16	Seed length	SEL	cm
	17	Seed diameter	SED	cm

A data matrix containing 25 variables was constructed for the 39 sites. The average of at least 10 measurements for each morphological descriptor and the corresponding values of the 8 most informative variables, as detected by the DA, were recorded in the matrix. This matrix was standardized. A Principal Component Analysis (PCA) was conducted based on the correlation matrix in order to determine if variation in any of the morphological variables was associated with the climatic requirements of each subspecies.

2.4.6. Potential distribution and estimate of potential impact of climate change

This analysis used the Maxent v3.3.3 modeling program (Phillips, Anderson & Schapire, 2006) to predict potential distribution of black cherry under current and future climatic conditions. Distribution area was restricted using the 10 percentile as the threshold in training the model, which indicates the probability value at which 10 % of the presence points fall outside the potential area. Using the corresponding logistical value, current and future grid models were reclassified as presence/absence models and then combined to identify unsuitable areas (current and future), stable areas (currently suitable and suitable in the future), areas lost (currently suitable but not so in the future) and new areas (suitable in the future but not currently suitable). Quantitative estimates (in km^2) of potential changes in distribution area of *P. serotina* were calculated with ArcGIS v10.3 (ESRI, 2013), taking into account areas permanently occupied by masses of water. All layers were used with the Lambert Conformal Conic projection.

Climatic projections towards 2070 (average for 2061-2080) were estimated using two General Circulation Models (GCM): GFDL-CM3 (Geophysical Fluid Dynamics Laboratory Climate Model version 3) and HADGEM2-ES (Hadley Global Environment Model 2 - Earth System). The respective values were downloaded from the WorldClim data base (Hijmans *et al*., 2005). Both models were used in the 8.5 W/m2 (RCP 8.5) radiative forcing scenario of concentration pathways. In this scenario, emission of greenhouse gases of anthropogenic origin will continue

to increase until the end of the twenty-first century, in spite of mitigation efforts (IPCC, 2014).

2.5. RESULTS

2.5.1. Environmental conditions in which the subspecies thrive

According to TNC's classification, the 474 collection sites of *P. serotina* are distributed in two ecozones: Neotropical and Nearctic. The Neotropical zone included 109 Mexican presence points, while the other 125 points were distributed in the Nearctic. These 234 points (ssp. *capuli*, *serotina* and *virens*) were representative of four types of dominant vegetation and 22 ecoregions (Appendix II). The 240 USA points (ssp. *eximia*, *hirsuta*, *serotina* and *virens*) belong to the Nearctic ecozone, and are distributed in 26 ecoregions and five types of vegetation (Appendix II). The Apache Highlands and Chihuahuan Desert ecoregions, and one dominant vegetation type, denominated Deserts and Xeric Shrublands, are present in both countries.

The 137 presence points of ssp. *capuli* were distributed in 17 Mexican ecoregions, concentrated in the Trans-Mexican Volcanic Belt (59 %), the Western (20 %) and Eastern (9 %) Sierra Madre and the Central Mexican Matorral (10 %). The 24 Mexican points of ssp. *serotina* were distributed in 12 ecoregions; however, 10 of these coincide with those of ssp. *capuli* and concentrated 88 % of these points. In turn, the 89 USA points of ssp. *serotina* were distributed in 18 ecoregions, mainly in the *Ozarks* (38 %), Central Tallgrass Prairie (23 %), Osage Plains/Flint Hills Prairie (9 %) and Southern Blue Ridge (5 %).

The 73 Mexican presence points of ssp. *virens* were distributed in nine ecoregions, mainly (60 %) in the Western and Eastern Sierra Madre and in the Trans-Mexican Volcanic Belt; in addition, three ecoregions were exclusive for this subspecies–Apalache highlands (16 %), Sierra de la Laguna Pine-Oak Forests (6 %) and Sonoran-Sinaloan Transition Subtropical Dry Forest (3 %). In turn, the 131 USA points for ssp. *virens* were distributed in six ecoregions–Apalache highlands

(70 %), Arizona-New Mexico Mountains (15 %), Chihuahuan Desert (8 %), Sonoran Desert (5 %), Southern Shortgrass Prairie (<2 %) and Colorado Plateau. The 13 points of ssp. *eximia* belonged to a single ecoregion (Edwards Plateau savanna), while the 7 points of ssp. *hirsuta* were distributed in the Cumberlands and Southern Valley (3), South Atlantic Coastal Plain (2), and Lower England/Northern Piedmont, and Piedmont.

The DA indicated that eight variables were sufficient in the last step to construct discriminant functions that would separate subspecies (Table 3).

Table 3. Summary of the influence of bioclimatic variables on the two main functions that differentiated the five subspecies. The most relevant contributions for each function appear in bold face. The eight variables are organized following the sequence in which they were entered in the construction of the model. Steps = 8, λ (last step) = 0.048, F(32, 1709) = 68.618, p <0.000.

Code	Function 1	Function 2	Variable
BIO17	**-0.572**	0.473	Precipitation of driest quarter
BIO04	-0.188	**0.852**	Temperature seasonality
BIO19	-0.249	0.488	Precipitation of the coldest quarter
BIO12	**-0.423**	-0.223	Annual precipitation
ALT	0.403	-0.498	Altitude
BIO13	-0.062	-0.397	Precipitation of wettest month
BIO15	0.377	**-0.600**	Precipitation seasonality
BIO11	0.115	-0.547	Mean temperature of coldest quarter
% of Variance	65.9	26.9	
capuli	-0.139	-2.030	
eximia	-1.675	1.298	
hirsuta	-3.713	1.517	Scores of centroids
serotina	-3.003	0.830	
virens	1.981	0.765	

Here, climate data was well summarized by the first two discriminant functions, which together explained 93 % of differences in climate requirements of the subspecies (Table 3). Functions correctly classified 84 % of cases studied (Table 4), that is to say, that these were perfectly assigned to their respective climate niches. Classification errors were asymmetrically distributed, showing that *capuli* presence points get confused primarily (11 %) with those of *virens*, and to a lesser extent (2 %) with those of *serotina* and *eximia*. These percentages of confusion can be the result of domestication promoted by Mexican peoples who encouraged the introduction of *capuli* in several environments. Furthermore, 23 % of presence points of the subspecies *serotina* get confused with those of the other four subspecies; this can be explained by the fact that this subspecies is distributed throughout several regions in Mexico and the United States, meaning it is present in a great variety of niches, many of which could be similar to those of the other subspecies.

Table 4. Classification of presence points based on a priori conclusions of belonging to a subspecies. *p: a priori* probability.

From / To	*capuli* $p = 0.288$	*eximia* $p = 0.027$	*hirsuta* $p = 0.015$	*serotina* $p = 0.238$	*virens* $p = 0.432$	Percent correct
capuli	117	2	0	3	15	85.402
eximia	0	13	0	0	0	100.00
hirsuta	0	0	6	1	0	85.714
serotina	16	5	7	82	3	72.566
virens	22	0	0	0	183	89.268
TOTAL	155	20	13	86	201	84.421

As shown graphically, these two functions were sufficient to separate four of the five better represented subspecies in the presence points data base (Figure 3). The first function was dominated by precipitation during the driest quarter (BIO17) and annual precipitation (BIO12) (Table 3). When projecting original values of the eight variables in this function (data not shown), the more humid environments throughout the year (>800 mm) are grouped in the negative extreme and during

the driest quarter (>70 mm), in which ssp. *hirsuta* and *serotina* thrive; and the drier ones in the positive extreme (typical of spp. *virens*) (Figure 3). In the second function, the greatest weightings are derived from temperature seasonality (BIO04) and precipitation seasonality (BIO15); when interpreted with their opposite sign, the positive segment of this function would be associated with environments characterized by greater variability in monthly temperatures (convenient for ssp. *eximia*, *hirsuta*, *serotina* and *virens*), while the negative segment would be associated with environments exhibiting high variability in relation to monthly mean precipitation (primarily those of ssp. *capuli*).

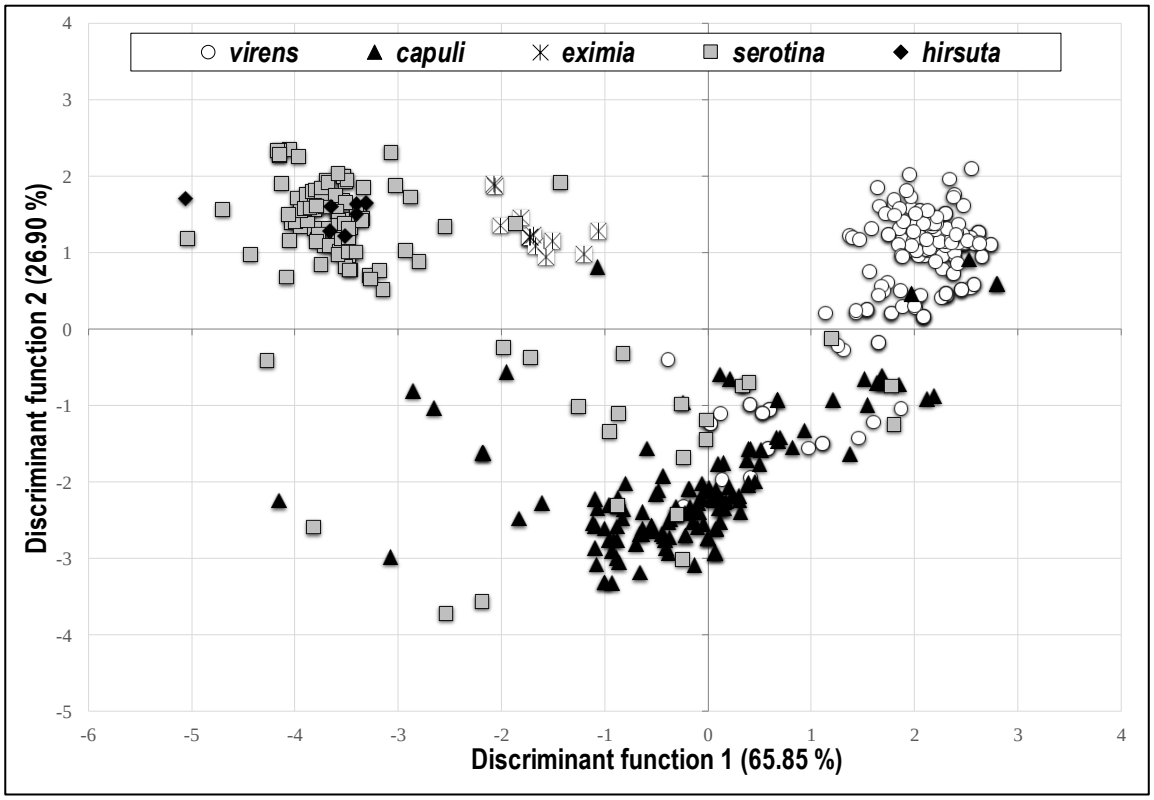

Figure 3. Projection of individuals in the environmental space constituted by the first two discriminant functions.

On the other hand, absolute weighting value of the altitude variable is equivalent in both functions (Table 3). Nonetheless, their opposite signs cause, in Figure 3, that: i) the lower elevation (<1000 masl) environments, suitable for ssp. *eximia*, *hirsuta* and *serotina*, concentrate in quadrant IV, ii) the intermediate elevation (1000< to <2000 masl) environments in quadrant I, where ssp. *virens* is mainly present, and iii) the greater elevation (2000< to <3450 masl) environments in

quadrants II and III, suitable for ssp. *capuli*. In turn, scores of means of canonical variables (Table 3) indicated that the first function discriminates well among subspecies *capuli*, *eximia, serotina* and *virens*, and indicates that subspecies *hirsuta* overlaps ssp. *serotina*. On its part, the second function clearly promotes separation of ssp. *capuli* from the rest.

The original values of the eight variables selected for the DA distribute this complex from sea level to altitudes of 3400 masl (Figure 4). Within this range, presence points of subspecies *eximia* and *hirsuta* are located in areas below 690 masl, while presence points of the other three subspecies are distributed throughout the overall altitude range. Additionally, temperature variables suggest that the complex's area of natural distribution exhibits a broad range of minimum temperatures (-6 to 21 ºC) during the coldest quarter (BIO11). In this range, the colder environments in winter are occupied by subspecies *serotina* (-6 ºC) and *virens* (-3 ºC). In addition, subspecies *eximia* and *hirsuta* occupy a narrow portion (8 to 11 ºC and 0 to 8 ºC, respectively) and ssp. *capuli* is found in areas with minimum temperatures above 5 ºC.

In terms of precipitation variables, the complex is distributed in areas with annual precipitation (BIO12) between 189 and 2,334 mm (Figure 4). Even though subspecies precipitation values overlap, ssp. *virens* inhabits the drier environments throughout the year (BIO12) and during the driest quarter (BIO17), and exhibits high variation of monthly precipitation (BIO15). On the other hand, spp. *serotina* is found in environments with higher annual precipitation regimes (BIO12) and during the driest quarter (BIO17), and those having precipitation seasonality (BIO15).

Subspecies *eximia* inhabits dry environments, basically different from those of ssp. *virens* in that they have more rainfall throughout the year (BIO12) and during the driest quarter (BIO17); but less precipitation during the period with greater rainfall (BIO13). In turn, ssp. *hirsuta* is found in areas annually as humid as those inhabited by ssp. *serotina* but with greater precipitation during the driest (BIO17) and coldest (BIO19) quarters, and with less variation in monthly precipitation (BIO15).

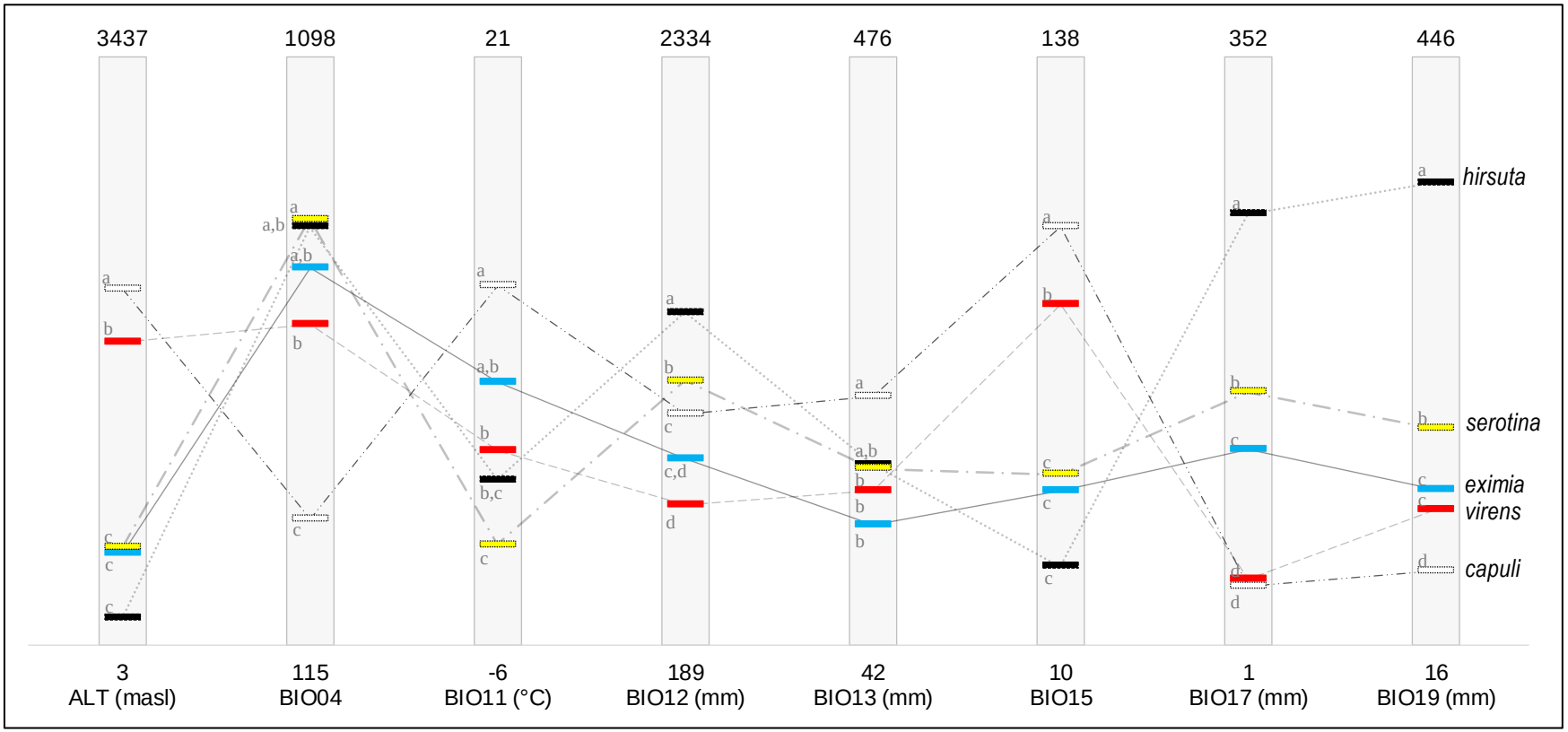

Figure 4. Environmental variation of the *Prunus serotina* complex. Vertical bars represent the more discriminant variables, and their extremes represent their respective minimum and maximum values. Horizontal bars show the average value of each subspecies; letters next to these are the result of Tukey's test for subspecies. Averages with different letters are significantly different (p <0.001). ALT: Altitude. BIO04: Temperature seasonality. BIO11: Average temperature in the coldest quarter. BIO12: Annual precipitation. BIO13: Precipitation of wettest month. BIO15: Precipitation seasonality. BIO17: Precipitation during the driest quarter. BIO19: Precipitation during the coldest quarter.

Since the second function of the DA discriminated ssp. *capuli* well, original values of precipitation variables (data not shown) were projected on this function; resulting in this subspecies being observed in areas with the more humid environments throughout the year (BIO12) and exhibiting the greatest precipitation seasonality (BIO15).

Additionally, the first two functions also discriminated between Mexican and US presence points for subspecies *serotina* and *virens* (Figure 5).

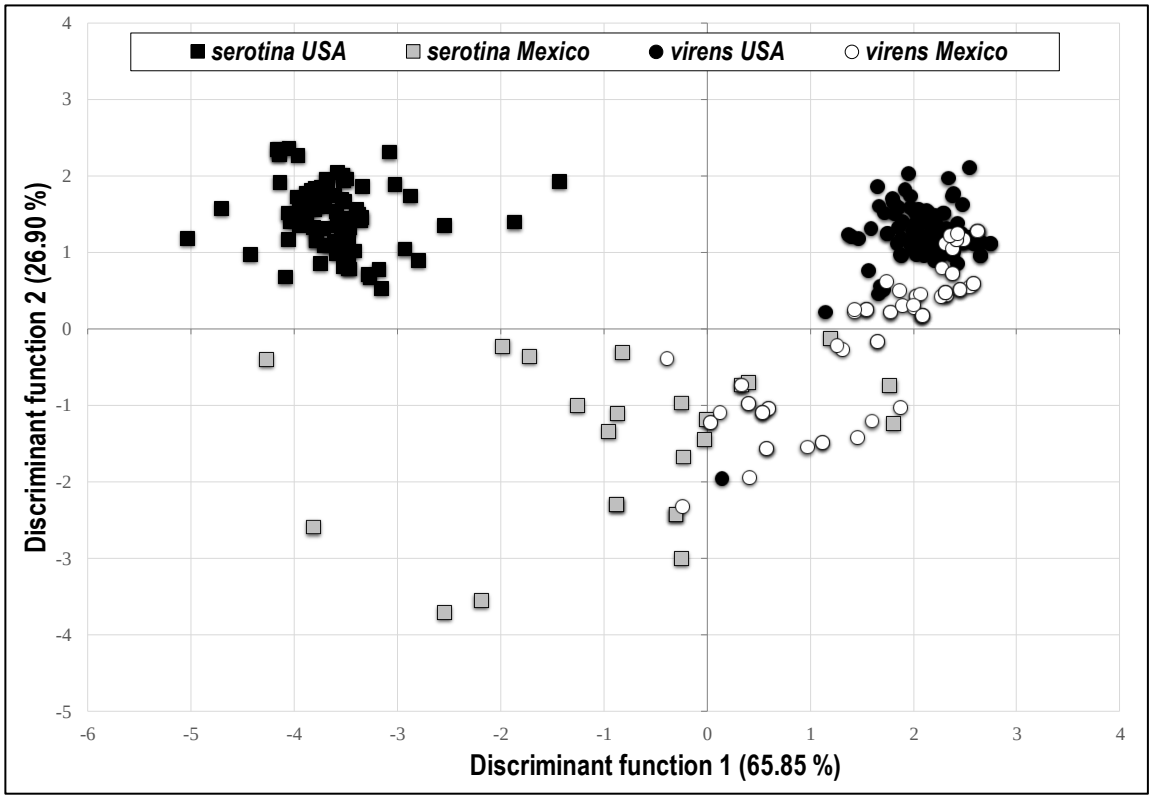

Figure 5. Geographical differentiation of *Prunus serotina* presence points using the two-dimensional space constituted by the first two discriminant functions.

Overall, dispersion of points indicates that environments in the United States are more homogeneous than those in Mexico (Figures 3 and 5). This can be explained by the fact that native Mexican peoples have used black cherries with different purposes (medicinal, nutritional and timber), thus favoring their introduction and survival in heterogeneous environments. Black cherries are used in the United

States mainly as a source of timber and Native Americans have used the species for medicinal or nutritional ends less intensely than Mexican peoples.

2.5.2. Influence of environmental factors on morphological variation of *P. serotina*

Pearson's bivariate correlation analysis detected highly significant correlations (absolute value >0.5) between the variation of four bioclimatic variables and six morphological descriptors [three leaf descriptors (2BL: Longitude of the second basal leaf, LT2: Length/thickness quotient length of second basal leaf flowering branch. LPQ: L2H/LP2 quotient); one flower descriptor (STL: Stamen length), one fruit descriptor (TFP: Thickness of fruit pedicel), and one stem and branch descriptor (ILS: Internode length in young stems)] (Figure 6). Positive correlations were detected between: 1) seasonality of precipitation (BIO15) and five morphological descriptors (leaf: 2BL, LT2; flower: STL; fruit: TFP; stem-branch: ILS), and 2) between altitude and four descriptors (leaf: 2BL, LT2, LPQ; fruit: TFP). Negative correlations were obtained between: 1) temperature seasonality (BIO04) and five descriptors (leaf: 2BL, LT2; flower: STL; fruit: TFP), 2) precipitation of driest quarter (BIO17) and two descriptors (leaf: 2BL; fruit: TFP), and 3) between precipitation of coldest quarter (BIO19) and one fruit descriptor (TFP).

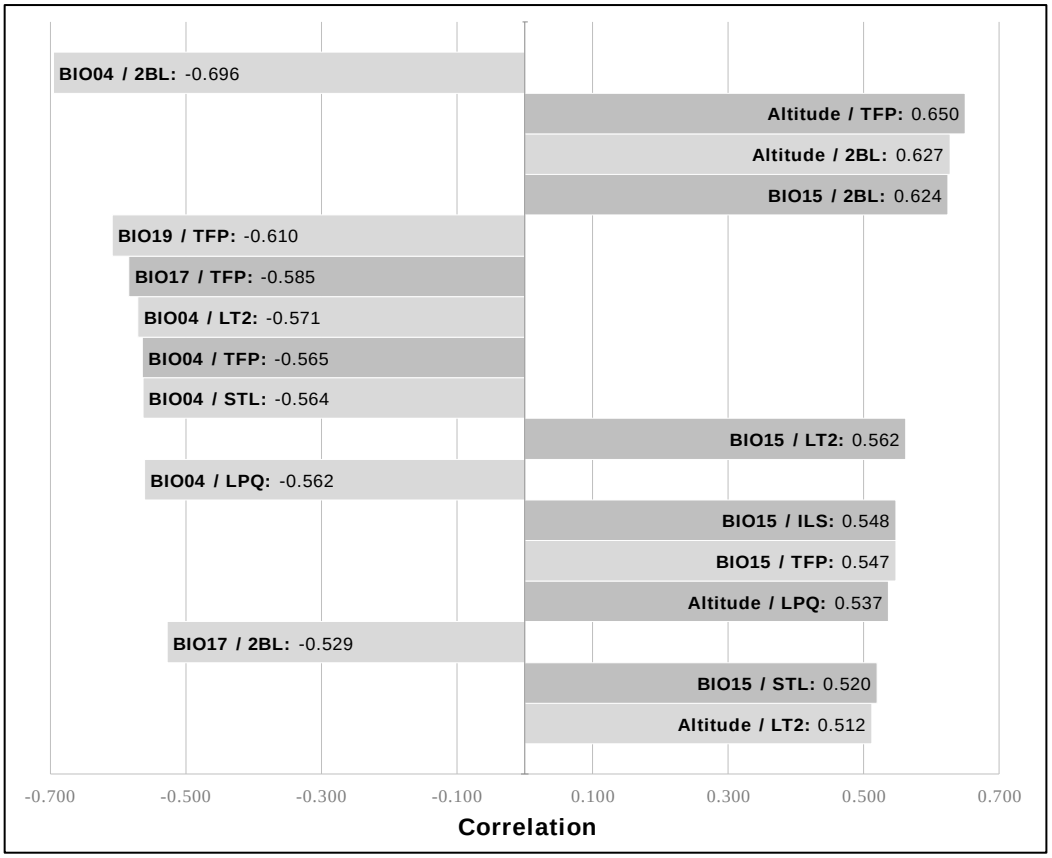

Figure 6. Correlation coefficients (-0.5> r >0.5) with statistical significance (*p* <0.05) between morphological descriptors and bioclimatic variables selected by the DA. ILS: Internode length in young stems. 2BL: Second basal leaf of flowering branch length. LT2: Length/thickness quotient length of second basal leaf of flowering branch. LPQ: L2H/LP2 quotient. STL: Stamen length. TFP: Thickness of fruit pedicel. BIO04: Temperature seasonality. BIO15: Precipitation seasonality. BIO17: Precipitation of driest quarter. BIO19: Precipitation of the coldest quarter.

Additionally, a PCA was conducted, combining the most informative eight variables found in the DA and 17 morphological descriptors. The PCA indicated that the space constituted by the first three components explained 58 % of total variance in the sample analyzed (Table 5). This three-dimensional space primarily explains the variability of the sample based on bioclimatic variables (Table 6). Since communality associated to a variable is the proportion of variability of this variable explained by a specific number of principal components, it can be deduced that the three first components acceptably explain (communality >0.6) the variability of 10 variables (Table 6). This three-dimensional space was useful in explaining variability of three morphological descriptors [Second basal leaf of flowering branch length (2BL), Petiole length of second basal leaf of flowering

branch (PL2) and number of flowers per branch (NFB)], altitude and six bioclimatic variables (BIO04, BIO12, BIO13, BIO15, BIO17 and BIO19).

Table 5. Results of the PCA of 17 morphological descriptors and eight bioclimatic variables selected by the DA. Values shown are the weight of each variable on the first three components. Code of morphological descriptors was assigned following Fresnedo-Ramírez *et al*. (2011).

Variable	Code	Component		
		1	2	3
Internode length in young stems	ILS	0.212	-0.188	0.133
Thickness of young stems	TYS	0.160	-0.041	0.333
Second basal leaf of flowering branch length	2BL	0.289	-0.075	0.208
Second basal leaf of flowering branch thickness	2BT	0.185	0.025	0.316
Length/thickness quotient length of second basal leaf of flowering branch	LT2	0.231	-0.174	-0.121
Petiole length of second basal leaf of flowering branch	PL2	0.103	0.120	0.430
Teeth quantity in second basal leaf margin of flowering branch per 1 cm^2	TQM	-0.092	-0.276	0.054
2BL/PL2 quotient	LPQ	0.201	-0.158	-0.143
Flower branch length	FBL	0.189	-0.064	0.292
Flower pedicel length	FPL	0.001	0.051	0.219
Stamen length	STL	0.233	-0.132	-0.118
Flower diameter	FLD	-0.057	0.230	-0.086
Number of flowers per branch	NFB	-0.099	0.134	0.409
Fruit pedicel length	FPL	0.002	0.206	0.229
Thickness of fruit pedicel	TFP	0.240	0.071	0.014
Seed length	SEL	-0.111	0.249	-0.177
Seed diameter	SED	0.105	-0.009	-0.109
Altitude	ALT	0.305	0.154	-0.113
Temperature seasonality	BIO04	**-0.313**	0.003	0.133
Mean temperature of coldest quarter	BIO11	0.204	-0.087	-0.179
Annual precipitation	BIO12	-0.074	-0.500	0.067
Precipitation of wettest month	BIO13	0.168	-0.395	0.000
Precipitation seasonality	BIO15	**0.316**	0.074	-0.102
Precipitation of driest quarter	BIO17	-0.283	-0.274	0.078
Precipitation of the coldest quarter	BIO19	-0.262	-0.298	0.060
% of variance		33.581	12.724	11.233
% cumulative		33.581	46.305	57.538

Table 6. Proportion of the variance explained by the three-dimensional space constituted by the first three components. Variables are organized in descending order based on their communalities. *PC:* principal component. *com:* communalities.

Variable	PC-variable correlation			Communalities = $PC1^2+PC2^2+PC3^2$ (>0.6)	% of variance accounted for each PC		
	PC1	PC2	PC3		$PC1^2$/com	$PC2^2$/com	$PC3^2$/com
BIO17	-0.820	-0.489	0.131	**0.929**	**0.724**	0.258	0.018
ALT	0.884	0.274	-0.189	**0.892**	**0.876**	0.084	0.040
BIO15	0.916	0.131	-0.172	**0.885**	**0.947**	0.019	0.033
BIO04	-0.907	0.005	0.222	**0.872**	**0.943**	0.000	0.057
BIO19	-0.760	-0.531	0.100	**0.870**	**0.664**	0.325	0.012
BIO12	-0.214	-0.892	0.112	**0.854**	0.054	**0.932**	0.015
2BL	0.837	-0.134	0.349	**0.840**	**0.834**	0.021	0.145
BIO13	0.485	-0.704	0.001	**0.731**	0.322	**0.678**	0.000
PL2	0.297	0.214	0.721	**0.655**	0.135	0.070	**0.795**
NFB	-0.286	0.239	0.686	**0.609**	0.134	0.094	**0.772**
LT2	0.669	-0.310	-0.202	0.584	0.766	0.164	0.070
2BT	0.536	0.044	0.530	0.570	0.503	0.003	0.493
FBL	0.549	-0.115	0.489	0.553	0.544	0.024	0.432
STL	0.676	-0.235	-0.197	0.552	0.829	0.100	0.071
ILS	0.614	-0.336	0.222	0.540	0.699	0.210	0.091
TYS	0.463	-0.073	0.558	0.532	0.404	0.010	0.586
TFP	0.695	0.126	0.023	0.499	0.967	0.032	0.001
LPQ	0.582	-0.281	-0.239	0.476	0.713	0.167	0.120
BIO11	0.591	-0.155	-0.299	0.463	0.75	0.052	0.194
SEL	-0.322	0.444	-0.297	0.389	0.267	0.507	0.227
TQM	-0.266	-0.492	0.090	0.321	0.221	0.754	0.025
FPL	0.007	0.367	0.383	0.282	0.000	0.478	0.522
FLD	-0.166	0.411	-0.144	0.217	0.126	0.778	0.096
FPL	0.003	0.090	0.368	0.143	0.000	0.057	0.943
SED	0.303	-0.016	-0.183	0.126	0.730	0.002	0.268

The first component in this space explained the greater percentage of variability of one morphological variable (Second basal leaf of flowering branch length (2BL: 83 %), and altitude; and of four bioclimatic variables [one for temperature (BIO04) and three for precipitation (BIO15, BIO17 and BIO19)]. The second was useful for explaining variability of two bioclimatic variables of precipitation: annual (BIO12) and for the wettest month (BIO13). The third component explained the variability of two morphological variables: one for the leaf (PL2: 80 %) and one for the flower (NFB: 77 %). Figure 7 illustrates the separation between two main groups of presence points, one constituted by spp. *eximia*, *hirsuta* and *serotina* that would represent the longer and thinner leaves (2BL, LT2 and LPQ), shorter stamens (STL) and thinner fruit pedicel (TFP). The other group, mainly made up of ssp. *capuli* and *virens*, in essence would have shorter and wider leaves. Subspecies *capuli* would have longer stamens (STL) and thicker fruit pedicel (TFP).

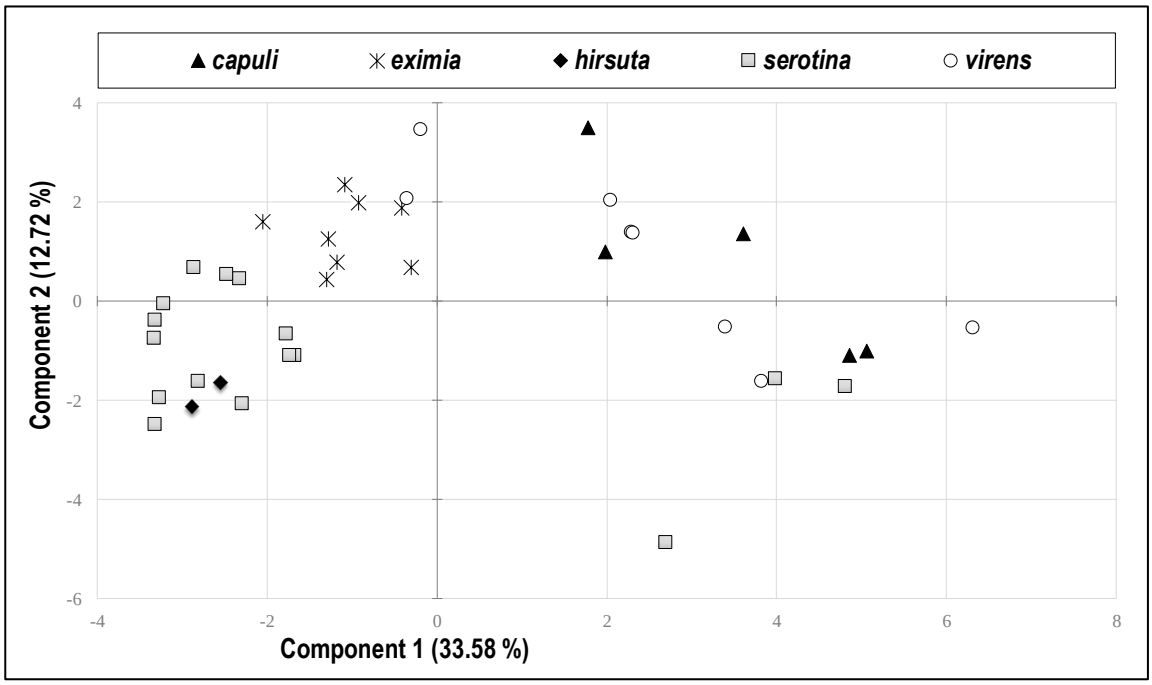

Figure 7. Projection of the 39 individuals in the two-dimensional space of the PCA that combined morphological descriptors and bioclimatic variables.

Overall, these results suggest that differences in altitude along the natural distribution range of black cherries, together with variation of extreme temperature (BIO04) and precipitation (BIO15, BIO17 and BIO19) variables are associated

with a portion of variation of its leaves (2BL, LT2 and LPQ), flowers (STL) and fruits (TFP).

2.5.3. Potential change in areas of probable habitats

Climate change is altering ecological niche areas of the species and this will probably affect the natural distribution of many of them (IPCC, 2014). Therefore, *P. serotina* will almost inevitably face change in the areas it inhabits. This work estimated those changes, comparing its areas of potential distribution under current climatic conditions with potential distribution areas under future climatic conditions. In spite of its limitations, niche modeling is a useful tool for establishing an initial perspective of potential impact of climate change on species distribution (Pearson & Dawson, 2003). Comparison in this study revealed that increases in temperature would imply altitudinal and latitudinal displacements from suitable areas (Figure 8). The two models used indicated that in Mexico (GFDL: 38 % and HADGEM-CM3: 33 %) and in the United States (GFDL: 45 % and HADGEM-CM3: 37 %) a large proportion of areas currently suitable would not be so in the future. Nonetheless, both models also suggested that new areas would appear whose climatic conditions would be apt for black cherries. In Mexico these areas would be equivalent to 26 % (GFDL) and 20 % (HADGEM) of current potential distribution area and the species would move to mountain zones at higher altitudes, since, overall, temperatures will increase in the lower altitudes. In the United States, suitable areas would represent 151 % (GFDL) and 120 % (HADGEM) of current area and will be located mainly in the northernmost zones.

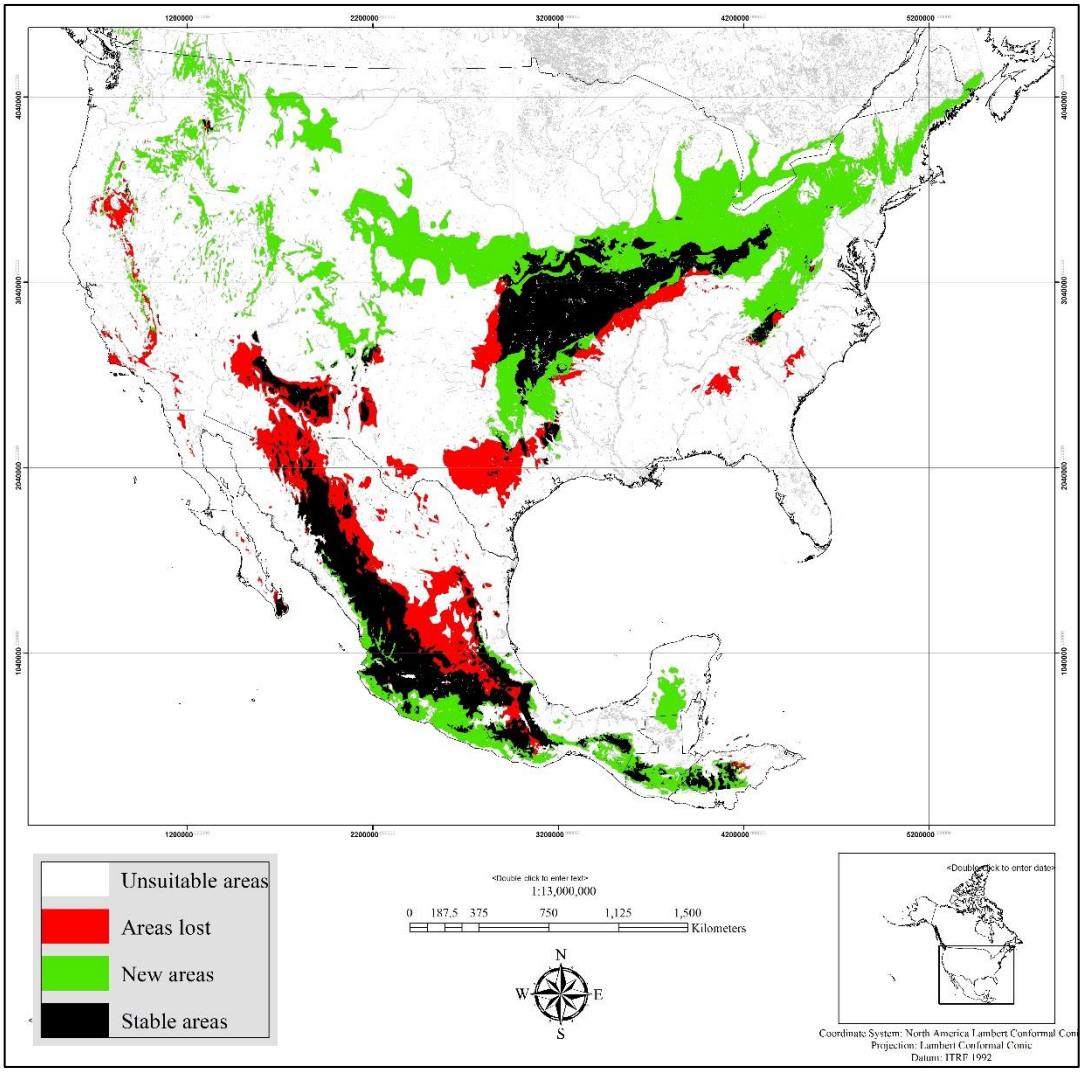

Figure 8. Potential effect of climate change on potential distribution of the *Prunus serotina* complex. Model: GDFL.

2.6. DISCUSSION

After the taxonomic and distribution analyses of *P. serotina* done by McVaugh (1951, 1952), this is the first study to use information of collections conducted throughout North America to associate the species' morphological variations and the climatic conditions of its distribution. To date, few studies have evaluated morphological variation associated to local climatic conditions (Auclair & Cottam, 1971; Fresnedo-Ramírez *et al*., 2011) or to its ethnobotanic value (Avendaño-Gómez *et al*., 2015).

In the present study, distribution in two ecozones and 46 ecoregions is indicative of the capacity of black cherry subspecies to adapt to climatic conditions, which in Mexico range from humid to subhumid temperate climates and in the United States from temperate to very severe climates (temperate and rainy in the southern states, flat with variable climate in the Midwest, and hot and dry in the southwest).

The high percentage of variation explained by the first two canonical functions of the DA enables a significative interpretation of distribution areas' climatic variation for the five subspecies (Figure 3). Representation of multivariate niches showed that ssp. *capuli* is adapted to heterogeneous environmental conditions; furthermore, it occupies ecoregions that broadly coincide with those of ssp. *serotina* in Mexico. Similarity of these distributions possibly is an effect of domestication carried out by Mexican peoples who have used both subspecies. Subspecies *serotina* is considered the wild form of ssp. *capuli* (Hernández-Xolocotzi, 1993); therefore, humans have favored the introduction of the latter subspecies in different environments, thus broadening its niche and resulting in overlapping with that of the other subspecies, especially ssp. *serotina*.

Dispersion of presence points of ssp. *serotina* and *virens* suggest that their niches are more homogeneous, yet contrasting since they occupy opposite extremes of the two-dimensional representation of the multivariate niche (Figure 3). The niche done for ssp. *virens* is located in zones with the lowest annual precipitation and whose annual average temperature is 19 ºC. This subspecies is considered wild and typical of dry zones (McVaugh, 1951). In spite of the small number of presence points for subspecies *hirsuta* and *eximia,* by comparison, their niche can be said to be especially narrow, with ssp. *eximia* inhabiting characteristically drier and hotter areas and spp. *hirsuta* clearly occupying a subset of environments specific to ssp. *serotina*. McVaugh (1952) associated the origin of these differences with climate changes occurring during the end of the tertiary era.

Climate contributes largely to morphological variation in plants (Guerin *et al*., 2012). Nonetheless, in this study, bioclimatic parameters of collection sites

explained a relatively small portion of the morphological variation evaluated, suggesting that the latter is not easily explained by climate. This low influence of bioclimatic parameters may be due to the fact that climate is not the only factor affecting the morphology of black cherry subspecies. The difficulty in differentiating black cherry subspecies is evident in the study conducted by Fresnedo-Ramírez *et al*. (2011). These authors use morphological descriptors and geographic distribution to differentiate populations of the three Mexican subspecies; however, they found two large groups: one made up of individuals of the subspecies *capuli* and *serotina*, and another by individuals of the ssp. *serotina* and *virens*. Consequently, other environmental factors such as topography and soil properties (type, moisture, nutrient content, among others) must be considered as potential predictors of morphological variation of these subspecies. Since soil type and its properties vary significantly throughout climatic zones in North America, this could have contributed to morphological variation of *P. serotina* in Mexico and the United States. The present study also revealed that average annual temperature and precipitation do not have direct impact on the more discriminant morphological descriptors, but rather its extremes (for example, temperature and precipitation of coldest quarters) and other related parameters such as daily annual temperature amplitude, and temperature and precipitation seasonality.

Phenotypic plasticity is the capacity of a same genotype to express different phenotypes in different environments (Gratani, 2014). This adaptive capacity in response to diverse environmental changes results in the alteration of the plant's morphological traits, allowing it to prosper in a broader range of habitats that would be possible if all traits remained genetically fixed (Sultan, 2000). Alternatively, species with a broad range of distribution must face many environmental situations in which natural selection can give rise to genotypic differentiation (Gaston, 2003). Therefore, morphological variation in black cherry, associated to heterogeneous environmental conditions could be a consequence of genetic differentiation of subspecies or of phenotypic plasticity inherent to the species. Nonetheless, based on results of this study, these two alternatives

cannot be clearly distinguished. Consequently, in order to differentiate between the two alternatives, it would be useful to do morphological description of individuals typical of the five subspecies in the same experimental site and use molecular techniques to evaluate populations representative of the natural range of geographical distribution.

In this study, estimates of climate change impact are considered a tool to enable objective and sound conservation decisions. Even though it is impossible to predict the future accurately, using bioclimatic modeling improves comprehension of probable effects of future climate conditions on biodiversity (Araújo & Rahbek, 2006). Consequently, results of this study are a hypothesis of the magnitude of climate change impact, and indicators of sensibility and of possible vulnerabilities. In addition, they represent the first step to understand potential impacts of climate change on black cherry habitats and to establish an initial vision on what could happen in its areas of distribution. To this end, the disappearance of suitable areas is a warning on possible genetic erosion that would inevitably result in the loss of variability of the complex.

Modeling done in this study not only indicates that–due to inevitable global warming–currently suitable areas will disappear, but also that areas will arise whose climatic conditions will be favorable for black cherry. The larger proportion of these new areas is concentrated in the northern latitudes of the United States, suggesting that in this country, the use of the species for timber would be favored since suitable zones would appear where commercial timber plantations could be established. In Mexico, the new areas would be in higher altitudes, essentially implying altitudinal displacement of the species.

Genetic diversity increases biological efficiency of populations because it enables them to respond in different ways to environmental changes (Hughes & Stachowicz, 2004). Morphological heterogeneity can be interpreted as the result of the adaptation of species to different habitats, thus increasing their probability of surviving in the context of climate change (Visser, 2008). In this sense, *P. serotina* is shown to be adapted to different climatic conditions (Figures 3 and 4);

therefore, it is possible that its genetic variability may allow it to adapt to future climate conditions (Figure 8). A relevant aspect–which reinforces this assumption– is its invasive behavior in different parts of the planet (Starfinger *et al*., 2003) and also its capacity to tolerate and adapt itself to a broad range of climates. In any case, even though climate is the main factor that will determine the distribution of plants in the future (Woodward, 1987), other factors, such as soil conditions, competition and predators, also affect the presence of any species and represent additional limitations to is current distribution and to possible displacements in the future.

2.7. CONCLUSION

Nineteen climate and altitude parameters were used to determine that ssp. *eximia*, *hirsuta* and *serotina* thrive in humid and cold environments, while *virens* thrives in distinctly dry environments. Even though ssp. *capuli* prefers humid environments, it was also evident that its distribution has no well-defined pattern and is frequently found growing sympatrically with ssp. *serotina* in middle and western Mexico. The study revealed that climate is partially related with morphological variation of subspecies of *P. serotina*, especially variation of the second floral leaf, fruit pedicel, and longitude of the stamens with the topographic variable (altitude), two temperature variables (BIO04 and BIO11), two precipitation variables (BIO15 and BIO17), and one that combines precipitation and temperature (BIO19). Additionally, it suggests that extreme parameters of future climate change will affect the morphology of the species. Potential black cherry conservation programs can be defined taking into account its morphological variation, its climatic preferences, and the potential impact of climate change.

2.8. LITERATURE CITED

Adriano-Morán, C.C., & McClung de Tapia, E. (2008). Trees and shrubs: the use of wood in prehispanic Teotihuacan. *Journal of Archaeological Science*, 35(11), 2927-2936.

Aguerrebere, I.A., Rojas Molina, A., Oomah, B.D., & Drover, J.C.G. (2011). Characteristics of *Prunus serotina* seed oil. *Food Chemistry, 124*, 983-990.

Araújo, M.B., & Rahbek, C. (2006). How does climate change affect biodiversity?, *Science*, 313, 1396-1397.

Auclair, A.N., & Cottam, G. (1971). Dynamics of black cherry *(Prunus serotina* Ehrh.) in Southern Wisconsin oak forests. *Ecological Monographs*, 41, 153-177.

Avendaño-Gómez, A., Lira-Saade, R., Madrigal-Calle, B., García-Moya, E., Soto-Hernández, M., & Romo de Vivar-Romo, A. (2015). Management and domestication syndromes of capulin (*Prunus serotina* Ehrh. ssp. *capuli* (Cav.) Mc Vaugh) in communities of the state of Tlaxcala. *Agrociencia*, 49, 189-204.

Barnd, B.D., & Ginzel, M.D. (2008). Causes of gummosis in black cherry (*Prunus serotina*). USDA Forest Service and Purdue University. FNR-229-W. 6 p.

Busby, J.R. (1991). BIOCLIM a bioclimatic analysis and prediction system. In C.R Margules & M.P. Austin (Eds.), *Nature conservation: cost effective biological surveys and data analysis*. East Melbourne, Melbourne. CSIRO. pp. 64-68.

Ellison, A.M., Buckley, H.L., Miller, T.E., & Gotelli, N.J. (2004). Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): geographic, environmental and taxonomic correlates. *American Journal of Botany*, 91(11), 1930-1935.

ESRI. (2013). ArcGIS Desktop: Release 10.2. Redlands, CA. Environmental Systems Research Institute. Consulted in http://desktop.arcgis.com/en/

Fresnedo-Ramírez, J., Segura, S., & Muratalla-Lúa, A. (2011). Morphovariability of capulín (*Prunus serotina* Ehrh.) in the central-western region of Mexico from a plant genetic resources perspective. *Genetic Resources and Crop Evolution*, 58(4), 481-495, doi:10.1007/s10722-010-9592-2.

Gaston, J.K. (2003). The structure and dynamics of geographic ranges. Ed. 1. New York, Oxford University Press. 267 p.

Global Biodiversity Information Facility – GBIF. (2017). *Prunus serotina*. 154 records from 8 published datasets. 20-February-2017. GBIF occurrence download. Consulted online http://doi.org/10.15468/dl.xggt4u

Gratani, L. (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in Botany*, 2014, 1-17.

Guerin, G.R., Wen, H., & Lowe, A.J. (2012). Leaf morphology shift linked to climate change. *Biology Letters*, 8(5), 882-886, doi:10.1098/rsbl.2012.0458.

Hernández-Xolocotzi, E. (1993). Aspects of plant domestication: A personal view. In T. P. Ramamoorthy, R. Bye, A. Lot, and J. Fa (Eds.), *Biological Diversity*

of Mexico, Origins and Distribution. Oxford University Press, New York. 733 p.

Hijmans, R.J., Guarino, L., Cruz, M., & Rojas, E. (2001). Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resources Newsletter*, 127, 15-19.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25(15), 1965-1978.

Hughes, A.R., & Stachowicz, J.J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences*, 101(24), 8998-9002.

Ibarra-Alvarado, C., Rojas, A., Luna, F., Rojas, J.I., Rivero-Cruz B., & Rivero-Cruz, J.F. (2009). Vasorelaxant constituents of the leaves of *Prunus serotina* "capulín". *Revista Latinoamericana de Química*, 37, 164-173.

Ibarra-Alvarado, C., Rojas, A., Mendoza, S., Bah, M., Gutiérrez, D.M., Hernández-Sandoval, L., & Martínez, M. (2010). Vasoactive and antioxidant activities of plants used in Mexican traditional medicine for the treatment of cardiovascular diseases. *Pharmaceutical Biology*, 48(7), 732-739, doi:10.3109/13880200903271280.

IPCC. (2014). Climate change 2014: Synthesis Report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change [Core writing team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland. 151 p.

Jiménez, M., Castillo, I., Azuara, E., & Beristain, C.I. (2011). Antioxidant and antimicrobial activity of capulin (*Prunus serotina* subsp. *capuli*) extracts. *Revista Mexicana de Ingeniería Química*, 10(1), 29-37.

Luna-Vázquez, F.J., Ibarra-Alvarado, C., Rojas-Molina, A., Rojas-Molina, J.I., Yahia, E.M., Rivera-Pastrana, D.M., Rojas-Molina, A., & Zavala-Sánchez, A.M. (2013). Nutraceutical value of black cherry *Prunus serotina* Ehrh. fruits: Antioxidant and antihypertensive properties. *Molecules*, 18, 14597-14612, doi:10.3390/molecules181214597.

Luna-Vázquez, F.J., Ibarra-Alvarado, C., Rojas-Molina, A., Romo-Mancillas, A., López-Vallejo, F.H., Solís-Gutiérrez, M., Rojas-Molina, J.I., & Rivero-Cruz, F. (2016). Role of nitric oxide and hydrogen sulfide in the vasodilator effect of ursolic acid and uvaol from black cherry *Prunus serotina* fruits. *Molecules*, 21, 78, doi:10.3390/molecules21010078.

Marquis, D.A. (1990). *Prunus serotina* Ehrh. Black Cherry. In R.M. Burns & B.H. Honkala, *Silvics of North America*. Volume 2, Hardwoods. USDA Forest Service. Agriculture Handbook 654, pp. 594-604, Washington, D.C. Consulted in: https://www.srs.fs.usda.gov/pubs/misc/ag_654_vol2.pdf.

Maynard, C.A., Kavanagh, K., Fuernkranz, H., & Drew, A.P. (1991). Black Cherry (*Prunus serotina* Ehrh.). In Y. P. S. Bajaj (Ed.). Trees III: Biotechnology in Agriculture and Forestry 16. Heidelberg, Berlin: Springer. pp. 3-22.

McVaugh, R. (1951). A revision of the North American black cherries (*Prunus serotina* Ehrh., and relatives). *Brittonia*, 7(4), 279-315, doi:10.2307/2804698.

McVaugh, R. (1952). Suggested phylogeny of *Prunus serotina* and other wide-ranging phylads in North America. *Brittonia*, 7(5), 317-346, doi:10.2307/2804965.

Olszewska, M. (2007). Quantitative HPLC analysis of flavonoids and chlorogenic acid in the leaves and inflorescences of *Prunus serotina* Ehrh. *Acta Chromatographica*, 19, 253-269.

Palomares-Alonso, F., Rojas-Tomé, I.S., Palencia-Hernández, G., Jiménez-Arellanes, M.A., Macías-Rubalcava, M.L., González-Maciel, A., Ramos-Morales, A., Santiago-Reyes, R., Castro, N., González-Hernández, I., Rufino-González, Y., & Jung-Cook, H. (2017). *In vitro* and *in vivo* cysticidal activity of extracts and isolated flavanone from the bark of *Prunus serotina*: A bio-guided study. *Acta Tropica*, 170, 1-7, doi:10.1016/j.actatropica.2017.02.023.

Pearson, R.G., & Dawson, T.P. (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*, 12, 361-371, doi:10.1046/j.1466-822X.2003.00042.x.

Phillips, S.J., Anderson, R.P., & Schapire, R.E. (2006). Maximum entropy modeling of species geographic distributions. Ecological Modeling, 190, 231-259, doi:10.1016/j.ecolmodel.2005.03.026.

Popenoe, W., & Pachano, A. (1922). The capulín cherry: A superior form of the Northern black cherry develop in the highlands of tropical America. *Journal of Heredity, 13*(2)*,* 51-62.

Raya-Pérez, J.C., Aguirre-Mancilla, C.L., Tapia-Aparicio, R., Ramírez-Pimentel, J.G., & Covarrubias-Prieto, J. (2012). Storage proteins characterization and mineral composition of black cherry seed (*Prunus serotina*). *Polibotánica*, *34*, 203-215.

Rohrer, J.R. (2014). *Prunus* (Rosaceae). In Flora of North America Editorial Committee (Eds.), *Flora of North America North of Mexico*. New York, Oxford. 20+ vols. and. Vol 9, 1993+. pp. 363-365.

Rzedowsky, J., & Calderón de Rzedowski, G. (2005). Flora del Bajío y de Regiones Adyacentes. *Rosacea,* fascículo 135. Instituto de Ecología A. C. y CONABIO, Morelia, Michoacán. 157 p.

Scheldeman, X., & Van Zonneveld, M. (2010). *Training Manual on Spatial Analysis of Plant Diversity and Distribution*. Bioversity International, Rome, Italy. 179 p.

Starfinger, U., Kowarik, I., Rode, M., & Schepker, H. (2003). From desirable ornamental plant to pest to accepted addition to the flora? – the perception of an alien tree species through the centuries. *Biological Invasions*, 5, 323-335.

StatSoft. Inc. (2004). STATISTICA (Data Analysis Software System), Version 7.0.

Sultan, S.E. (2000). Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*, 5, 537–542, doi:10.1016/S1360-1385(00)01797-0.

Visser, M.E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings on the Royal Society of London B*, 275, 649-659, doi:10.1098/rspb.2007.0997.

Wang, Y., & Pijut, P.M. (2014). Can *Prunus serotina* be genetically engineered for reproductive sterility and insect pest resistance?. *Springer Science Reviews*, 2, 75-93, doi:10.1007/s40362-014-0024-0.

Woodward, F.I. (1987) Climate and Plant Distribution. Cambridge University Press. 177 p.

CHAPTER III. LACK OF MOLECULAR DISTINCTIVENESS IN FOUR SUBSPECIES OF THE MULTIPURPOSE FRUIT TREE BLACK CHERRY (*Prunus serotina* EHRH.)

CAPÍTULO III. ESCASA DIFERENCIACIÓN MOLECULAR EN CUATRO SUBESPECIES DEL FRUTAL MULTIPROPÓSITO CAPULÍN (*Prunus serotina* EHRH.)

3.1. ABSTRACT

Black cherry (*Prunus serotina* Ehrh.) is a fruit tree native to North America, and almost all parts of this plant have some use. This species is a complex of five subspecies with morphological differences and distinctive habitats. The genetic structure of 18 natural populations of black cherry was evaluated with 16 microsatellite markers. One hundred sixty-four individuals were collected across seven states of México, and another 14 in Texas. These individuals represented subspecies *capuli* (38), *eximia* (14), *serotina* (53), and *virens* (73). A total of 246 alleles was detected for the 16 markers. A Neighbor-Joining tree and a principal coordinate analysis (PCoA) revealed two major groups of individuals while Bayesian clustering analysis detected six main clusters that do not clearly correspond to the four putative subspecies studied. At the $p \geq 0.900$ cut off values, the composition of these clusters indicates geographical structuring of the samples; their genetic differentiation suggests gene flow among populations with geographical proximity. This is the first time that molecular markers have been used to assess the genetic structure present in natural populations of four subspecies of black cherry with the goal of evaluating their taxonomic classification and providing guidelines for *in situ* conservation of genetic resources of the species. Based on our results, we conclude that these four taxa should be managed as one conservation or biological entity.

Keywords: *Prunus serotina*, capulín, genetic structure, molecular taxonomic discrimination, subspecies complex.

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3.2. RESUMEN

El capulín (*Prunus serotina* Ehrh.) es un árbol frutal nativo de Norteamérica, y casi todas sus partes tienen algún uso. Esta especie conforma un complejo de cinco subespecies con diferencias morfológicas y hábitats distintivos. En este trabajo se evaluó la estructura genética de 18 poblaciones naturales de capulín con 16 microsatélites. Para hacerlo, se evaluaron 164 individuos recolectados en siete estados mexicanos y 14 que lo fueron en Texas. Estos individuos representaron a las subespecies *capuli* (38), *eximia* (14), *serotina* (53) y *virens* (73). Los 16 marcadores detectaron 246 alelos. El árbol construído con el método *Neighbor-Joining* y el análisis de coordenadas principales (PCoA) revelaron dos grupos principales de individuos, mientras que el análisis Bayesiano de agrupamientos detectó seis grupos principales que claramente no correspondieron a las cuatro subespecies estudiadas. Usando valores de probabilidad de pertenencia mayores o iguales a 0.900, la composición de estos grupos indica estructuración genética de la muestra; y su diferenciación genética sugiere flujo génico entre las poblaciones geográficamente más cercanas. Este es la primera vez que se usan marcadores moleculares para evaluar la estructura genética de poblaciones naturales de cuatro subespecies del capulín, y para evaluar su clasificación taxonómica y proporcionar directrices para la conservación *in situ* de los recursos genéticos de la especie. Los resultados permiten concluir que estos cuatro taxones deberían manejarse como una sola unidad de conservación o entidad biológica.

Palabras clave: *Prunus serotina*, black cherry, estructura genética, discriminación taxonómica molecular, complejo de subespecies.

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3.3. INTRODUCTION

Black cherry (*Prunus serotina* Ehrh.) is a fruit tree native to North America, almost all parts of which have some use. In México, where the species is commonly known as capulín, its fruits are eaten fresh, dried, or as ingredients in other preparations (e.g., jellies, tamales, and liqueurs), and the seed is consumed after toasting the stony endocarp (Raya-Pérez, Aguirre-Mancilla, Tapia-Aparicio, Ramírez-Pimentel & Covarrubias-Prieto, 2012). Its leaves and fruits are believed to have expectorant, sedative, and antispasmodic properties; additionally, inflorescences and leaves are considered to be an excellent source of antioxidants (Olszewska, 2007). Its wood has been used since pre-Columbian times as firewood (Adriano-Morán & McClung de Tapia, 2008). In the United States of America, the wood of *P. serotina* is appreciated because of its hardness and incorruptibility (Maynard, Kavanagh, Fuernkranz & Drew, 1991; Rohrer, 2014), and owing to the thickness of the trunks and reddish color of the wood, it has a commercial value similar to sweet cherry (*Prunus avium* L.) (Barnd & Ginzel, 2008; Rohrer, 2014; Wang & Pijut, 2014). Historical accounts show that black cherry was introduced to South America and Europe in the 17th century by colonizers; in Ecuador it reached some importance for commercial trading, and in several European countries it became an invasive species (Popenoe & Pachano, 1922; Starfinger, Kowarik, Rode & Schepker, 2003).

McVaugh (1951) described black cherry as a botanical complex of five native subspecies of North America: *Prunus serotina* ssp. *capuli* (Cav.) McVaugh (southern México to Guatemala), *Prunus serotina* ssp. *eximia* (Small) Little (Edwards Plateau of Texas), *Prunus serotina* ssp. *hirsuta* (Elliot) McVaugh (Georgia and Alabama), *Prunus serotina* ssp. *serotin*a (Ehrh.) McVaugh (eastern United States and Canada, re-appearing in eastern México), and *Prunus serotina* ssp. *virens* (Wooton et Standl.) McVaugh (western Texas to Arizona and northern México). This author stated that the five subspecies are geographical races whose morphological characters and habitats are distinctive, but that instant visual field recognition of the different subspecies would be unfeasible if they were not

geographically segregated in nature due to continuous variation and some degree of overlap among subspecies in nearly all distinguishing characters.

More recently, in México some morphological partial evaluations of the botanical complex have been carried out, barely differentiating the three Mexican subspecies (*capuli*, *serotina* and *virens*) (Fresnedo-Ramírez, Segura & Muratalla-Lúa, 2011; Rzedowski & Calderón de Rzedowski, 2005). On the other hand, Rohrer (2014) delimited four botanical varieties (var. *alabamensis* = ssp. *hirsuta*, var. *capuli* = ssp. *capuli*, var. *rufula* = ssp. *virens*, and var. *serotina* = ssp. *serotina* + ssp. *eximia*), and suggested that the taxonomic differences among the infraspecific groups are even subtler than classically proposed by McVaugh (1951).

It has been established that black cherry is an allotetraploid ($2n = 4x = 32$) for which there is no certainty about its progenitor species (Pairon & Jacquemart, 2005). Diploid individuals had also been reported by Forbes (1969), and pentaploid and hexaploid individuals were referenced by Dickson, Arumuganathan, Kresovich and Doyle (1992). The first published study of molecular variability in this species focused on demonstrating the usefulness of DNA markers developed from peach (*Prunus persica* (L.) Batsch), sweet cherry (*Prunus avium* (L.) L.), and sour cherry (*Prunus cerasus* L.) in the evaluation of black cherry diversity (Downey & Iezzoni, 2000). Subsequently, some molecular genetic analyses have been conducted with various objectives, including: to improve the understanding of its invasive behavior in European forests and to establish efficient control strategies (Pairon, Jonard & Jacquemart, 2006; Pairon *et al*., 2010; Petitpierre *et al*., 2009); to evaluate genetic variability of the species in Ecuador (Guadalupe *et al*., 2015); and to test the Abundant Center Model in central North America using black cherry populations (Beck, Ferguson, Mayfield & Shaw, 2014). In the context of timber use, in the USA it has been included in genetic breeding projects that sought to obtain transgenic black cherries more resistant to insect attack, to reduce the occurrence of gummosis, and to improve the economic gains from the use of its wood (Wang & Pijut, 2014).

In México, there are some selections of *P. serotina* intended for horticultural research (Segura-Ledesma, Zavala-Robles, Equihua-Cervantes, Andrés-Agustín & Yepez-Torres, 2009); however, to date, there is a lack of formal *ex situ* collections of its germplasm. The Germplasm Resources Information Network (GRIN) in its public website (GRIN-Global; GRIN, 2015), catalogs 41 accessions of *P. serotina* in the United States (4), México (16), Ecuador (1), Poland (6), and the United Kingdom (14). Besides, it indexes 37 accessions belonging to three conspecific taxa. Nonetheless, all these accessions are referenced as "Historical record only", and therefore they are not available. These observations highlight that *ex situ* conservation is urgently needed and strongly recommended for the natural populations of black cherry.

Similarly, at the present time, there are no published studies of population genetic structure across the natural geographic distribution range of the species. This study was undertaken with the main goal of evaluating for first time the genetic structure within and among subspecies of black cherry, in order to improve the understanding of the geographic and taxonomic distribution of genetic diversity within the species. To do this, 178 individuals representing four out the five subspecies were collected in México and the United States.

In addition to informing decisions about the number and rank of infraspecific taxa that should be recognized, understanding the patterns of genetic diversity of black cherry can shed light on its intraspecific genetic variation and will also guide decisions about sustainable genetic resources conservation and use. In this context, this study is intended to answer three key questions: 1) Do intraspecific genetic groups of black cherry clearly correspond to the four subspecies studied?, 2) To what extent is the genetic structure within black cherry explainable by geographic distribution?, and 3) Is there evidence of gene flow among subspecies and/or other (e.g., geographically based) genetic clusters?

3.4. MATERIALS AND METHODS

3.4.1. Field sampling and DNA extraction

In 2015 and 2016, 178 individuals representing four black cherry subspecies were collected (Table 7). The determination of the 18 collection sites and the taxonomic identities were aided by consulting herbarium specimens from Herbario del Instituto de Ecología A.C. – IE-BAJÍO (IEB, Pátzcuaro, México) and The University of Texas at Austin Herbarium (LL, Austin, U.S.A.) and botanical reports (e.g., Fresnedo-Ramírez *et al*., 2011; McVaugh, 1951; Rzedowski & Calderón de Rzedowski, 2005). At least three individuals per site were collected and labeled, pressed, and mounted as herbarium voucher specimens, which were deposited at Universidad Autónoma Chapingo Herbarium (CHAP, Chapingo, México). Young leaves were collected directly in the field, immediately put in silica gel and later lyophilized for at least 60 hours using the Labconco Freezone 2.5 Lyophilizer (Labconco Corporation, Kansas City, Missouri). For DNA extraction, 30-40 mg of desiccated leaf tissue was pulverized with a Mini- Beadbeater-1 (BioSpec Products Inc., Bartlesville, Oklahoma). DNA samples were obtained with *DNEasy Plant Mini Extraction* (Qiagen Inc., Valencia, California) following the directions provided by the manufacturer. Modifications suggested for improving this method of extraction by Costa and Roberts (2014) were implemented.

Table 7. Natural populations of the four black cherry subspecies studied. [a] Country: [1] México, [2] The United States of America. [b] Subspecies taxonomic identity reported by: [1] McVaugh (1951); [2] Rzedowski & Calderón de Rzedowski (2005).

State[a]	Municipality/ Locality	Geographic location and elevation	Subspecies[b]	Population ID	Population code	Individuals
Coahuila[1]	Arteaga/ Chapultepec	25º14'03.0"N 100º49'57.5"W 2153 m	*virens*[1]	Coah1_vir	6	9
	Arteaga/ Los Lirios	25º22'37.1"N 100º47'32.6"W 2194 m	*virens*[1]	Coah2_vir	5	9
	Arteaga/ Guardarraya	25º21'50.7"N 100º28'24.4"W 1965 m	*virens*[1]	Coah3_vir	4	9
Guanajuato[1]	San Miguel de Allende/ Cañada de la Virgen	21º08'52.8"N 101º11'34.3"W 2335 m	*virens*[2]	Gto_vir	16	14
México[1]	Chapingo/ Collection in experimental fruit orchard	19º29'58.7"N 99º52'43.6"W 2261 m	*capuli*[2]	Mex_cap	10	14
Michoacán[1]	Pátzcuaro/ Pátzcuaro	19º30'19.4"N 101º38'02.8"W 2210 m	*serotina*[2]	Mich1_ser	13	12
	Pátzcuaro/ Cerro del Estribo	19º30'39.6"N 101º38'33.7"W 2380 m	*serotina*[2]	Mich2_ser	14	5
	Erongarícuaro/ Road from Erongarícuaro to Napízaro	19º35'58.4"N 101º42'59.7"W 2075 m	*serotina*[2]	Mich3_ser	12	12
	Cuanajo/ Road from Tupátaro to Cuanajo	19º30'01.5"N 100º30'10.4"W 2267 m	*serotina*[2]	Mich4_ser	1	10
	Huaniqueo de Morales/ Road to Huaniqueo	19º52'56.7"N 101º30'13.0"W 2031 m	*virens*[2]	Mich5_vir	15	14

Table 7. Continuation.

State[a]	Municipality/ Locality	Geographic location and elevation	Subspecies[b]	Population ID	Population code	Individuals
Nuevo León[1]	Santiago/ Laguna de Sánchez	25º20'12.1"N 100º16'20.4"W 1884 m	*virens*[1]	NL1_vir	7	9
	Santiago/ Ciénega de González	25º22'28.3"N 100º12'23.0"W 1511 m	*virens*[1]	NL2_vir	8	6
	Aramberri/ La Trinidad	25º12'59.1"N 100º05'26.6"W 839 m	*virens*[1]	NL3_vir	9	3
Querétaro[1]	Pinal de Amoles/ Pinal de Amoles	21º08'11.5"N 99º37'32.1"W 2345 m	*serotina*[2]	Qro_ser	11	14
Tlaxcala[1]	Españita/ Españita	19º29'52.7"N 98º26'15.3"W 2693 m	*capuli*[1]	Tlax1_cap	2	9
	Nanacamilpa/ Francisco I. Madero	19º31'22.2"N 98º29'16.4"W 2642 m	*capuli*[1]	Tlax2_cap	3	15
Texas[2]	Austin/ Bullcreek	30º22'35.4"N 97º47'02.4"W 171 m	*eximia*[1]	TX1	17	3
	Kerrville/ Native of Texas	29º55'59.9"N 99º14'07.0"W 556 m	*eximia*[1]	TX2	18	11

3.4.2. Microsatellites amplification

Twenty-two microsatellite markers from five economically important *Prunus* species were PCR amplified with FAM-, NED- or HEX-labeled forward primers and unlabeled reverse primers (Table 8). The primers were run in triplexes, based on their fluorescence dye and allele sizing. PCR conditions were 0.06 ul of each primer (10 pmol stock), 1.5 ul of 10X buffer, 1.5 ul of $MgCl_2$ (25 mM), 1.2 ul dNTPs (2.5 mM each nucleotide), 0.15 ul of Taq polymerase (5000 U/ml, New England Biolabs), and 15 ng of DNA in a 15.0 uL volume. The PCR conditions were as follows: one initial denaturation step of 5 min at 94 ºC; 30 cycles of 30 s at 94 ºC, 30 s at 55 ºC, 40 s at 72 ºC; and a final extension of 7 min at 72 ºC. Replicate samples were tested in separate experiments to confirm repeatability of results. Negative controls were run with every PCR to check for DNA contamination.

Out of the 22 SSR markers, six were dropped after preliminary evaluation because they produced poor amplification (Table 8). PCR products were run on an ABI PRISM 3130 Fragment Analyzer (Applied Biosystems, Foster City, California) using the size standard 400H ROX, and allele sizes were determined by means of GeneMapper Software Version 4.0 (Applied Biosystems, Foster City, California). The observed allele sizes were then adjusted for the discrete allele sizing using the bins databased in the laboratory of the USDA National Clonal Germplasm Repository in Davis (California). Alleles sizes were scored as di-allelic for each assigned locus (1 = band present, 0 = band absent), and a binary matrix was produced.

Table 8. Allelic richness detected in the 16 SSRs used for the evaluation of the 178 individuals. [a] Source species: [1] Peach, [2] Wild cherry (*Prunus avium* L.), [3] Apricot (*Prunus armeniaca* L.), [4] Sweet cherry, and [5] Japanese plum (*Prunus salicina* Lindl.).

SSR	Reference[a]	Primers (5' → 3')	Dye	Total alleles	Max. alleles per individual	Size (bp)			PIC
						Min	Max	Range	
BPPCT014	Dirlewanger *et al*. (2002)[1]	ttgtctgcctctcatcttaacc catcgcagagaactgagagc	FAM	8	2	169	201	32	0.74
EMPaS06	Vaugh & Russell (2004)[2]	aagcggaaagcacaggtag ttgctagcatagaaaagaattgtag	HEX	4	3	193	202	9	0.51
UDP98-409	Cipriani *et al*. (1999)[1]	gctgatgggttttatggttttc cggactcttatcctctatcaaca	NED	28	4	108	166	58	0.935
ssrPaCITA07	Lopes *et al*. (2002)[3]	cttttgtgcctcagcttcccaacac cctggcctgaccctaagcaattcg	FAM	24	4	159	217	58	0.74
AMPA118	Hagen *et al*. (2004)[3]	ggagagaggcaaatgttattgc aactcacaatctccaccgttac	HEX	Dropped. Poor amplification					
UDP97-402	Cipriani *et al*. (1999)[1]	tcccataaccaaaaaaaacacc tggagaagggtgggtacttg	NED	Dropped. Poor amplification					
BPPCT001	Dirlewanger *et al*. (2002)[1]	aattcccaaaggatgtgtatgag caggtgaatgagccaaagc	FAM	3	2	121	125	4	0.57
MA027a	Yamamoto *et al*. (2002)[1]	gggcagtgaagaatctatga gatagcataaaccccgtgaa	HEX	14	3	110	151	41	0.77
EMPA015	Clarke & Tobutt (2003)[4]	ttttggtcaatctgctgctg ctctcatcttccccctcctc	NED	15	2	196	234	38	0.86
CPSCT012	Mnejja *et al*. (2004)[5]	gtggccggacgagagaac cgatcgaatgaagctcagtg	FAM	8	3	112	128	16	0.57
BPPCT034	Dirlewanger *et al*. (2002)[1]	ctacctgaaataagcagagccat caatggagaatggggtgc	HEX	23	4	187	239	52	0.86

Table 8. Continuation.

SSR	Reference[a]	Primers (5' → 3')	Dye	Total alleles	Max. alleles per individual	Size (bp)			PIC
						Min	Max	Range	
BPPCT040	Dirlewanger *et al*. (2002)[1]	atgaggacgtgtctgaatgg agccaaacccctcttatacg	NED	15	4	132	157	25	0.83
pchgms3	Sosinski *et al*. (2000)[1]	ctgcagaacactactga gctttgcaaccaccagc	FAM	24	4	167	232	65	0.91
ssrPaCITA15	Lopes *et al*. (2002)[3]	gagatttgcaatgtcggaataagac cagacagctgctggttataggctcg	HEX	2	2	227	229	2	0.01
BPPCT042	Dirlewanger *et al*. (2002)[1]	aaccctactggttcctcagc gaccagtcctttagttggagc	NED	14	4	214	259	45	0.75
M12a	Yamamoto *et al*. (2002)[1]	aggtgcctcatcttcttctcttg gtgtggtgaggggtgagagc	FAM	22	4	166	215	49	0.92
BPPCT-039	Dirlewanger *et al*. (2002)[1]	attacgtaccctaaagcttctgc gatgtcatgaagattggagagg	HEX	24	2	98	146	48	0.92
CPSCT026	Mnejja *et al*. (2004)[5]	tctcacacgctttcgtcaac aaaaagccaaaaggggttgt	NED	18	4	162	201	39	0.86
EMPaS12	Vaugh & Russell (2004)[2]	tgtgctaatgccaaaaatacc acatgcatttcaacccactc	FAM	Dropped. Poor amplification					
BPPCT-007	Dirlewanger *et al*. (2002)[1]	tcattgctcgtcatcagc cagatttctgaagttagcggta	HEX	Dropped. Poor amplification					
MA017a	Yamamoto *et al*. (2002)[1]	aaggcatatagcgcaggt atctgaggccttcaacactt	NED	Dropped. Poor amplification					
CPPCT022	Aranzana *et al*. (2002)[1]	caattagctagagagaattattg gacaagaagcaagtagtttg	NED	Dropped. Poor amplification					

3.4.3. SSR genetic diversity analysis

Each band was considered to represent a di-allelic locus; thus, presence of the band present is scored as one allele and its absence is the alternative allele. The polymorphism information content (PIC) value for each SSR was estimated on an Excel spreadsheet using the following formula: $PIC = 1 - \sum x_i^2$, where x_i is the relative frequency of the *i*th allele of the SSR loci. Markers were classified as informative when PIC $\geq$ 0.5.

GenAlEx 6.5b3 (Peakall & Smouse, 2012) was used to assess the genetic diversity of each population and subspecies by calculating the percentage of polymorphic loci, allelic richness (number of alleles, number of private alleles, and number of locally common alleles), and expected heterozygosity (He). In GenAlEx, the genetic structure was studied by determining the number of private alleles for each population and subspecies, and by analyses of molecular variance (AMOVA; 999 permutations) for populations and subspecies. Additionally, correlation between geographic distance and genetic distance between individuals was tested in GenAlEx using Mantel tests (999 replicates).

In PAST software (Hammer, Harper & Ryan, 2001), the binary matrix was used to estimate a distance matrix using Nei´s unbiased measure of genetic distance (Nei, 1987), which bases on the proportion of alleles shared between two samples for all possible pairwise combinations of individuals and populations. The resultant matrix was subjected to a cluster analysis using the Neighbor-Joining method to obtain a tree that depicted the genetic relationships among individuals; a bootstrap analysis (10,000 replicates) was carried out to measure branch support by the data. Additionally, in GenAlEx, with the genetic distance matrix, a principal coordinate analysis (PCoA) was performed. The output of this test allows the representation of the distribution of the individuals in a multidimensional metric space in such a way that it reflects the samples' relationships based on their similarity in banding profiles, facilitating the visualization of their dispersion and possible structuring of the populations. In this study, two dimensions were chosen for illustration.

3.4.4. Analysis of population structure

The genetic structure was further investigated with Bayesian model-based clustering using the software STRUCTURE 2.3.4, which differentiates subgroups of individuals that have distinctive allele frequencies without *a priori* groupings (Pritchard, Stephens & Donnelly, 2000) and estimates the most likely number of genetic groups (K). STRUCTURE was run for K values ranging from 1 to 18. Each run was performed using the admixture model with 10,000 replicates for burn-in and 100,000 during the analysis (Falush, Stephens & Pritchard, 2007; Pritchard *et al*., 2000), and 10 simulations per K value. The STRUCTURE output was summarized using STRUCTURE HARVESTER (Earl & von Holdt, 2012). Both, the Evanno, Regnaut, and Goudet (2005) delta K test and the method of Pritchard *et al*. (2000) (K with the highest Pr (X|K) were performed to estimate the optimal value of K. CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg & Mayrose, 2015) was used for the summation and graphical presentation of the STRUCTURE results.

3.5. RESULTS

3.5.1. Molecular variation with respect to collection sites and subspecies

A total of 246 alleles were identified, giving an average of 15.4 alleles per locus for the 16 microsatellites evaluated (Table 8). The number of alleles ranged from 2 to 28, with the marker UDP98-409 having the highest number of alleles, followed by ssrPaCITA07, pchgms3, and BPPCT-039, each with 24 alleles. There was significant correlation (r = 0.936; p < 0.000) between the number of alleles per marker and the size range, and between the number of alleles per marker and PIC values (r = 0.794; p < 0.000). Based on PIC values, 15 of the SSR can be considered informative (PIC > 0.5), four of these being highly informative (PIC > 0.9).

Table 9 presents the allelic patterns across the 18 collection sites (populations) studied. This description of the 18 populations is based on the number of alleles and their observed frequencies. Five populations did not have private alleles, the other 13 had between one and six alleles unique to a single population. The NL1 and Coah3 populations had the highest gene diversity.

Table 9. Allelic values recorded in the 18 sampled natural populations of black cherry. Alleles freq. ≥ 5 %: number of alleles with a frequency ≥ 5 %. Private alleles: number of alleles unique to a single population. LComm alleles (≤ 25 %): number of locally common alleles (Freq. ≥ 5 %) found in 25 % or fewer populations. LComm alleles (≤ 50 %): number of locally common alleles (Freq. ≥ 5 %) found in 50 % or fewer populations. He: expected heterozygosity = 2pq. uHe: unbiased expected heterozygosity = (2N/[2N-1])*He. For diploid binary data and assuming Hardy-Weinberg Equilibrium: q = (1 - Band freq.)^0.5, and p = 1 - q.

Population		n	Alleles	Alleles freq. ≥ 5 %	Private alleles	LComm alleles (≤ 25 %)	LComm alleles (≤ 50 %)	Mean He (SE)	Mean uHe (SE)
Code	ID								
1	Mich4_ser	10	97	97	1	6	32	0.083 (0.009)	0.088 (0.009)
2	Tlax1_cap	9	97	97	2	4	29	0.092 (0.009)	0.097 (0.010)
3	Tlax2_cap	15	113	113	0	11	45	0.087 (0.008)	0.090 (0.009)
4	Coah3_vir	9	101	101	4	2	28	0.102 (0.010)	0.108 (0.011)
5	Coah2_vir	9	94	94	4	6	32	0.088 (0.009)	0.093 (0.010)
6	Coah1_vir	9	78	78	4	5	20	0.095 (0.011)	0.101 (0.012)
7	NL1_vir	9	117	117	5	9	46	0.106 (0.009)	0.112 (0.010)
8	NL2_vir	6	94	94	2	3	31	0.092 (0.009)	0.100 (0.010)
9	NL3_vir	3	67	67	0	5	20	0.075 (0.010)	0.090 (0.011)
10	Mex_cap	14	107	107	4	10	36	0.089 (0.009)	0.092 (0.009)
11	Qro_ser	14	145	145	6	20	64	0.100 (0.008)	0.104 (0.009)
12	Mich3_ser	12	122	122	2	12	46	0.098 (0.009)	0.102 (0.010)
13	Mich1_ser	12	107	107	2	5	38	0.100 (0.010)	0.104 (0.010)
14	Mich2_ser	5	72	72	0	0	16	0.085 (0.010)	0.094 (0.011)
15	Mich5_ser	14	86	86	0	3	30	0.083 (0.009)	0.086 (0.010)
16	Gto_vir	14	121	121	2	16	51	0.100 (0.009)	0.104 (0.010)
17	TX1_exi	3	52	52	0	6	18	0.059 (0.009)	0.070 (0.011)
18	TX2_exi	11	92	92	2	12	38	0.072 (0.008)	0.075 (0.008)

The total diversity (H = Hs + Dst) is the average locus heterogeneity over the total number of evaluated loci; within-group diversity (Hs) is the weighted average of diversity within each group, the diversity among groups (Dst) is the difference between H and Hs, and the ratio Dst/H corresponds to the genetic differentiation coefficient (Gst). Thus, the comparison of genetic diversity of the alleles obtained across the 18 populations showed that the amount of diversity in these was moderately similar; that is, the proportion of total variation explained by the difference among populations, their genetic differentiation (Gst), accounted for 25% of the total diversity. Figure 9 describes the four subspecies based on their allelic patterns, number of bands and their observed frequencies. The line above the bars indicates pattern of gene diversity among them, and depicts that ssp. *virens* had the highest gene diversity. The comparison of genetic diversity across the four subspecies showed that the genetic differentiation (Gst) was even lower (16 %) than the one for populations. In this context, the genetic differentiation between *eximia* and the other three subspecies (15 %) was higher than the differentiation among these three (6 %).

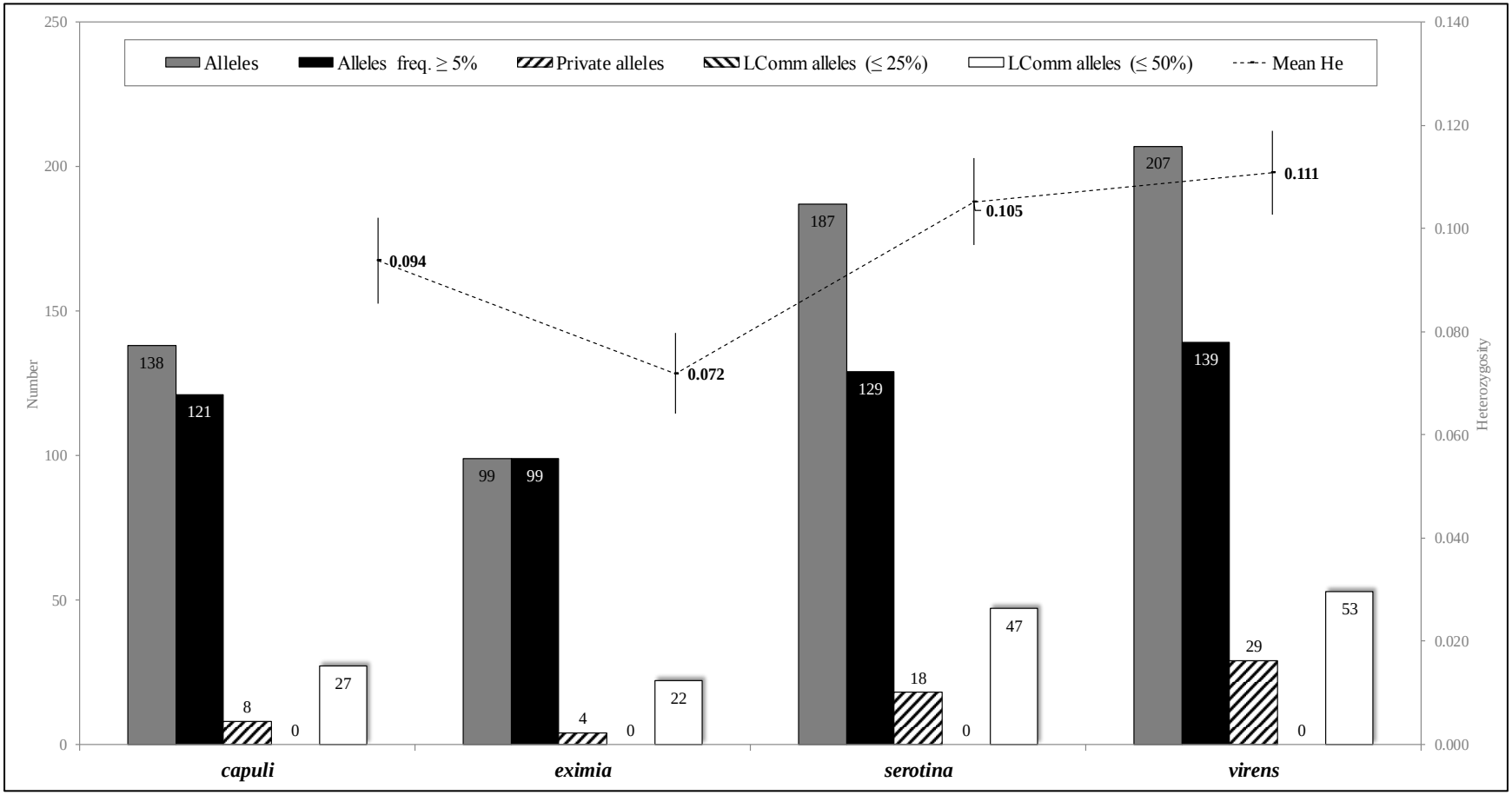

Figure 9. Allelic patterns across the four subspecies of black cherry studied. Allele freq. ≥ 5 %: number of alleles with a frequency ≥ 5 %. LComm alleles (≤ 25 %): number of locally common alleles (Freq. ≥ 5 %) found in 25 % or fewer populations. LComm alleles (≤ 50 %): number of locally common alleles (Freq. ≥ 5 %) found in 50 % or fewer populations. He: expected heterozygosity = 2pq. For diploid binary data and assuming Hardy-Weinberg Equilibrium: q = (1 - Band freq.)^0.5 and p = 1 - q.

The AMOVA results showed that 79 % of genetic variation was attributed to individuals within the 18 populations ($p < 0.001$), and the remaining was attributed to the diversity among populations (Table 10). On the other hand, while the amount of genetic differentiation detected among subspecies was also highly significant ($p < 0.001$), this differentiation was mainly due to genetic variation within the subspecies (86 %).

Table 10. Results of the AMOVA conducted for populations and for subspecies. Permutations: 999. df: degree of freedom; SS: sum of square; MS: mean square; Est. Var.: estimated variation. Stat: statistics.

Source of variation	df	SS	MS	Est. Var.	Value (%)	Stat.	*p* value
Among populations	17	999.820	58.813	4.312	20.701	0.207	0.001
Within populations	160	2642.838	16.518	16.518	79.299		
Total	177	3642.657		20.830	100.0		
Among ssp.	3	413.349	137.783	2.906	13.539	0.135	0.001
Within ssp.	174	3229.308	18.559	18.559	86.461		
Total	177	3642.657		21.466	100.0		

3.5.2. Neighbor-Joining and PCoA clusterings

Figure 10 shows the Neighbor-Joining clustering pattern for individuals. Two main groups were identified (Texans = ssp. *eximia*, and Mexicans = ssp. *capuli*, *serotina* and *virens*). Furthermore, within the Mexican cluster, individuals from different collections sites and subspecies grouped together, impeding a conclusive interpretation based on their taxonomic identities.

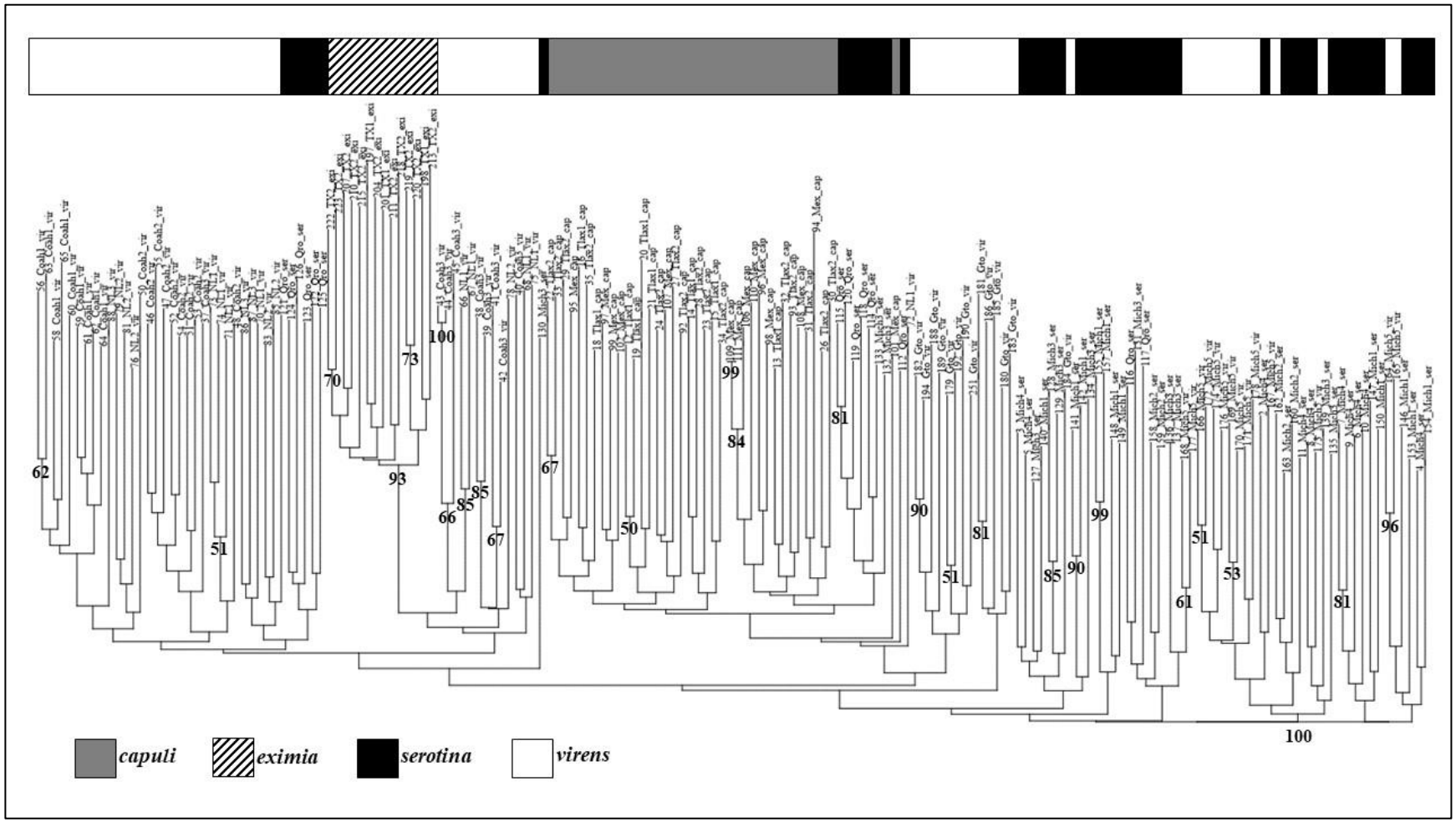

Figure 10. Neighbor-Joining tree, based on Nei´s genetic distance, depicting the genetic relationships among the 178 individuals studied. Numbers at the nodes provide branch support ≥ 50 % based on 10,000 bootstrap replicates.

In the PCoA conducted using the 18 natural populations, the first three axes accounted together 23.17 % of the total variation, with 9.17, 8.90 and 5.10 % explained, respectively, by PC axis 1, 2, and 3. The results of the PCoA reflect the samples' relationships based on their similarity in banding profiles. Nonetheless, the representation of the distribution of the individuals in a bi-dimensional PCoA space suggested a structuring of three main clusters (Figure 11), corresponding to one group that included the 14 *eximia* individuals collected in Texas, a second group clustering 38 *capuli* individuals sampled in the two populations from Tlaxcala and eight *serotina* individuals collected in Michoacán (3) and Querétaro (5); and a third group composed of 126 samples representing subspecies *serotina* and *virens*. Congruently with clustering analysis, individuals from Texas clearly grouped together while those from México were intermixed with no evident separation based on collection site and/or subspecies. Additionally, the Mantel tests resulted in a positive significant correlation (r = 0.6732, $p < 0.001$), indicating that there is a pattern of isolation by distance among the populations (collection sites).

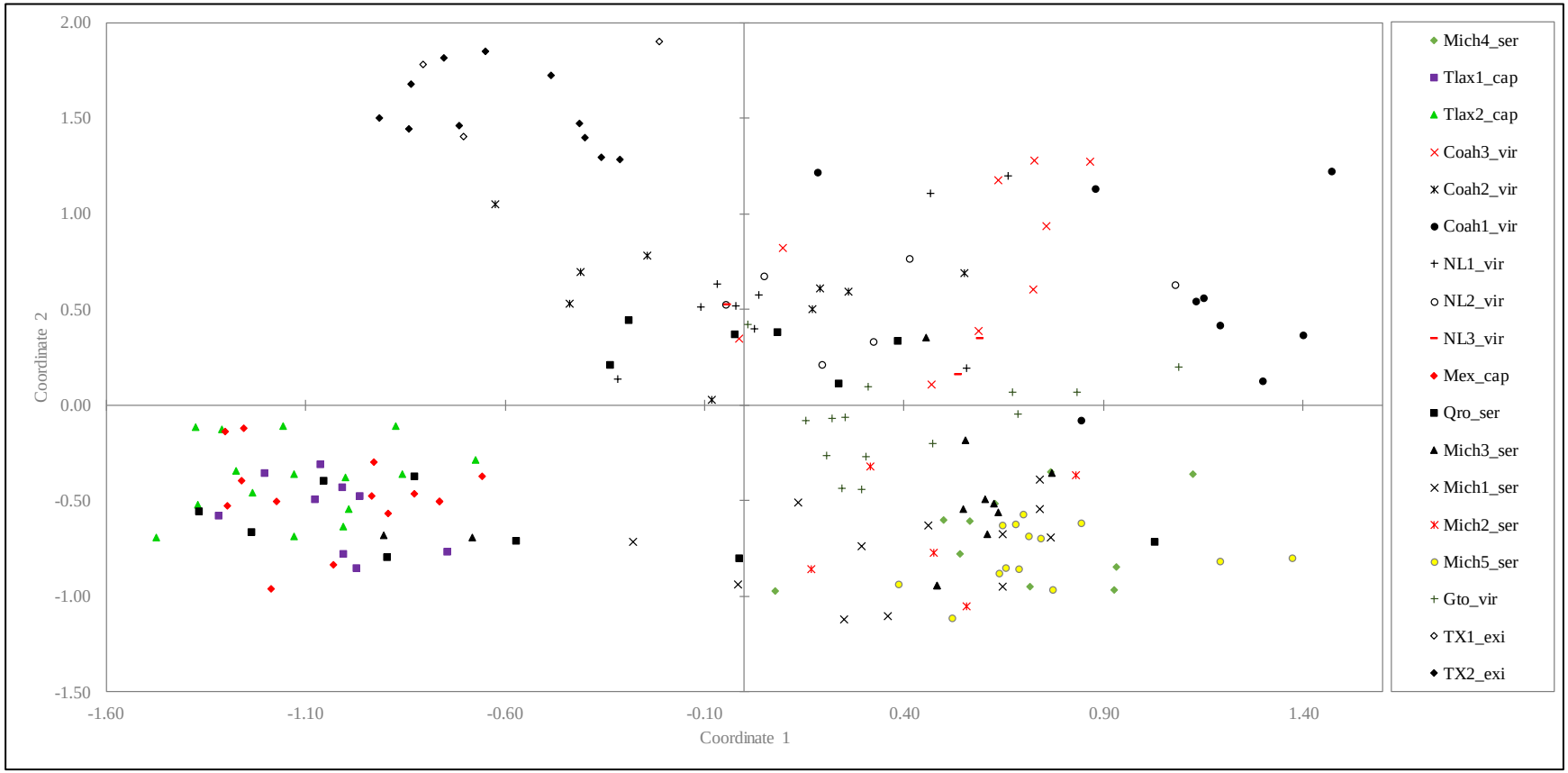

Figure 11. PCoA bi-dimensional projection of the 18 populations studied. cap: *capuli*, exi: *eximia*, ser: *serotina*, and vir: *virens*.

3.5.3. Analysis of populations by STRUCTURE

The delta K statistic in the Evanno test showed that K = 3 was the optimal number of subpopulations in this analysis. This split was congruent with the Neighbor-Joining clustering and PCoA analyses performed by populations, because clearly grouped together all individuals representing ssp. *eximia*, tended to include all individuals from ssp. *capuli* into a single group, and still depicted intermixed clusters of ssp. *capuli*, *serotina* and *virens* (Figure 12).

Among these highly similar 10 runs (mean similarity score: 0.900), the run with the highest likelihood value (lnL = -13921.1) was chosen to classify samples based on posterior assignment probabilities ($p \geq 0.900$), and explore the possibility of a geographical explanation of these three genetic clusters. Thirteen Texan individuals representing ssp. *eximia* were classified into a single group, which also included samples collected in Coahuila (22 *virens*), Nuevo León (12 *virens*), and Querétaro (three *serotina*). Another cluster grouped together 23 individuals collected in Tlaxcala, all of them representing ssp. *capuli*, as well as individuals from México (13 *capuli*), Michoacán (two *serotina*), and Querétaro (five *serotina*). A third cluster included 43 samples from Michoacán (29 *serotina* and 14 *virens*) and one *serotina* sample collected in Querétaro. In brief, this K = 3 value suggested that populations with geographical proximity (Table 7) tended to group together. However, it did not show clear segregation among subspecies, impeding an interpretation of its subgroups based on taxonomic identities.

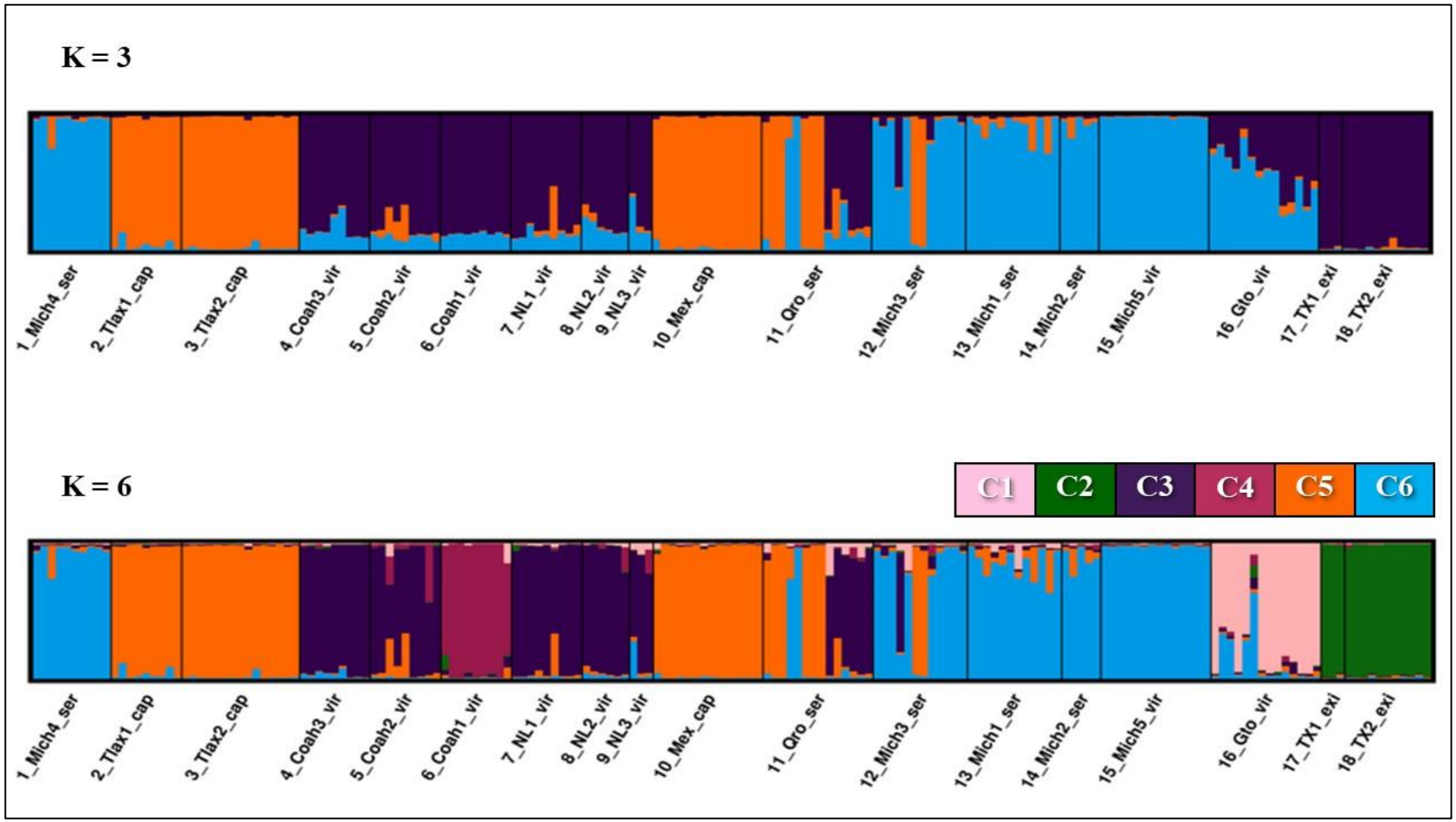

Figure 12. Population structure for the 178 individuals studied at K = 3 and K = 6. cap: *capuli*, exi: *eximia*, ser: *serotina*, and vir: *virens*.

A second peak in the Evanno test suggested that at K = 6 there was a further meaningful subdivision of Mexican samples; moreover, using median values of ln(Pr Data), this K = 6 had the highest lnProb (-13515.1). For K= 6, the clustering in different runs was almost identical (similarity coefficient 0.981). The run with the highest likelihood value (lnL = -12954.7) was selected among these 10 runs (Figure 12), and the individuals with more than 90 % posterior assignment probability were assigned to each of these six clusters. At the $p \geq 0.900$ cut off values, the proportion of non-hybrid individuals in each collection site ranged from 0 % in one population (NL3_vir) to 100 % in three (Tlax2_cap, Mich5_vir, TX1-exi and TX2_exi) (Table 11). Lower posterior assignment probability values less than 90 % were taken as indicative of hybrids or admixed individuals.

The six clusters were identified as *C1:* Gto_virens, *C2:* TX1-2_eximia, *C3:* Coah2-3 + NL1-2_virens, *C4:* Coah1_virens, *C5:* Tlax1-2_Mex_cap + QroMich3_ser, and *C6:* Mich1-2-3-4_ser + Mich5_vir (Table 11, Figure 12). These clusters merged geographically nearby populations. Four out the five populations from the Northern Mexican states Coahuila (2) and Nuevo León (2) were included in the cluster C3, the fifth population (Coah1_virens) conformed one single group (C4). Five populations from Tlaxcala (2), México (1), Querétaro (1), and Michoacán (1) integrated the cluster C5; these are states from the central-western region of México. Each of Clusters C1 and C2 was constituted by one population from only one state (Guanajuato and Texas, respectively).

Table 11. Composition of the six main genetic clusters at $p \geq 0.900$ cut off values. H = putative hybrid individual.

Sample	Pop	*p*	Cluster	Sample	Pop	*p*	Cluster	Sample	Pop	*p*	Cluster	Sample	Pop	*p*	Cluster
1	1	0.936	C6	2	1	0.977	C6	3	1	0.737	H	4	1	0.972	C6
5	1	0.972	C6	6	1	0.945	C6	7	1	0.938	C6	8	1	0.974	C6
9	1	0.964	C6	10	1	0.937	C6	11	2	0.967	C5	12	2	0.875	H
13	2	0.986	C5	14	2	0.975	C5	15	2	0.930	C5	16	2	0.968	C5
17	2	0.963	C5	18	2	0.897	H	19	2	0.975	C5	20	3	0.974	C5
21	3	0.968	C5	22	3	0.976	C5	23	3	0.979	C5	24	3	0.985	C5
25	3	0.981	C5	26	3	0.979	C5	27	3	0.978	C5	28	3	0.944	C5
29	3	0.916	C5	30	3	0.985	C5	31	3	0.972	C5	32	3	0.984	C5
33	4	0.929	C3	34	4	0.940	C3	35	4	0.902	C3	36	4	0.926	C3
37	4	0.952	C3	38	4	0.893	H	39	4	0.980	C3	40	4	0.974	C3
41	4	0.982	C3	42	5	0.938	C3	43	5	0.942	C3	44	5	0.383	H
45	5	0.864	H	46	5	0.635	H	47	5	0.972	C3	48	5	0.966	C3
49	5	0.545	H	50	5	0.931	C3	51	6	0.817	H	52	6	0.985	C4
53	6	0.989	C4	54	6	0.933	C4	55	6	0.984	C4	56	6	0.979	C4
57	6	0.957	C4	58	6	0.982	C4	59	6	0.679	H	60	7	0.926	C3
61	7	0.962	C3	62	7	0.959	C3	63	7	0.907	C3	64	7	0.960	C3
65	7	0.609	H	66	7	0.969	C3	67	7	0.974	C3	68	7	0.964	C3
69	8	0.883	H	70	8	0.940	C3	71	8	0.924	C3	72	8	0.959	C3
73	8	0.955	C3	74	8	0.771	H	75	9	0.620	H	76	9	0.875	H
77	9	0.746	H	78	3	0.968	C5	79	3	0.977	C5	80	10	0.869	H
81	10	0.985	C5	82	10	0.980	C5	83	10	0.961	C5	84	10	0.973	C5
85	10	0.980	C5	86	10	0.940	C5	87	10	0.961	C5	88	10	0.983	C5
89	10	0.978	C5	90	10	0.988	C5	91	10	0.978	C5	92	10	0.986	C5
93	10	0.978	C5	94	11	0.827	H	95	11	0.981	C5	96	11	0.987	C5
97	11	0.719	H	98	11	0.967	C6	99	11	0.955	C5	100	11	0.979	C5

Table 11. Continuation.

Sample	Pop	*p*	Cluster	Sample	Pop	*p*	Cluster	Sample	Pop	*p*	Cluster	Sample	Pop	*p*	Cluster
101	11	0.986	C5	102	11	0.697	H	103	11	0.672	H	104	11	0.826	H
105	11	0.862	H	106	11	0.859	H	107	11	0.944	C3	108	12	0.949	C6
109	12	0.895	H	110	12	0.936	C6	111	12	0.744	H	112	12	0.741	H
113	12	0.948	C5	114	12	0.925	C5	115	12	0.753	H	116	12	0.928	C6
117	12	0.970	C6	118	12	0.973	C6	119	12	0.936	C6	120	13	0.974	C6
121	13	0.896	H	122	13	0.765	H	123	13	0.853	H	124	13	0.830	H
125	13	0.928	C6	126	13	0.717	H	127	13	0.890	H	128	13	0.711	H
129	13	0.961	C6	130	13	0.607	H	131	13	0.929	C6	132	14	0.944	C6
133	14	0.753	H	134	14	0.969	C6	135	14	0.854	H	136	14	0.892	H
137	15	0.978	C6	138	15	0.971	C6	139	15	0.984	C6	140	15	0.979	C6
141	15	0.982	C6	142	15	0.968	C6	143	15	0.987	C6	144	15	0.975	C6
145	15	0.989	C6	146	15	0.967	C6	147	15	0.983	C6	148	15	0.985	C6
149	15	0.975	C6	150	15	0.971	C6	151	16	0.969	C1	152	16	0.679	H
153	16	0.668	H	154	16	0.952	C1	155	16	0.697	H	156	16	0.608	H
157	16	0.945	C1	158	16	0.969	C1	159	16	0.953	C1	160	16	0.850	H
161	16	0.875	H	162	16	0.966	C1	163	16	0.969	C1	164	17	0.979	C2
165	17	0.979	C2	166	17	0.936	C2	167	18	0.945	C2	168	18	0.983	C2
169	18	0.980	C2	170	18	0.950	C2	171	18	0.973	C2	172	18	0.976	C2
173	18	0.969	C2	174	18	0.966	C2	175	18	0.973	C2	176	18	0.959	C2
177	18	0.983	C2	178	16	0.911	C1								

On the other hand, the first three axes of the PCoA performed using these six clusters, accounted together 28.06 % of the total variation, with 11.51, 10.77 and 5.78 % explained by the PC axis 1, 2, and 3, respectively. Figure 13 depicts the bi-dimensional (axes 1 and 2) scattering of these six main genetic clusters.

The six clusters did not correspond to the four subspecies, similar to the results from Neighbor-Joining, PCoA, and K = 3. However, they reinforced that *eximia* individuals are the most distinct ones, since they comprised one exclusive cluster (C2). In turn, cluster C5 included samples representing ssp. *capuli* and *serotina*, and C6 grouped *serotina* and *virens* individuals; moreover, ssp. *virens* was split into three other clusters (C1, C3 and C4).

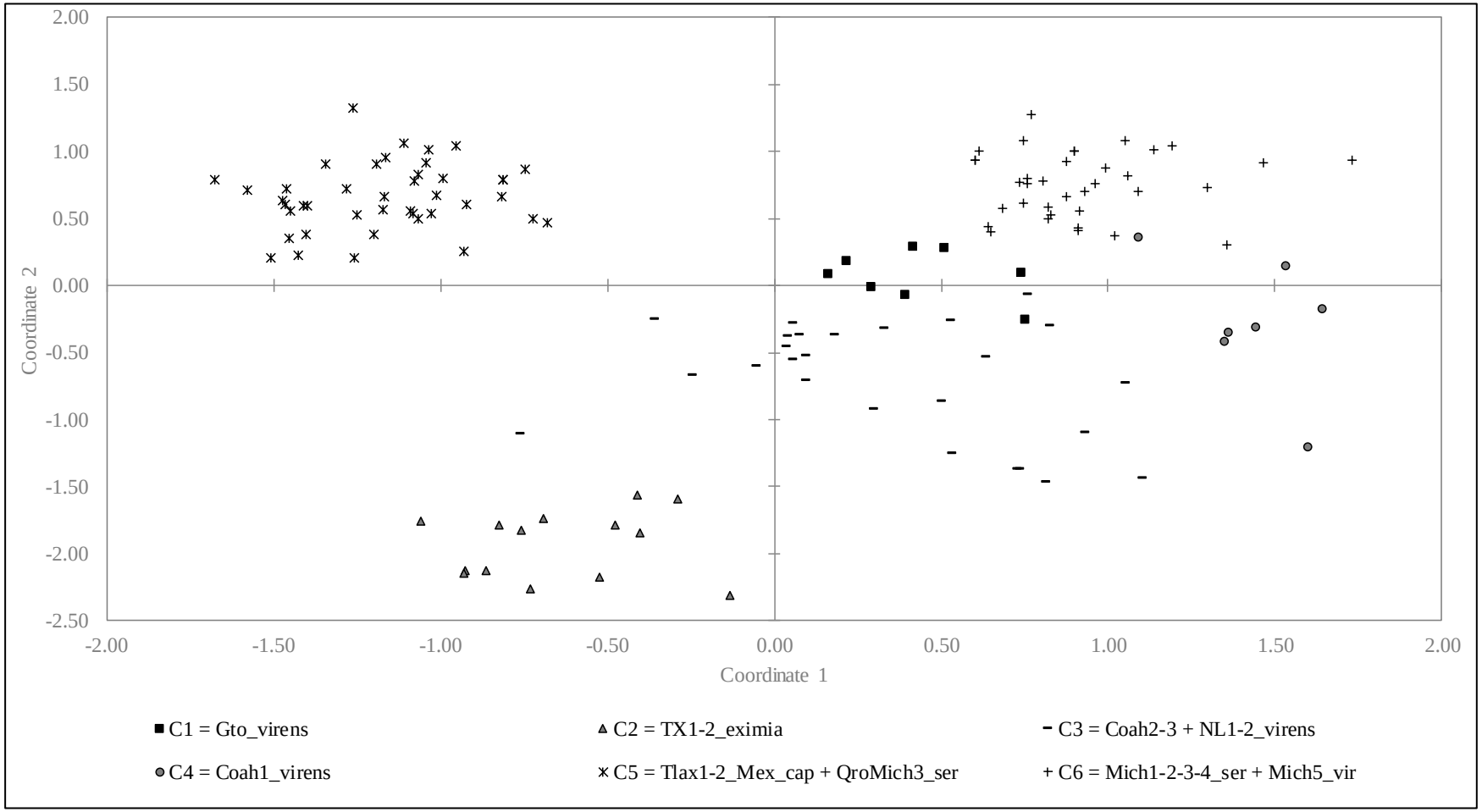

Figure 13. PCoA bi-dimensional projection of the six main clusters detected by STRUCTURE. cap: *capuli*, ser: *serotina*, and vir: *virens*.

3.5.4. Genetic distinctiveness among clusters in K = 6

On average, C2 and C4 had higher Fst value (0.377) than C1, C5 and C6 (0.1964); in turn, C3 had the lowest Fst (0.091) (Table 12). At the $p \geq 0.900$ cutoff value, the proportion of non-hybrid individuals ranged from 61 % (C1 and C3, both wholly *virens*) to 100 % in C2 (the exclusive *eximia* cluster). In consequence, the proportions of polymorphic loci were 49 % in the C3 individuals; 43 % and 44 % in the clusters C5 (*capuli* + *serotina*) and C6 (*serotina* + *virens*); 36 % and 40 % in the groups C1 (*virens*) and C2, respectively; and 26 % in the *virens* cluster C4.

Table 12. Composition of the six main genetic clusters detected by STRUCTURE. ssp.: subspecies.

Cluster	Fst	n total	$p \geq 0.900$ cutoff		Populations within the cluster	ssp.
			n	% from total		
C1	0.191	13	8	61.5 %	Gto	*virens*
C2	0.358	14	14	100.0 %	TX1, TX2	*eximia*
C3	0.091	43	26	60.5 %	Coah2, Coah3, NL1, NL2	*virens*
C4	0.396	9	7	77.8 %	Coah1	*virens*
C5	0.207	46	42	91.3 %	Tlax1, Tlax2, Mex + Qro, Mich3	*capuli* + *serotina*
C6	0.191	53	36	67.9 %	Mich1, Mich2, Mich3, Mich4 + Mich5	*serotina* + *virens*

The allelic patterns across these six clusters are presented in Figure 14. This figure describes the six groups based on the number of bands and their observed frequencies.

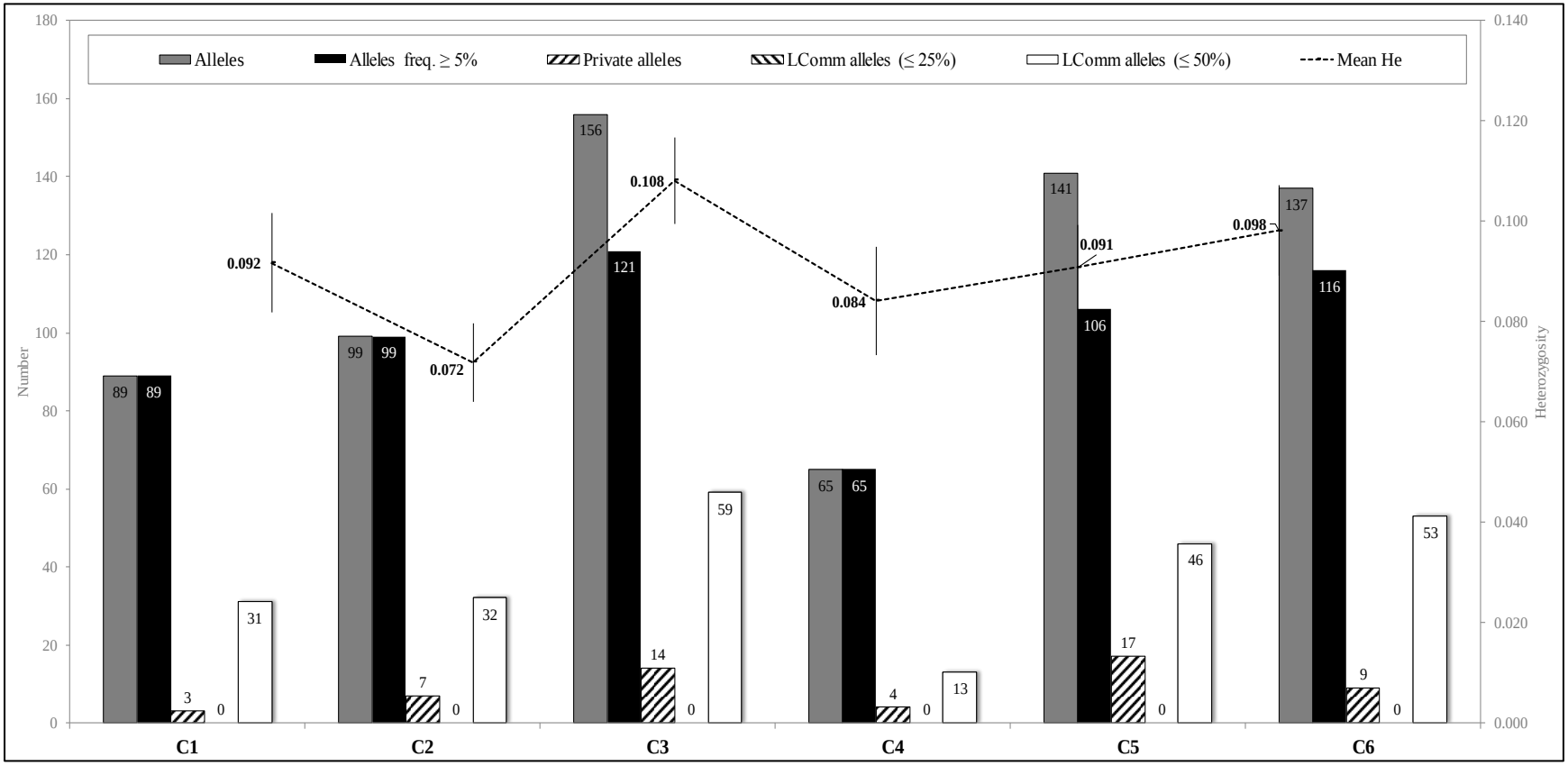

Figure 14. Allelic patterns across the six main genetic clusters after classifying individuals at the p ≥ 0.900 cut off values. Allele freq. ≥ 5 %: number of alleles with a frequency ≥ 5 %. LComm alleles (≤ 25 %): number of locally common alleles (Freq. ≥ 5 %) found found in 25 % or fewer populations. LComm alleles (≤ 50 %): number of locally common alleles (Freq. ≥ 5 %) found in 50 % or fewer populations. He: expected heterozygosity = 2pq. For diploid binary data and assuming Hardy-Weinberg Equilibrium: q = (1 - Band freq.)^0.5 and p = 1 - q.

The C3 cluster had the highest number of different alleles, common alleles, and heterogeneity; whereas C4 had the lowest values for these parameters, except for number of private bands that corresponded to C1. The line above the bars indicates pattern of gene diversity among the different clusters. In terms of genetic diversity, clusters C1 and C4 (both *virens*) brought together the lowest numbers of individuals (8 and 7, respectively), but cluster C2 (*eximia*) with 14 individuals was the least diverse. In contrast, cluster C3 was the most diverse despite the fact that the biggest number of individuals were grouped by the admixture clusters C5 (*capuli* and *serotina*) and C6 (*serotina* and *virens*), which were the biggest ones (42 and 36, respectively). Complementarily, the allelic patterns across subspecies, after classifying individuals at the $p \geq 0.900$ cutoff values, are presented in Figure 15.

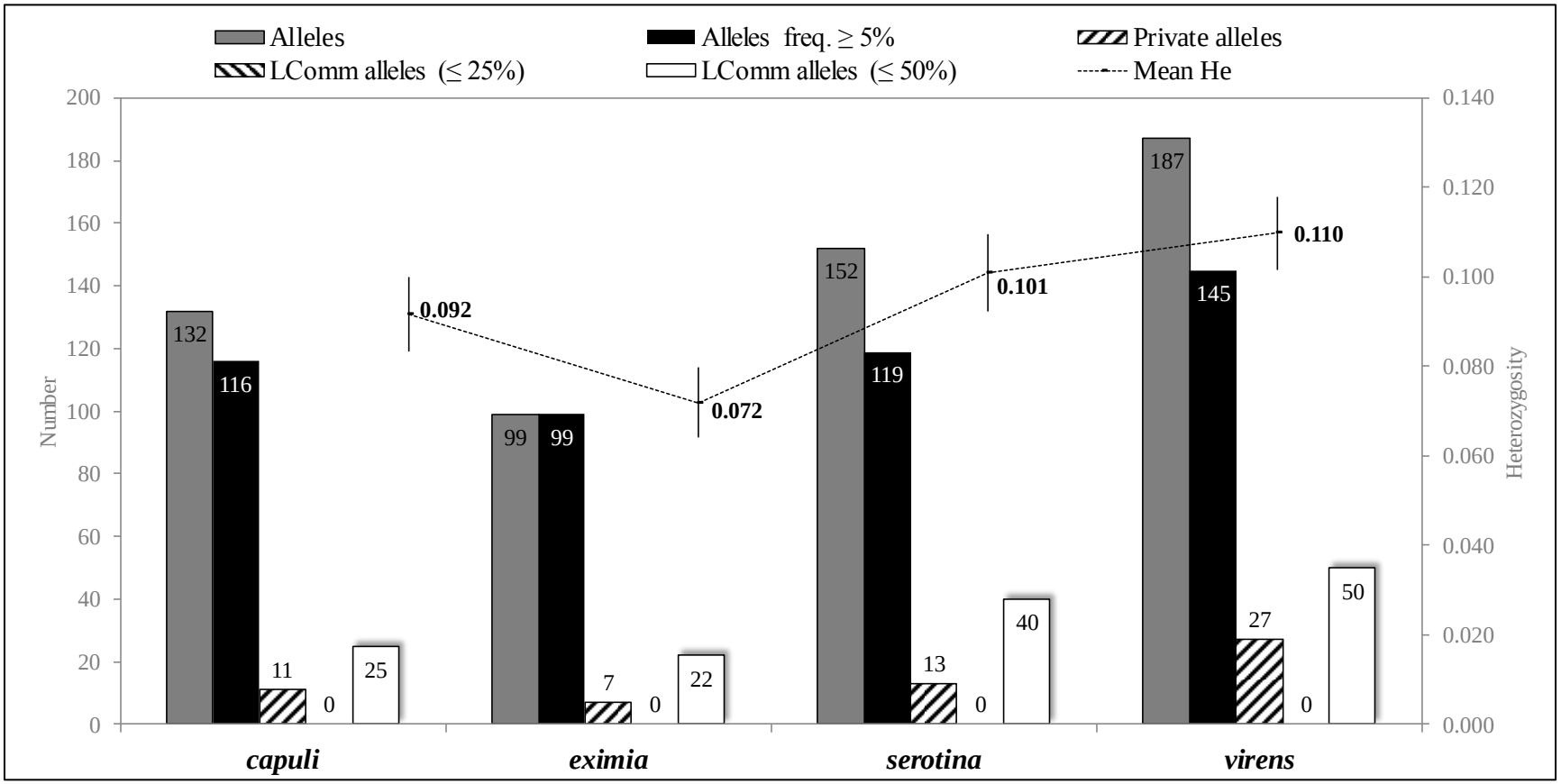

Figure 15. Allelic patterns across the four subspecies after classifying individuals at the p ≥ 0.900 cut off values. Allele freq. ≥ 5 %: number of alleles with a frequency ≥ 5 %. LComm alleles (≤ 25 %): number of locally common alleles (Freq. ≥ 5 %) found in 25 % or fewer populations. LComm alleles (≤ 50 %): number of locally common alleles (Freq. ≥ 5 %) found in 50 % or fewer populations. He: expected heterozygosity = 2pq. For diploid binary data and assuming Hardy-Weinberg Equilibrium: q = (1 - Band freq.)^0.5 and p = 1 - q.

3.6. DISCUSSION

3.6.1. Genetic diversity across populations

As far as is known, this is the first molecular study that includes four out of the five black cherry subspecies. Preceding studies have included only one subspecies; for instance, *serotina* by Beck *et al*. (2014), and *capuli* by Gordillo, Tobar, Arahana, and Torres (2015), and Guadalupe *et al*. (2015). In the raw data, the maximum number of peaks per individual was four in eight SSRs (Table 8), this clearly matches with the fact that black cherry is tetraploid, as previously indicated by Pairon and Jacquemart (2005); although diploid, pentaploid and hexaploid individuals have been reported by Forbes (1969) and Dickson *et al*. (1992).

The comparison of the banding patterns of the samples collected from 18 populations shows that they differed moderately in terms of their total genetic diversity (25 %). Populations studied showed a low percentage of unique bands (Table 9; 0 % ≤ Private alleles ≤ 5 %), which could mean that these 18 populations have a low frequency of rare genetic variants and there has been significant gene flow among them historically. Despite the fact we detected a distance-structured genetic divergence causing more similarity between neighboring populations. In general, these findings indicate that few populations would be sufficiently representative of the total diversity currently present in Mexican populations, or at least of that portion of the seven states included in the present study.

The hierarchical classification scheme into two main clusters obtained in this study, segregating ssp. *eximia* from the three Mexican subspecies, was in disagreement with McVaugh's (1951) proposal. The Texan group was exclusively formed by individuals belonging to ssp. *eximia*; however, the results of this study do not validate the taxonomic distinctness of spp. *capuli*, *serotina* and *virens*. The obtained genetic differentiation between ssp. *eximia* and the others (15 %) indicates that this can be considered distinct from them, and does not support that Mexican populations correspond to three distinct biological subspecies (Gst = 6

%). We expected that true taxonomic groups would have a striking number of specific molecular markers that allow their molecular identification. Subspecies-specific markers found were insufficient to support the molecular identity of *capuli*, *serotina* and *virens*, testifying the absence of significant differences between these taxa. Therefore, it seems that the genetic clusters detected do not correspond to subspecies.

The observed genetic differentiation parameters were also validated by clustering using Bayesian modelling (Figure 12), where again ssp. *capuli*, *serotina* and *virens* populations did not form corresponding clusters, thus pointing to an absence of significant molecular differentiation between these three taxa.

The difficulties in taxonomic differentiation of these taxa have been previously reported by McVaugh (1951), and Rzedowski and Calderón de Rzedowski (2005). Similarly, Fresnedo-Ramírez *et al*. (2011) examined 39 morphological characters from different plant organs and found that they barely differentiated two main geographical and morphological groups, which did not clearly correspond to the three Mexican subspecies; according to them, morphological similarities within groups are a possible consequence of human selection related to the fruit consumption, which is differential among the two geographical Mexican regions studied by them.

3.6.2. Subspecies genetic distinctiveness is subtle

Based on Bayesian modelling, six genetic clusters (K = 6) with moderate percentage of hybrid individuals (25 %) were observed in the present study. These putative hybrid individuals will be important in future studies aimed at shedding light on the evolutionary history of the black cherry in its natural geographical range of distribution, and as baseline information in future decisions on the conservation of its genetic resources. The configuration of the sample in six main clusters confirms that there is genetic structure among the 18 populations analyzed; however, this does not correspond to the four subspecies studied.

For the six clusters at the minimal cutoff value of 0.900, subspecies *eximia* separates significantly from the rest of the subspecies, as expected according to the classical classification indicated by morphology (McVaugh, 1951). This subspecies is geographically isolated from the other three subspecies studied due to it is restricted to Edwards Plateau in Texas, and morphologically is the most distinct of any of them (McVaugh, 1951). Clusters C1, C3, and C4 were composed of ssp. *virens* only; but they split populations according to their geographical proximity (C1: Guanajuato, C3: Coahuila and Nuevo León, and C4: Coahuila). It is noteworthy that group C2 was exclusively composed of ssp. *eximia*, the most distinct of any of the subspecies. The clustering of *capuli* together with *serotina* (C5) reflects the closeness of these two subspecies (Hernández-Xolocotzi, 1993; McVaugh, 1951); on the other hand, ssp. *serotina* grouped also with ssp. *virens* (C6). The authenticity of these two latter clusters relies on fact that they have a substantial percentage of unique or private alleles (Figure 14; 12 % and 7 %, respectively). Together, these two clusters may point to the fact that in México there has been recent gene flow among these three subspecies, and help explain the difficulties that they offer to taxonomists (McVaugh, 1951; Rzedowski & Calderón de Rzedowski, 2005). The presence of gene flow tending to even out the diversity among populations could explain why, other than *eximia*, there was no clear genetic differentiation among the subspecies.

Thus, a strategy of *in situ* conservation of black cherry genetic resources would require the identification of areas where more than one subspecies is present, such as the Mexican states of México, Michoacán, and Tlaxcala. This conservation option allows evolutionary processes to continue, and adds the advantage that it goes together with continued use by local people. Since *in situ* conservation is considered to be a complementary approach to *ex situ* conservation, it is important to promote the establishment of formal comprehensive black cherry *ex situ* collections. This would allow further characterization and evaluation of black cherries from different geographical areas and, in consequence, they would be available for breeders and general

users. *Ex situ* conservation would also guarantee the conservation of these valuable genetic resources in the event that they disappear in the future.

In conclusion, this study offers new insights about the pattern and extent of genetic diversity and population structure of *P. serotina* in North America. The results in the context of five subspecies elucidate the presence of adequate genetic diversity organized into two main groups, Texan and Mexican, the latter with five subgroups. In turn, the interpretation of these six genetic clusters identified by STRUCTURE, can be strengthened using morphological information.

3.7. LITERATURE CITED

Adriano-Morán, C.C., & McClung de Tapia, E. (2008). Trees and shrubs: the use of wood in prehispanic Teotihuacan. *Journal of Archaeological Science*, 35(11), 2927-2936.

Aranzana, M.J., Carbó, J., & Arús, P. (2002). Microsatellite variability in peach [*Prunus persica* (L.) Batsch]: cultivar identification, marker mutation, pedigree inferences and population structure. *Theoretical and Applied Genetics*, 106(8),1341-1352, doi:10.1007/s00122-002-1128-5.

Barnd, B.D., & Ginzel, M.D. (2008). Causes of gummosis in black cherry (*Prunus serotina*). USDA Forest Service and Purdue University. FNR-229-W. 6 p.

Beck, J.B., Ferguson, C.J., Mayfield, M.H., & Shaw, J. (2014). Reduced genetic variation in populations of Black Cherry (*Prunus serotina* subsp. *serotina*, Rosaceae) at its western range limit in Kansas. *Northeastern Naturalist*, 21(3), 472-478.

Cipriani, G., Lot, G., Huang, W.G., Marrazzo, M.T., Peterlunger, E., & Testolin, R. (1999). AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. *Theoretical and Applied Genetics*, 99, 65-72, doi:10.1007/s001220051209.

Clarke, J.B., & Tobutt, K.R. (2003). Development and characterization of polymorphic microsatellites from *Prunus avium* 'Napoleon'. *Molecular Ecology Notes*, 3(4),578-580, doi:10.1046/j.1471-8286.2003.00517.x.

Costa, C.M., & Roberts, R.P. (2014). Techniques for improving the quality and quantity of DNA extracted from herbarium specimens. *Phytoneuron*, 2014-48, 1-8.

Dickson, E. E., Arumuganathan, K., Kresovich, S., & Doyle, J.J. (1992). Nuclear DNA content variation within the Rosaceae. *American Journal of Botany*, 79(9), 1081-1086.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M., Poizat, C., Zanetto, A., Arús, P., & Laigret, F. (2002). Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theoretical and Applied Genetics*, 105, 127-138, doi:10.1007/s00122-002-0867-7.

Downey, S. L., & Iezzoni, A.F. (2000). Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. *Journal of the American Society for Horticultural Science*, 125(1), 76-80.

Earl, D.A., & VonHoldt, B.M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359-361, doi:10.1007/s12686-011-9548-7.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14(8), 2611-2620, doi:10.1111/j.1365-294X.2005.02553.x.

Falush, D., Stephens, M., & Pritchard, J.K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, 7(4), 574-578, doi:10.1111/j.1471-8286.2007.01758.x.

Forbes, D. (1969). *Self incompatibility and cross-incompatibility in black cherry* (*Prunus serotina*) (Ph.D. dissertation). University of Florida, Gainesville, USA. 92 p.

Fresnedo-Ramírez, J., Segura, S., & Muratalla-Lúa, A. (2011). Morphovariability of capulín (*Prunus serotina* Ehrh.) in the central-western region of Mexico from a plant genetic resources perspective. *Genetic Resources and Crop Evolution*, 58(4), 481-495, doi:10.1007/s10722-010-9592-2.

Germplasm Resources Information Network – GRIN (2015). *Prunus serotina*. Consulted on-line https://npgsweb.ars-grin.gov/gringlobal/view2.aspx?dv=web_taxonomyspecies_view_accessionlist&params=:taxonomyid=30099.

Gordillo, M., Tobar, J., Arahana, V., & Torres, M.L. (2015). Identification of S alleles associated with self-incompatibility in capuli (*Prunus serotina* subsp. *capuli*) samples by amplification of the Intron I of the S-RNasa gene. *Avances en Ciencias e Ingenierías* 7(1), B17-B23.

Guadalupe, J.J., Gutiérrez, B., Intriago-Baldeón, D.P., Arahana, V., Tobar, J., Torres A.F., & Torres, M.L. (2015). Genetic diversity and distribution patterns of Ecuadorian capuli (*Prunus serotina*). *Biochemical Systematics and Ecology*, 60, 67-73, doi:10.1016/j.bse.2015.04.001.

Hagen, L.S., Chaib, J., Fady, B., Decroocq, V., Bouchet, J.P., Lambert, P., & Audergon, J.M. (2004). Genomic and cDNA microsatellites from apricot

(*Prunus armeniaca* L.). *Molecular Ecology Notes*, 4(4), 742-745, doi:10.1111/j.1471-8286.2004.00802.x.

Hammer, Ø., Harper, D.A.T., & Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1-9.

Hernández-Xolocotzi, E. (1993). Aspects of plant domestication: A personal view. In T. P. Ramamoorthy, R. Bye, A. Lot, and J. Fa (Eds.), *Biological Diversity of Mexico, Origins and Distribution*. Oxford University Press, New York. 733 p.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, 15(5), 1179-1191, doi:10.1111/1755-0998.12387.

Lopes, M.S., Sefc, K.M., Laimer, M., & Da Câmara Machado, A. (2002). Identification of microsatellite loci in apricot. *Molecular Ecology Notes*, 2, 24-26, doi:10.1046/j.1471-8286.2002.00132.x.

Maynard, C.A., Kavanagh, K., Fuernkranz, H., & Drew, A.P. (1991). Black Cherry (*Prunus serotina* Ehrh.). In Y. P. S. Bajaj (Ed.). *Trees III: Biotechnology in Agriculture and Forestry* 16 (pp. 3-22). Heidelberg, Berlin: Springer.

McVaugh, R. (1951). A revision of the North American black cherries (*Prunus serotina* Ehrh., and relatives). *Brittonia*, 7(4), 279-315, doi:10.2307/2804698.

Mnejja, M., Garcia-Mas, J., Howad, W., Badenes, M.L., & Arús, P. (2004). Simple-sequence repeat (SSR) markers of Japanese plum (*Prunus salicina* Lindl.) are highly polymorphic and transferable to peach and almond. *Molecular Ecology Notes*, 4(2), 163-166, doi:10.1111/j.1471-8286.2004.00603.x.

Nei, M. (1987). *Molecular Evolutionary Genetics*. USA: Columbia University Press. 512 p.

Olszewska, M. (2007). Quantitative HPLC analysis of flavonoids and chlorogenic acid in the leaves and inflorescences of *Prunus serotina* Ehrh. *Acta Chromatographica*, 19, 253-269.

Pairon, M.C., & Jacquemart, A.L. (2005). Disomic segregation of microsatellites in the tetraploid *Prunus serotina* Ehrh. (Rosaceae). *Journal of the American Society for Horticultural Science*, 130(5), 729-734.

Pairon, M., Jonard M., & Jacquemart, A.L. (2006). Modeling seed dispersal of black cherry, an invasive forest tree: how microsatellites may help? *Canadian Journal of Forest Research*, 36(6), 1385-1394.Pairon, M., B.

Pairon, M., Petitpierre, B., Campbell, M., Guisan, A., Broennimann, O., Baret, P.V., Jacquemart, A.L., & Besnard, G. (2010). Multiple introductions boosted genetic diversity in the invasive range of black cherry (*Prunus*

serotina; Rosaceae). *Annals of Botany*, 105(6), 881-890, doi:10.1093/aob/mcq065.

Peakall, R., & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, 28, 2537-2539, doi:10.1093/bioinformatics/bts460.

Petitpierre, B., Pairon, M., Broennimann, O., Jacquemart, A.L., Guisan, A., & Besnard. G. (2009). Plastid DNA variation in *Prunus serotina* var. *serotina* (Rosaceae), a North American tree invading Europe. *European Journal of Forest Research*, 128(5), 431-436.

Popenoe, W., & Pachano, A. (1922). The Capulín Cherry: A superior form of the Northern black cherry develop in the highlands of tropical America. *Journal of Heredity, 13*(2)*,* 51-62.

Pritchard, J.K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945-959.

Raya-Pérez, J.C., Aguirre-Mancilla, C.L., Tapia-Aparicio, R., Ramírez-Pimentel, J.G., & Covarrubias-Prieto, J. (2012). Storage proteins characterization and mineral composition of black cherry seed (*Prunus serotina*). *Polibotánica*, *34*, 203-215.

Rohrer, J.R. (2014). *Prunus* (Rosaceae). In Flora of North America Editorial Committee (Eds.), *Flora of North America North of Mexico*. New York, Oxford. 20+ vols. and. Vol 9, 1993+. pp. 363-365.

Rzedowski, J., & Calderón de Rzedowski, G. (2005). *Prunus serotina* Ehrh. In J. Rzedowski and G. Calderón de Rzedowski (Eds.), *Flora del Bajío y Regiones Adyacentes*. Instituto de Ecología A. C. Centro Regional del Bajío, Pátzcuaro, Michoacán, México. pp. 101-111.

Segura-Ledesma, S., Zavala-Robles, D., Equihua-Cervantes, C. Andrés-Agustín, J., & Yepez-Torres, E. (2009). Genetic resources of fruit trees in Michoacan. *Revista Chapingo Serie Horticultura*, 15, 207-305, doi:10.5154/r.rchsh.2009.15.041.

Sosinski, B., Gannavarapu, M., Hager, L.D., Beck, L.E., King, G.J., Ryder, C.D., Rajapakse, S., Baird, W.V., Ballard, R.E., & Abbott, A.G. (2000). Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. *Theoretical and Applied Genetics*, 101(3), 421-428, doi:10.1007/s001220051499.

Starfinger, U., Kowarik, I., Rode, M., & Schepker, H. (2003). From desirable ornamental plant to pest to accepted addition to the flora? – the perception of an alien tree species through the centuries. *Biological Invasions*, 5, 323-335.

Vaughan, S.P., & Russell, K. (2004). Characterization of novel microsatellites and development of multiplex PCR for large-scale population studies in wild cherry, *Prunus avium. Molecular Ecology Notes*, 4(3), 429-431, doi:10.1111/j.1471-8286.2004.00673.x.

Wang, Y., & Pijut, P.M. (2014). Can *Prunus serotina* be genetically engineered for reproductive sterility and insect pest resistance?. *Springer Science Reviews*, 2, 75-93, doi:10.1007/s40362-014-0024-0.

Yamamoto, T., Mochida, K., Imai, T., Shi, Y.Z., Ogiwara, I., & Hayashi, T. (2002). Microsatellite markers in peach (*Prunus persica* (L.) Batsch) derived from an enriched genomic and cDNA libraries. *Molecular Ecology Resources*, 2(3), 298-301, doi:10.1046/j.1471-8286.2002.00242.x.

CHAPTER IV. GENERAL DISCUSSION

CAPÍTULO IV. DISCUSIÓN GENERAL

4.1. CONTRIBUTING TO A DESCRIPTION OF BLACK CHERRY GENETIC RESOURCES

A comprehensive description of the genetic organization of species requires analyzing data from multiple sources (e.g., morphologic, climatic, topographic, physiologic, molecular, cytogenetic, edaphic). The relationship among different types of data of the same set of individuals is an issue partially resolved in different studies. More recent studies tend to combine morphologic and climatic data (Hernández-Nicolás, Córdova-Téllez, Luna-Cavazos, Romero-Manzanares & Jiménez-Ramírez, 2017; Hounkpèvi *et al*., 2016; Lopez-Laphitz, Ezcurra & Vidal-Russell, 2015), or focus on each one separately (Fresnedo-Ramírez, Segura & Muratalla-Lúa, 2011; López-Álvarez *et al*., 2015). Generally, studies that do not include molecular data conclude that confirming stability of discriminating morphological characteristics requires genetic evaluation to determine if it is a case of phenotypic plasticity or if the discriminating characters are really stable.

In the present work, the overall strategy to contribute to describing black cherry genetic resources consisted in analyzing morphologic, climatic and molecular data. Morphologic and climatic data were refined in the first stage, before conducting factorial analysis and automatic classification in order to synthesize relationships between variables and individuals evaluated. This phase of the study used a set of data from the *Prunus serotina* collection sites, the most compete to date (474 presence points), for describing climatic preferences of the five subspecies, associating their morphologic variation with climatic factors, and estimating the potential effect of climate change on the distribution of the complex under future environmental conditions.

Black cherries were found to have their natural distribution range in two ecozones and 46 ecoregions, indicating that their subspecies are adaptable to conditions of humid and subhumid temperate climates in Mexico; and to temperate and very severe climates in the United States where they can be found in the southern states (temperate and rainy), in the Midwest (lowlands with variable climate) and

in the Southwest (hot and dry). Adaptation of black cherries to different environmental conditions in Europe has been confirmed through observation of their invasive behavior (Camenen, Porte & Benito Garzon, 2016; Starfinger, Kowarik, Rode & Schepker, 2003).

Analysis of the 19 climatic parameters and the altitude showed that ssp. *eximia*, *hirsuta* and *serotina* thrive in humid and cold environments, while *virens* prefers purely dry environments. Even though ssp. *capuli* thrives in humid environments, its distribution pattern is not as well-defined and generally this subspecies can be found growing sympatrically with ssp. *serotina* in central and western Mexico.

This part of the study also revealed that climate is partially associated to morphologic variation of subspecies of *P. serotina*. Variation of the second floral leaf, fruit pedicel and longitude of stamen of the five subspecies was related to altitude, two temperature variables (BIO04 and BIO11), two precipitation variables (BIO15 and BIO17), and one variable that combines precipitation and temperature (BIO19). Even though other correlated characteristics such as longitude of internodes, and diameter and width of seed also showed significant differences among the five subspecies, multivariate analyses and geographic distribution did not support their being defined as useful in differentiating infraspecific groups. Additionally, results suggest that extreme parameters of a changing future climate would affect the species' morphology and cause genetic erosion due to the loss of suitable areas.

In the second phase, 16 microsatellite markers were used to determine, for the first time, the genetic structure of 18 populations of the natural geographic distribution range of four black cherry subspecies. A total of 178 individuals were evaluated, representing the subspecies: *capuli* (38), *eximia* (14), *serotina* (53) and *virens* (73). The objectives of this phase were evaluating their taxonomic classification and providing guidelines for *in situ* and *ex situ* conservation of the species' genetic resources. Results allow us to conclude that the four taxa evaluated must be managed as one biological or conservation entity. Estimated

molecular variability represents the first real approximation to the genetic structure of natural black cherry populations.

Molecular variability analyses detected two main genetic groups: Texas and México (subdivided in five groups) that clearly do not correspond to the four subspecies studied. In other words, Mexican subspecies (*capuli*, *serotina* and *virens*) did not separate into excluding groups, indicating the absence of significative molecular differentiation among the three taxa.

An explanation for these results–which would seem to contradict the existence of different taxonomic groups–is that black cherry subspecies have been delimited and described based on morphologic data, and the main problem is that these variables are somehow influenced by the environment. The environment has an important influence on plant growth, such that many times changes in size of leaves or length of racemes are not due to genetic mutations (inheritable variation), but rather to environmental adaptation (ecotypes or variants induced by the environment) which creates taxonomic confusions. Additionally, some morphologic characteristics may vary among the different stages of plant growth, and the variation of other characteristics depends on the conditions in which the plant develops (in other words, they are adaptive). Another limiting factor of morphologic characteristics is that their importance depends to a large extent on the taxonomist's point of view.

Obtainment of two main genetic groups (Texas and Mexico) suggests that there has been gene flow among subspecies (*capuli*, *serotina* and *virens*) in Mexico, and explains the difficulty of taxonomists in separating them (McVaugh, 1951; Rzedowski & Calderón de Rzedowski, 2005). Gene flow tends to even out the diversity among populations and this could be one reason for not having a clear genetic differentiation among Mexican subspecies.

Nonetheless, this molecular evaluation's estimates and conclusions are relative to the sample analyzed, and to the set of microsatellites used. Therefore, in spite of trying to have geographic representativeness and broad genomic coverage,

questions arise such as: Is all of black cherry's diversity represented in the sample? Are field explorations required to identify other potential collection sites? Is genetic uniformity detected a consequence of a specific management practice, of gene flow, or simply a relative result of the sample analyzed? Are microsatellites used associated to genomic regions with low change rates? And more. Some of these questions could be resolved by evaluating more populations or individuals with the 16 markers used in this study, or using more discriminating markers, such as single-copy regions or the chloroplast sequence recently published by Luan, Gao, He, Bi & He (2017). However, a certain degree of uncertainty will always accompany studies on biological entities.

The different tools applied in this research confirm the difficulty in separating black cherry infraspecific groups. In the same way, techniques used proved to be useful and effective in studying their morphologic variation associated to climatic conditions and their molecular variability, and in contributing to broaden knowledge on the genetic organization of this botanic complex. Additionally, results will complement additional studies on the evolution of this species while undergoing domestication, such as those conducted by Avendaño-Gómez *et al*. (2015).

Overall, this study does not present evidences supporting the classic proposal of five subspecies proposed by McVaugh (1951). The discrepancy may be due to several confusing and inconsistent infraspecific classifications that used taxonomic nomenclature based only on morphology and geographic distribution.

McVaugh (1951) delimited five subspecies based on morphologic differences among groups of populations that occupied excluding geographic areas, following the definition of subspecies. McVaugh pointed out that due to black cherry's continuous morphologic variation, discriminating its infraspecific groups in the field would almost be impossible were it not for the species' geographic segregation. In turn, Rohrer (2014) delimited four varieties, suggesting that black cherry's infraspecific groups present more subtle morphologic differences than

those identified by McVaugh, and admits there can be sympatry in their distribution.

In essence, both proposals accept evidence of morphologic variation; however, in evolutionary terms, McVaugh supposes we are in the face of a speciation phenomenon in black cherries; while Rohrer is more conservative and takes into account only those population groups having distinguishing morphologic characteristics. Maybe delimiting black cherry (and other species) infraspecific groups should focus more on establishing standardized criteria that can be repeatable and used logically, instead of getting caught up in the controversy on validity of terms used.

Results of this study suggest that an *in situ* conservation strategy of black cherry's genetic resources would require identifying those areas with more than one subspecies, such as the states of Michoacán and Tlaxcala in Mexico. This conservation option gives continuity to evolutionary processes, at the same time that it enables sustained use of this resource by inhabitants in the protected area.

Since *ex situ* conservation is considered a complementary approach to *in situ* conservation, promoting the formal establishment of comprehensive black cherry *ex situ* collections is important, enabling broader characterization and evaluation of populations and subspecies in different geographic areas. Also, germplasm would be available for breeders and other users. *Ex situ* conservation would also guarantee the survival of these valuable genetic resources in case they disappear in the future from their current distribution areas, as suggested by the potential impact of climate change modelled in this study, or land-use change.

Consequently, black cherry conservation and breeding programs to be established should take into consideration the species' morphologic and molecular variability, its climatic preferences and the potential impact of climate change. Heterogeneous but trustworthy information should be examined comprehensively to find novel and unexpected evidences, favoring a rigorous definition of black cherry conservation and sustainable use strategies.

4.2. POTENTIATING AND VALUING BLACK CHERRY GENETIC RESOURCES

Different approaches can be used for valuing this species; yet it is important to do so from the agricultural perspective, since this is the more traditional anthropocentric use of black cherries. The main focus would be to divulge its nutritional qualities, which–as discussed in the first chapter of this dissertation– are as important as those of other fruit species of the *Prunus* genus.

Value as a source of food would increase the species' economic importance and would restore its cultural value, important because it helps increase the sense of belonging and awakens interest in its conservation and sustainable use. From a gastronomic perspective, black cherries have been used to prepare liquor, among other uses, suggesting it can undergo agroindustrial transformation processes, thus adding an aggregate value to the species in traditional markets. Additionally, multidisciplinary research studies have broadened the species' potential therapeutic uses (Ibarra-Alvarado *et al*., 2010; Palomares-Alonso *et al*., 2017), another key factor favoring interest in further market studies.

Specifically in Mexico, black cherries could be included in the *Macro Red Frutales* (Servicio Nacional de Inspección y Certificación de Semillas, 2017); interdisciplinary research in this area is very important. To be part of the network, active and coordinated participation of different areas (agronomy, botany, taxonomy, sociology, economy, among others) is needed to promote planning and execution of *ex situ* and *in situ* black cherry germplasm conservation, as well as to carry out typification of the species' production systems in order to introduce it in the productive scenario in the short and medium terms. Also, the intervention of producers and dwellers in the areas of distribution of black cherries is indispensable in order to develop proposals that suit their living conditions and to mobilize resources for conservation, research and production projects.

Forestry is another important aspect for fomenting conservation and sustainable use of this species. In several Mexican states, such as Tlaxcala and Michoacán,

black cherry wood is used in artisanal carpentry. Therefore, characterization of its wood and qualities is important for discovering other potential uses of this resource. This would increase black cherry's economic value since, both in Mexico and the United States, this native species provides good timber that can be easily managed. An alternative for enhancing use of its timber is by introducing germplasm–considered elite by the US timber industry–in Mexican environments with suitable environmental conditions.

Finally, another known potential use for cultivating black cherry and that could enhance its use if properly divulged, is as rootstock for sweet cherry with low chill requirement. Another is to use black cherry to distract birds from consuming the fruits of other species.

To conclude, even though climatic, morphologic and molecular black cherry variations used as parameters in this study did not clearly discriminate the infraspecific groups defined taxonomically, the species presents different anthropocentrically interesting aspects that make it worth promoting, coordinating, supporting and conducting activities addressed at enhancing research, conservation and sustainable use for the benefit of the communities where black cherries are distributed.

4.3. LITERATURE CITED

Avendaño-Gómez, A., Lira-Saade, R., Madrigal-Calle, B., García-Moya, E., Soto-Hernández, M., & Romo de Vivar-Romo, A. (2015). Management and domestication syndromes of capulin (*Prunus serotina* Ehrh. ssp. *capuli* (Cav.) Mc Vaugh) in communities of the state of Tlaxcala. *Agrociencia*, 49, 189-204.

Camenen, E., Porte, A.J., & Benito Garzon, M. (2016). American trees shift their niches when invading Western Europe: evaluating invasion risks in a changing climate. *Ecology and Evolution*, 6(29), 7263-7275, doi:10.1002/ece3.2376.

Fresnedo-Ramírez, J., Segura, S., & Muratalla-Lúa, A. (2011). Morphovariability of capulín (*Prunus serotina* Ehrh.) in the central-western region of Mexico from a plant genetic resources perspective. *Genetic Resources and Crop Evolution*, 58(4), 481-495, doi:10.1007/s10722-010-9592-2.

Hernández-Nicolás, N.Y., Córdova-Téllez, L., Luna-Cavazos, M., Romero-Manzanares, A., & Jiménez-Ramírez, J. (2017). Morphological variation related with environmental factors in endemic and threatened *Jatropha* species of Tehuacan-Cuicatlan, Mexico. *Genetic Resources and Crop Evolution*, 64(3), 557-568, doi:10.1007/s10722-016-0383-2.

Hounkpèvi, A., Akomian, F., Azihou, E., Kouassi, D.K., Porembski, S., & Kakaï, R.G. (2016). Climate-induced morphological variation of black plum (*Vitex doniana* Sw.) in Benin, West Africa. *Genetic Resources and Crop Evolution*, 63, 1073-1084, doi:10.1007/s10722-016-0409-9.

Ibarra-Alvarado, C., Rojas, A., Mendoza, S., Bah, M., Gutiérrez, D.M., Hernández-Sandoval, L., & Martínez, M. (2010). Vasoactive and antioxidant activities of plants used in Mexican traditional medicine for the treatment of cardiovascular diseases. *Pharmaceutical Biology*, 48(7), 732-739, doi:10.3109/13880200903271280.

López-Alvarez, D., Manzaneda, A., Rey, R., Giraldo, P., Benavente, E., Allainguillaume, J., Mur, L., Caicedo, A.L., Hazen, S.P., Breiman, A., Ezrati, S., & Catalán, P. (2015). Environmental niche variation and evolutionary diversification of the *Brachypodium distachyon* grass complex species in their native circum-Mediterranean range. *American Journal of Botany*, 102(7), 1-16, http://www.amjbot.org/content/102/7/1073.

Lopez-Laphitz, R.M., Ezcurra, C., & Vidal-Russell, R. (2015). Morphological variation in *Quinchamalium* (Schoepfiaceae) is associated with climatic patterns along Its Andean distribution. *Systematic Botany*, 40(4), 1045-1052, doi:10.1600/036364415X690085.

Luan, A., Gao, A., He, J., Bi, G., & He, Y. (2017). Characterization of the complete chloroplast genome of black cherry (*Prunus serotina* Ehrh.). *Conservation Genetics Resources*, published online: 22-August-2017, doi:10.1007/s12686-017-0826-x.

McVaugh, R. (1951). A revision of the North American black cherries (*Prunus serotina* Ehrh., and relatives). *Brittonia*, 7(4), 279-315, doi:10.2307/2804698.

Palomares-Alonso, F., Rojas-Tomé, I.S., Palencia-Hernández, G., Jiménez-Arellanes, M.A., Macías-Rubalcava, M.L., González-Maciel, A., Ramos-Morales, A., Santiago-Reyes, R., Castro, N., González-Hernández, I., Rufino-González, Y., & Jung-Cook, H. (2017). *In vitro* and *in vivo* cysticidal activity of extracts and isolated flavanone from the bark of *Prunus serotina*: A bio-guided study. *Acta Tropica*, 170, 1-7, doi:10.1016/j.actatropica.2017.02.023.

Rohrer, J.R. (2014). *Prunus* (Rosaceae). In Flora of North America Editorial Committee (Eds.), *Flora of North America North of Mexico*. New York, Oxford. 20+ vols. and. Vol 9, 1993+. pp. 363-365.

Rzedowski, J., & Calderón de Rzedowski, G. (2005). *Prunus serotina* Ehrh. In J. Rzedowski and G. Calderón de Rzedowski (Eds.), *Flora del Bajío y Regiones Adyacentes*. Instituto de Ecología A. C. Centro Regional del Bajío, Pátzcuaro, Michoacán, México. pp. 101-111.

Servicio Nacional de Inspección y Certificación de Semillas. (2017). Macro Red Frutales. *Cultivos nativos de México para la alimentación y la agricultura*. Consulted in https://www.gob.mx/snics/acciones-y-programas/macro-red-frutales.

Starfinger, U., Kowarik, I., Rode, M., & Schepker, H. (2003). From desirable ornamental plant to pest to accepted addition to the flora? – the perception of an alien tree species through the centuries. *Biological Invasions*, 5, 323-335.

Appendix I. Data base of presence points used in the study.

ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude
capuli	16.8500	-92.6800	*capuli*	19.3500	-99.3330	*capuli*	19.5300	-101.6980	*capuli*	20.0180	-98.6350
capuli	17.4333	-97.1500	*capuli*	19.3670	-100.4330	*capuli*	19.5380	-101.6950	*capuli*	20.0670	-103.7350
capuli	18.7170	-97.1830	*capuli*	19.3740	-99.2850	*capuli*	19.5670	-97.0170	*capuli*	20.0830	-98.6170
capuli	18.7480	-110.9470	*capuli*	19.3900	-96.9200	*capuli*	19.5800	-98.5600	*capuli*	20.0950	-104.5980
capuli	18.7500	-110.9500	*capuli*	19.4100	-101.6050	*capuli*	19.5820	-97.0100	*capuli*	20.1000	-98.6200
capuli	19.0180	-99.2670	*capuli*	19.4180	-103.3520	*capuli*	19.5970	-103.9130	*capuli*	20.1250	-98.6520
capuli	19.0670	-98.4830	*capuli*	19.4300	-98.7700	*capuli*	19.6170	-99.4000	*capuli*	20.1300	-101.7170
capuli	19.0670	-99.0670	*capuli*	19.4330	-98.7670	*capuli*	19.6200	-101.3400	*capuli*	20.1400	-98.6700
capuli	19.0700	-98.4880	*capuli*	19.4380	-102.3930	*capuli*	19.6510	-100.2760	*capuli*	20.1500	-98.7500
capuli	19.1090	-99.6210	*capuli*	19.4400	-98.7600	*capuli*	19.6860	-101.9550	*capuli*	20.1670	-101.9970
capuli	19.1310	-99.0830	*capuli*	19.4570	-100.4230	*capuli*	19.7000	-100.9150	*capuli*	20.1700	-98.7300
capuli	19.1670	-99.0000	*capuli*	19.4886	-98.4389	*capuli*	19.7050	-103.4630	*capuli*	20.2150	-98.7320
capuli	19.1700	-98.9900	*capuli*	19.4900	-101.3000	*capuli*	19.7200	-98.6300	*capuli*	20.2880	-100.7580
capuli	19.1800	-99.0700	*capuli*	19.4900	-98.8800	*capuli*	19.7330	-99.4500	*capuli*	20.2980	-100.3950
capuli	19.1830	-99.0670	*capuli*	19.4980	-98.4376	*capuli*	19.7550	-101.2570	*capuli*	20.4170	-103.3920
capuli	19.1900	-99.1200	*capuli*	19.4986	-98.4389	*capuli*	19.7670	-96.8170	*capuli*	20.8060	-100.1180
capuli	19.2170	-98.9830	*capuli*	19.4994	-98.8789	*capuli*	19.7700	-96.7900	*capuli*	20.8520	-99.5880
capuli	19.2430	-99.3970	*capuli*	19.5000	-101.8500	*capuli*	19.7710	-104.3690	*capuli*	20.8690	-100.7040
capuli	19.2500	-99.3330	*capuli*	19.5000	-99.1330	*capuli*	19.7820	-100.6580	*capuli*	21.0720	-101.1940
capuli	19.2670	-99.1330	*capuli*	19.5000	-98.8830	*capuli*	19.8170	-98.6170	*capuli*	21.1750	-99.9800
capuli	19.2800	-99.2500	*capuli*	19.5020	-101.8520	*capuli*	19.8180	-101.7880	*capuli*	21.2120	-100.2150
capuli	19.2830	-101.4500	*capuli*	19.5070	-101.5280	*capuli*	19.8400	-100.7720	*capuli*	21.4160	-104.9690
capuli	19.3101	-100.2580	*capuli*	19.5160	-101.6090	*capuli*	19.8420	-104.3450	*capuli*	21.4230	-99.6950
capuli	19.3140	-102.4110	*capuli*	19.5214	-98.5089	*capuli*	19.8550	-101.4550	*capuli*	21.4380	-100.5080
capuli	19.3330	-99.2000	*capuli*	19.5228	-98.4879	*capuli*	19.8780	-101.7720	*capuli*	21.4430	-104.9020
capuli	19.3370	-102.3630	*capuli*	19.5230	-101.7070	*capuli*	19.9000	-98.5600	*capuli*	21.4500	-104.7500

Appendix I. Continuation.

ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude
capuli	21.4620	-100.9550	*capuli*	25.5830	-106.3170	*hirsuta*	40.7589	-74.0881	*serotina*	29.4578	-95.0632
capuli	21.4700	-104.9200	*capuli*	25.7000	-106.0830	*serotina*	17.2500	-96.0300	*serotina*	30.4538	-95.8337
capuli	21.4770	-105.0010	*capuli*	27.3330	-108.6630	*serotina*	17.2700	-98.2800	*serotina*	31.2000	-89.2500
capuli	21.4800	-104.9800	*capuli*	27.3333	-108.6633	*serotina*	17.4000	-96.4800	*serotina*	31.4775	-81.2417
capuli	21.4940	-104.9920	*capuli*	29.1830	-108.1330	*serotina*	18.7500	-110.9500	*serotina*	31.7650	-96.0847
capuli	21.5050	-104.9830	*capuli*	29.1920	-108.1410	*serotina*	18.7670	-110.9500	*serotina*	32.0565	-94.6012
capuli	21.5170	-104.8950	*capuli*	29.5870	-108.3300	*serotina*	19.5044	-101.6422	*serotina*	32.7236	-95.5508
capuli	21.5220	-105.0480	*eximia*	29.6833	-98.9667	*serotina*	19.5108	-101.6417	*serotina*	33.1385	-96.1108
capuli	21.5550	-102.3080	*eximia*	29.7932	-99.4258	*serotina*	19.5500	-103.6300	*serotina*	33.1833	-87.5167
capuli	21.6200	-104.9700	*eximia*	29.9113	-99.8046	*serotina*	20.5200	-98.5500	*serotina*	33.6377	-98.6256
capuli	21.8800	-103.9200	*eximia*	29.9126	-99.2539	*serotina*	20.9500	-99.5000	*serotina*	34.9250	-85.0620
capuli	22.3830	-99.6000	*eximia*	29.9328	-99.2432	*serotina*	21.1340	-99.6250	*serotina*	35.1400	-83.4000
capuli	22.7100	-103.1230	*eximia*	29.9333	-99.2353	*serotina*	21.1367	-98.6256	*serotina*	35.5608	-87.4440
capuli	22.7620	-103.1010	*eximia*	29.9358	-99.2378	*serotina*	21.1820	-99.3200	*serotina*	35.8300	-82.8000
capuli	23.0330	-99.1670	*eximia*	30.0577	-100.1087	*serotina*	21.2170	-99.0750	*serotina*	35.8700	-82.8000
capuli	23.4830	-109.7830	*eximia*	30.0770	-99.5035	*serotina*	21.2220	-99.4730	*serotina*	36.0800	-81.7800
capuli	23.4833	-109.7833	*eximia*	30.1064	-98.6428	*serotina*	21.2320	-99.1410	*serotina*	36.7000	-91.7000
capuli	24.0000	-104.7000	*eximia*	30.2717	-99.9261	*serotina*	23.3330	-104.7120	*serotina*	36.8100	-93.4700
capuli	24.2330	-99.4330	*eximia*	30.3104	-97.8235	*serotina*	23.8333	-99.1667	*serotina*	36.8900	-90.2800
capuli	24.6980	-105.9920	*eximia*	30.3387	-97.7727	*serotina*	23.9786	-99.7419	*serotina*	36.8900	-89.9300
capuli	24.7670	-99.9000	*hirsuta*	33.8324	-81.2024	*serotina*	23.9900	-104.0180	*serotina*	36.9200	-90.4000
capuli	24.9470	-105.4070	*hirsuta*	32.9547	-86.4147	*serotina*	24.0000	-101.0000	*serotina*	37.0200	-94.3800
capuli	25.0830	-98.8170	*hirsuta*	33.4708	-81.9747	*serotina*	24.0333	-99.9667	*serotina*	37.0900	-93.2700
capuli	25.1020	-106.4450	*hirsuta*	33.5000	-86.7500	*serotina*	25.2310	-100.1430	*serotina*	37.0900	-93.7200
capuli	25.2450	-106.4220	*hirsuta*	33.7500	-86.3200	*serotina*	25.3000	-100.2200	*serotina*	37.1800	-94.3100
capuli	25.3330	-105.7170	*hirsuta*	33.6415	-85.7851	*serotina*	29.4497	-95.0240	*serotina*	37.3100	-89.5300

Appendix I. Continuation.

ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude
serotina	37.3300	-91.9500	*serotina*	38.7700	-90.7300	*serotina*	40.4500	-92.6000	*virens*	31.5330	-108.8840
serotina	37.3400	-93.4900	*serotina*	38.7711	-95.6649	*serotina*	40.4800	-95.6200	*virens*	31.6029	-108.7698
serotina	37.3500	-89.5200	*serotina*	38.8000	-93.2300	*serotina*	40.5200	-91.6400	*virens*	31.7128	-110.0669
serotina	37.4100	-91.2900	*serotina*	38.8100	-91.1400	*serotina*	40.5243	-81.2298	*virens*	31.7143	-110.0649
serotina	37.4100	-89.4400	*serotina*	38.8200	-90.3500	*serotina*	40.5500	-93.8400	*virens*	31.7169	-111.6488
serotina	37.5000	-94.5300	*serotina*	38.9400	-91.9500	*serotina*	40.5804	-83.0915	*virens*	31.7176	-111.6509
serotina	37.5500	-93.7000	*serotina*	38.9800	-92.1300	*serotina*	41.4049	-82.3235	*virens*	31.7436	-110.9348
serotina	37.6300	-91.2500	*serotina*	39.0100	-94.0700	*serotina*	41.4122	-82.3164	*virens*	31.7500	-110.8417
serotina	37.6400	-92.1300	*serotina*	39.0200	-92.1600	*serotina*	41.6197	-83.7305	*virens*	31.7745	-109.1203
serotina	37.7429	-97.2740	*serotina*	39.0700	-91.6400	*serotina*	43.3906	-88.0260	*virens*	31.7820	-111.0162
serotina	37.8039	-95.8446	*serotina*	39.0700	-90.9300	*virens*	28.0333	-108.1667	*virens*	31.8018	-110.8084
serotina	37.8300	-94.3000	*serotina*	39.2200	-92.6100	*virens*	28.0420	-108.1750	*virens*	31.8655	-109.3603
serotina	37.8400	-91.0100	*serotina*	39.2400	-91.0100	*virens*	28.1667	-108.7167	*virens*	31.8662	-109.3494
serotina	37.8800	-90.5800	*serotina*	39.2600	-92.4400	*virens*	28.1750	-108.7250	*virens*	31.8753	-110.7776
serotina	37.9400	-91.2100	*serotina*	39.3700	-93.1600	*virens*	31.3584	-110.2296	*virens*	31.9057	-109.2798
serotina	37.9500	-91.1700	*serotina*	39.6800	-91.6700	*virens*	31.3600	-110.3000	*virens*	31.9193	-109.9862
serotina	38.0000	-90.5200	*serotina*	39.7400	-92.4700	*virens*	31.3622	-110.3003	*virens*	31.9348	-109.2189
serotina	38.1300	-90.6800	*serotina*	39.7614	-84.1942	*virens*	31.3962	-108.7510	*virens*	31.9990	-109.8181
serotina	38.1700	-90.7000	*serotina*	40.1157	-82.8855	*virens*	31.4169	-111.0783	*virens*	32.1206	-110.5231
serotina	38.3400	-91.0800	*serotina*	40.2000	-91.6200	*virens*	31.4173	-108.9297	*virens*	32.3042	-110.7440
serotina	38.5250	-90.5577	*serotina*	40.2600	-93.8300	*virens*	31.4215	-110.2687	*virens*	32.4062	-110.7587
serotina	38.5500	-90.4300	*serotina*	40.2697	-82.3542	*virens*	31.4239	-110.2647	*virens*	32.5995	-110.7862
serotina	38.6000	-92.8100	*serotina*	40.2700	-94.0300	*virens*	31.4442	-109.8619	*virens*	32.6772	-106.5557
serotina	38.6100	-90.3100	*serotina*	40.3400	-92.0900	*virens*	31.4598	-110.2806	*virens*	32.7017	-109.8715
serotina	38.6100	-90.3000	*serotina*	40.3500	-94.8700	*virens*	31.4636	-110.2909	*virens*	32.7496	-109.8356
serotina	38.6300	-94.0700	*serotina*	40.4200	-92.5300	*virens*	31.5168	-108.9870	*virens*	33.6528	-109.4303

Appendix I. Continuation.

ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude
virens	33.7121	-108.7533	*virens*	31.7176	-111.6509	*virens*	32.7017	-109.8715	*virens*	34.2056	-112.3385
virens	33.8123	-110.9054	*virens*	31.7340	-111.5750	*virens*	32.7537	-109.8345	*virens*	34.5053	-112.6854
virens	34.4150	-112.4043	*virens*	31.7679	-110.8484	*virens*	32.7628	-109.7984	*virens*	34.5400	-112.4685
virens	23.9786	-99.7419	*virens*	31.8434	-109.2801	*virens*	32.9326	-105.8189	*virens*	34.5881	-112.0411
virens	23.9790	-99.7420	*virens*	31.9118	-109.9554	*virens*	33.0276	-108.1500	*virens*	34.7981	-112.8785
virens	24.4940	-99.9480	*virens*	31.9122	-109.9900	*virens*	33.0740	-110.2609	*virens*	34.9000	-113.8841
virens	24.4942	-99.9481	*virens*	31.9723	-109.0506	*virens*	33.1786	-109.8629	*virens*	34.9135	-112.8665
virens	24.8670	-100.2440	*virens*	32.0024	-109.3239	*virens*	33.1788	-109.8624	*virens*	34.9151	-111.7285
virens	24.8722	-100.2436	*virens*	32.0045	-109.3567	*virens*	33.2274	-108.2722	*virens*	34.9473	-111.7538
virens	25.3500	-100.4830	*virens*	32.0739	-107.6390	*virens*	33.2939	-106.5452	*virens*	34.9893	-111.7455
virens	25.3500	-100.4833	*virens*	32.1447	-104.6237	*virens*	33.2948	-106.5230	*virens*	35.0892	-113.8983
virens	31.3640	-110.7756	*virens*	32.1521	-110.4822	*virens*	33.3209	-108.7538	*virens*	35.1165	-113.8714
virens	31.3976	-111.2107	*virens*	32.1667	-110.4214	*virens*	33.3659	-109.7515	*virens*	35.1971	-108.0216
virens	31.4000	-111.2000	*virens*	32.1801	-104.4472	*virens*	33.3942	-110.7871	*virens*	35.8333	-112.0833
virens	31.4218	-111.1954	*virens*	32.2041	-110.5321	*virens*	33.4217	-111.1732	*virens*	36.0519	-104.3572
virens	31.4453	-110.4168	*virens*	32.2232	-104.7178	*virens*	33.4750	-109.4833	*virens*	36.7839	-103.9680
virens	31.4453	-110.3146	*virens*	32.2318	-104.5021	*virens*	33.6137	-109.0184	*virens*	19.6000	-101.7167
virens	31.4691	-109.9227	*virens*	32.3667	-110.7083	*virens*	33.6360	-108.9937	*virens*	19.8823	-101.5073
virens	31.5168	-108.9870	*virens*	32.5377	-110.7198	*virens*	33.7028	-111.2276	*virens*	21.1480	-101.1929
virens	31.5168	-108.9853	*virens*	32.6007	-110.7899	*virens*	33.7133	-108.7577	*virens*	23.4500	-104.3000
virens	31.5178	-110.3885	*virens*	32.6109	-110.7709	*virens*	33.7990	-109.9892	*virens*	23.4500	-109.9830
virens	31.5210	-109.0144	*virens*	32.6228	-109.8243	*virens*	33.8334	-111.4179	*virens*	23.5000	-110.0500
virens	31.5441	-109.3378	*virens*	32.6570	-108.4980	*virens*	33.8995	-112.2963	*virens*	23.5000	-109.9830
virens	31.5509	-110.3795	*virens*	32.6651	-109.8019	*virens*	33.9106	-109.5851	*virens*	23.5330	-110.0670
virens	31.5843	-108.7679	*virens*	32.6772	-106.5557	*virens*	34.0774	-107.0213	*virens*	23.5330	-109.9830
virens	31.7169	-111.6488	*virens*	32.6774	-106.5597	*virens*	34.1333	-109.9333	*virens*	23.5500	-109.9830

Appendix I. Continuation.

ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude	ssp.	Latitude	Longitude
virens	23.7560	-103.7670	*virens*	27.3330	-108.6670	*virens*	28.3700	-109.0280	*virens*	30.9417	-109.9583
virens	25.2320	-100.9130	*virens*	27.3333	-108.6667	*virens*	28.3750	-108.7500	*virens*	30.9420	-109.9500
virens	25.2355	-100.8326	*virens*	28.0430	-108.1770	*virens*	28.4250	-108.6060	*virens*	31.0561	-110.9683
virens	25.3367	-100.2723	*virens*	28.0433	-108.1767	*virens*	28.4580	-109.0390	*virens*	31.2690	-109.9920
virens	25.3390	-100.2710	*virens*	28.1000	-108.2833	*virens*	28.4610	-109.0250	*virens*	31.2695	-109.9917
virens	25.3641	-100.4733	*virens*	28.1080	-108.2920	*virens*	28.4611	-109.0250	*virens*	31.3167	-108.8333
virens	25.3740	-100.2080	*virens*	28.1110	-108.2830	*virens*	28.5000	-108.5000	*virens*	31.3170	-108.8330
virens	25.3770	-100.7924	*virens*	28.1111	-108.2833	*virens*	28.7790	-109.5310	*virens*	31.3231	-108.7575
virens	25.3830	-102.2170	*virens*	28.1667	-108.7289	*virens*	28.9600	-102.5780	*virens*	31.8659	-109.2800
virens	25.3833	-102.2167	*virens*	28.1670	-108.7290	*virens*	29.2833	-103.2667	*virens*	31.9165	-109.2737
virens	26.9830	-108.9830	*virens*	28.1830	-108.2110	*virens*	29.8578	-104.4467	*virens*	31.9509	-109.1790
virens	26.9833	-108.9833	*virens*	28.1833	-108.2111	*virens*	30.3583	-110.3625	*virens*	32.1667	-110.6083
virens	27.1430	-107.5720	*virens*	28.3160	-109.0070	*virens*	30.3903	-110.3989	*virens*	32.4000	-104.8000
virens	27.3250	-108.7417	*virens*	28.3670	-109.0330	*virens*	30.9330	-109.9580			
virens	27.3250	-108.7330	*virens*	28.3697	-109.0278	*virens*	30.9333	-109.9583			

Appendix II. Distribution of black cherry across ecoregions of North America.

	capuli	*eximia*	*hirsuta*	*serotina*	*virens*	Grand Total
MEXICO	**137**			**24**	**73**	**234**
Apache Highlands					12	12
Bajío Dry Forests	4					4
Balsas Dry Forests	1					1
Central American Pine-Oak Forests	1					1
Central Mexican Matorral	14					14
Chihuahuan Desert	2			2	4	8
Islas Revillagigedo Dry Forests	2			2		4
Jalisco Dry Forests	4					4
Meseta Central Matorral				2		2
Oaxacan Montane Forests	4			1		5
Sierra De La Laguna Dry Forests	2				2	4
Sierra De La Laguna Pine-Oak Forests					4	4
Sierra Madre De Oaxaca Pine-Oak Forests	1			1		2
Sierra Madre Del Sur Pine-Oak Forests	1			1		2
Sierra Madre Occidental Pine-Oak Forests	23			1	26	50
Sierra Madre Oriental Pine-Oak Forests	12			7	15	34
Sinaloan Dry Forests	3				5	8
Sonoran-Sinaloan Transition Subtropical Dry Forest					2	2
Tamaulipan Thorn Scrub	2			1		3
Trans-Mexican Volcanic Belt Pine-Oak Forests	59			3	3	65
Veracruz Moist Forests	2			2		4
Veracruz Montane Forests				1		1
UNITED STATES		**13**	**7**	**89**	**131**	**240**
Apache Highlands					92	92
Arizona-New Mexico Mountains					19	19
Central Tallgrass Prairie				20		20
Chihuahuan Desert					10	10
Colorado Plateau					1	1
Crosstimbers And Southern Tallgrass Prairie				3		3

Appendix II. Continuation.

	capuli	*eximia*	*hirsuta*	*serotina*	*virens*	Grand Total
Cumberlands And Southern Ridge And Valley			3	1		4
East Gulf Coastal Plain				1		1
Edwards Plateau		13				13
Great Lakes				3		3
Gulf Coast Prairies And Marshes				2		2
Interior Low Plateau				1		1
Lower New England / Northern Piedmont			1			1
Mississippi River Alluvial Plain				1		1
North Central Tillplain				3		3
Osage Plains/Flint Hills Prairie				8		8
Ozarks				34		34
Piedmont			1			1
Prairie-Forest Border				1		1
Sonoran Desert					7	7
South Atlantic Coastal Plain			2	1		3
Southern Blue Ridge				4		4
Southern Shortgrass Prairie				1	2	3
Upper East Gulf Coastal Plain				1		1
Upper West Gulf Coastal Plain				2		2
Western Allegheny Plateau				2		2
Grand Total	**137**	**13**	**7**	**113**	**204**	**474**