DIVERSITY ACROSS THE BORDER: GENETIC STUDY OF A HIGHLY DISJUNCT OCCURRENCE OF THE U.S. FEDERALLY-ENDANGERED PLANT SPECIES *PHYSARIA THAMNOPHILA*, BRASSICACEAE (ZAPATA BLADDERPOD) DISCOVERED IN MEXICO

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ABSTRACT

Taxonomy, genetics and biogeography each make key contributions to biological conservation. However, integrating these disciplines to obtain a coherent account of the status of a taxon of concern not always straightforward. This is the case for the cross-border endemic plant *Physaria thamnophila* (Brassicaceae). This US federally-listed endangered species is restricted to a set of unique geological sites just north of the Rio Grande (Rio Bravo del Norte) river in south Texas, USA. A single highly-disjunct occurrence of this species is found on a geologically and ecologically distinct site 260 km to the south, in Tamaulipas, Mexico. In this work, we quantify the genetic differentiation between the U.S. and Mexican populations using four microsatellite markers and sequences from three nuclear genes. In both sets of data, we find a high level of genetic divergence consistent with geographic isolation on a time scale of 1–2.5 million years. Further, we provide a hypothesis for the geological basis of this geographic isolation. Integrating our data with ecological, taxonomic and conservation considerations, we propose the sub-specific designation of **Physaria thamnophila** subsp. **loretensis** for the Mexican population. The evolutionary and conservation implications of this designation are presented.

RESUMEN

La taxonomía, la genética y la biogeografía contribuyen de manera importante a la conservación biológica. Sin embargo, la integración de estas disciplinas para lograr una descripción coherente del estado de un taxón de interés puede ser compleja. Este es el caso de la planta endémica transfronteriza *Physaria thamnophila* (Brassicaceae), una especie evaluada en peligro de extinción en la lista federal de los Estados Unidos, que está restringida a un conjunto de sitios geológicos únicos justo al norte del río Grande (Río Bravo del Norte) en el sur de Texas, EE.UU. Se encontró previamente un sitio adicional de ocurrencia esta especie a gran distancia (260 km al sur, en Tamaulipas, México) en una zona con características geológicas y ecológicas distintas. En este trabajo, cuantificamos la diferenciación genética entre las poblaciones de EE. UU. y de México, utilizando cuatro marcadores de microsatélites y secuencias de tres fragmentos de genes nucleares. Ambos tipos de marcadores revelaron un alto nivel de divergencia genética, que sugiere un aislamiento geográfico de aproximadamente 1–2.5 millones de años, para el cual desarrollamos una hipótesis con base geológica. Integrando nuestros datos con consideraciones ecológicas, taxonómicas y de conservación, proponemos la designación subespecífica de **Physaria thamnophila** subsp. **loretensis** para la población mexicana, y discutimos las implicaciones evolutivas y de conservación de dicha designación.

Key Words: Brassicaceae, geoendemic, transnational, molecular-clock, sibling species

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INTRODUCTION

The geographic ranges of wild species do not respect political borders. As a consequence, the scientific investigation and conservation management of trans-border species is often hindered by the political, social and physical barriers that borders impose. This daunting challenge is exemplified by the 3,145 km long border between the United States and Los Estados Unidos Mexicanos (Mexico), which bisects the biologically rich southwestern region of North America, as it divides the ranges of at least 1,506 native plant and animal species, including 62 species listed as "critically endangered," "endangered," or "vulnerable" on the IUCN Red List (Peters et al. 2018).

This study addresses the genetic diversity and geographical range of the Zapata bladderpod, *Physaria thamnophila* (Rollins & Shaw) O'Kane & Al-Shehbaz (Brassicaceae), a rare and endangered perennial plant that—within the U.S.—is endemic to a set of bluffs and uplands overlooking the lower Rio Grande (Rio Bravo del Norte) River valley on the U.S.A./Mexico border. The entire US range of this species lies within one of five "Borderlands Conservation Hotspots" identified as locations of high biological diversity that are threatened by ongoing and potential future border wall construction (Peters et al. 2018).

The U.S.A. distribution of *P. thamnophila* is limited to 14 dispersed geographic clusters in Starr and Zapata counties in extreme southern Texas (Fig. 1). All U.S.A. populations of *P. thamnophila* are found on or near outcrops of Eocene sedimentary geology (Jackson, Yequa and Laredo groups) exposed by the erosive forces of the Rio Grande and its local tributaries (Thompson 1972; Page et al. 2005). The plants are found on shallow, sandy, erosive, calcareous soils overlaying a massive, low-permeability sandstone aquiclude. On these eroded bluffs, the plants are distributed in a narrow band—often only a few meters wide—that follows a discrete stratigraphic layer characterized by abundant fossil oyster shells and small gypsum outcrops eroding over a thick sandstone layer (Jahrsdoerfer & Leslie 1988; US Fish & Wildlife Service 1999; Price et al. 2012; US Fish & Wildlife Service 2015).

Physaria thamnophila was listed in the U.S.A. as a federal endangered species after serious degradation of over 95% of its already-restricted habitat through urbanization, invasive species, overgrazing, root-plowing, highway and utility construction, and oil and gas development, leading to vulnerability from low population numbers (Jahrsdoerfer & Leslie 1988; US Fish & Wildlife Service 1999; Fowler et al. 2011). Further, several key populations have recently come under potential threat by border-wall construction projects (Fowler et al. 2018).

Outside of this cluster of U.S.A. populations, the only verified report of *P. thamnophila* is that of one small population located at the place name Rancho Loreto, a 25,000-ha privately-owned ranch in the Municipios of San Fernando, Abasolo, and Soto la Marina, in northeastern Tamaulipas, more than 260 km southeast of the U.S.A. populations (Fig. 1). Here the species is found in a geologically unusual coastal caliche-sand plain that occurs on outcrops of the Pliocene Goliad clastic formation (Sanches-Mehorada 1956; Baskin & Hurlbert 2008) and supports a biologically unique mosaic of small grassland prairies with interspersed mottes of thorn shrub (Johnston 1963).

At Rancho Loreto, Johnston described a *Physaria* in the prairie community, on deep sandy soils. A single herbarium specimen collected in 1960, *M.C. Johnston & J. Crutchfield 5556* (TEX-LL), was initially determined to be *P. argyraea* (A. Gray) O'Kane & Al-Shehbaz, but was later annotated to *P. thamnophila* by Guy Nesom, based on Rollins and Shaw (1973). In 2005, the Loreto *Physaria* population was rediscovered by a binational team (Contreras-Arquieta et al. 2005). New herbarium specimens were determined by Ishan Al-Shehbaz (Missouri Botanical Garden) to be *P. thamnophila*.

Because of the highly disjunct nature of the Loreto collections, as well as its starkly different habitat, we surmised that they might represent a highly distinct population of *P. thamnophila*, thus constituting an evolutionarily significant unit (ESU). Alternatively, the Loreto population might actually comprise a cryptic species that is morphologically indistinct from *P. thamnophila*. To examine these alternative hypotheses, we used four microsatellite markers and three exon-primed, intron-containing (EPIC) marker sequences from single-copy nuclear genes to quantify the level of genetic divergence between the Mexican and U.S.A. populations. Here we describe the evidence and its interpretation that led us to the designation of a new sub-specific taxon,

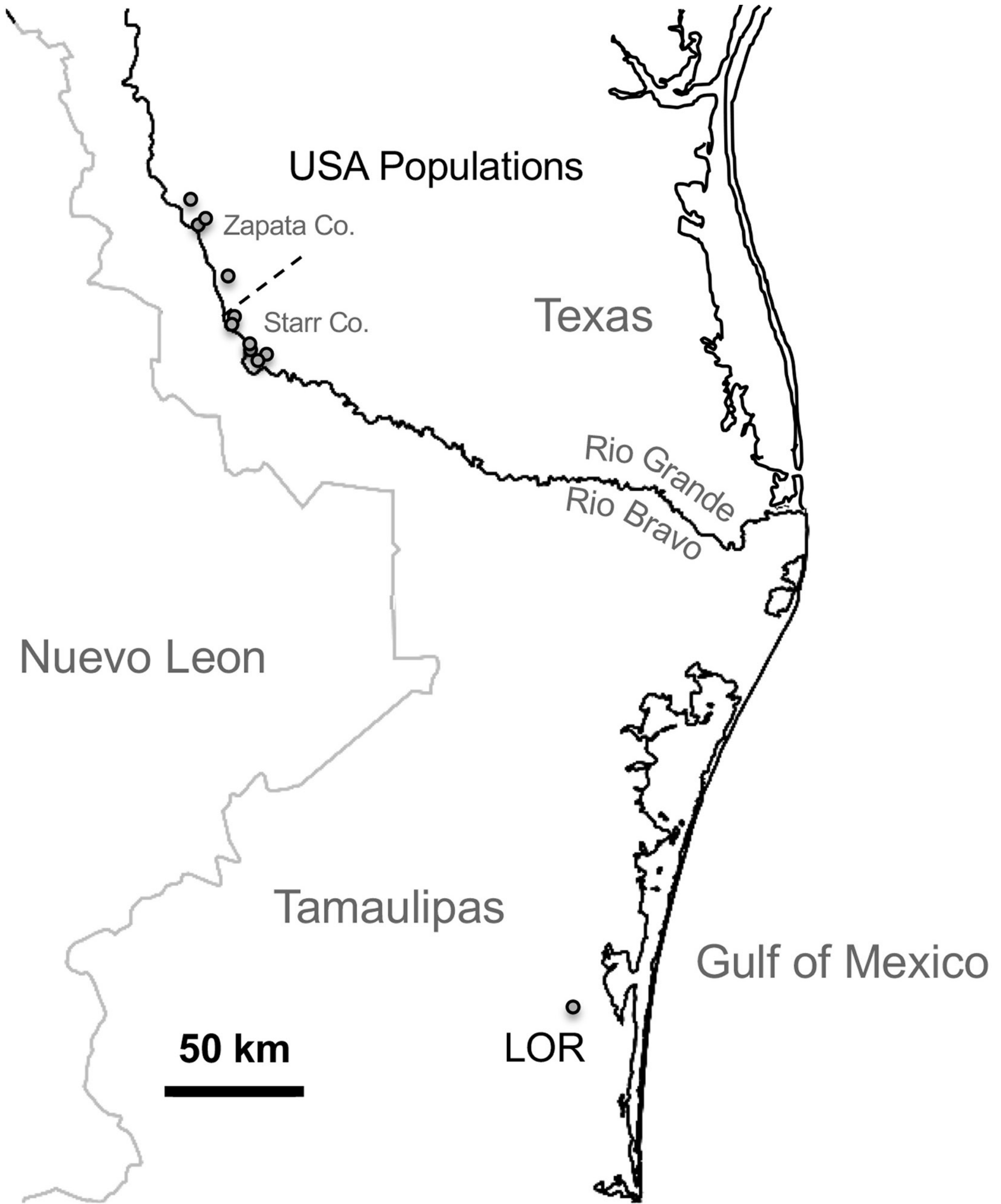

Fig. 1. Distribution of *Physaria thamnophila* in the U.S.A. and Mexico. Grey dots indicate locations of populations. Locations for individual populations will be made available for research and conservation purposes upon request to US Fish and Wildlife Service, Austin TX.

Physaria thamnophila ssp. *loretensis*, a rare and exceedingly vulnerable endemic plant of the U.S.A./Mexico borderlands.

MATERIALS AND METHODS

Plant collections and DNA sampling.—All *Physaria* species were formerly included in the genus *Lesquerella* (Al-Shebaz & O'Kane 2002). Tissues were obtained from 1) laboratory-germinated seedlings, and 2) herbaria specimens as listed in Appendix 1. Additional tissue collections were made from *P. thamnophila* populations under permit and in consultation with the US Fish and Wildlife Service. We sampled all known populations of *P. thamnophila* in which plants were present at the time of sampling. Four populations were in Zapata County, Texas, ten in Starr County, Texas, and one was in Tamaulipas, Mexico. Approximately 0.5 cm^2 tissue was used for each DNA isolation. DNAs were isolated from both fresh and dried tissue by a simple micro-scale preparation described previously (Pepper & Norwood 2001).

Microsatellite marker analyses.—Genomic DNAs from a single individual from the *P. thamnophila* SMR population (Starr County, Texas, U.S.A.) were used for microsatellite discovery. DNA fragments containing microsatellite (CT, CT and GG) repeats were captured using a biotinylated-oligonucleotide method described previously (Terry et al. 2006). All primers were designed to have a 45–60% GC content and a salt-adjusted (50 mM NaCl) melting temperature of 63–64°C. Amplifications were performed with Phusion Hi-Fi polymerase (New England Biolabs) using ±10 ng genomic DNA as template. An annealing temperature of 58°C was employed for all PCR reactions. PCR buffer and cycling conditions followed the manufacturer's recommendations (New England Biolabs). Four microsatellite markers (Appendix 2) were amplified using fluorescently labeled primers (6-HEX and 6-FAM). Multiplexed (HEX + FAM) fragment analysis was performed using the ABI 3130 capillary DNA sequencer as described (Tarin et al. 2014). Fragment sizes were determined using GeneScan ver. 3.1 software (Applied Biosystems Inc.). Microsatellite genotyping was performed using Genotyper ver. 2.5 software (Applied Biosystems Inc.). Unbiased genetic distances (Nei, 1978), as well as principal coordinates analyses (PCoA) were performed using GeneAlEx ver. 6.5 (Peakall & Smouse 2012).

Analysis of nuclear gene sequences.—Genomic DNA sequences of portions of *Physaria* orthologs of three *Arabidopsis thaliana* (L.) Heynh. nuclear genes, *COP1*(AT4G38620), *DET1*(AT2G32950), and *MYB4* (AT4G10180) were determined through direct sequencing of PCR amplification products. Conserved Brassicaceae cross-species PCR primers for *COP1* and *DET1* have been described previously (Kuittinen et al. 2002). Primers for *MYB4* were designed using an alignment of conserved regions of *Arabidopsis thaliana* and *Brassica rapa* ssp. *pekininsis MYB4* sequences obtained from Phytozome (Goodstein et al. 2012) (Appendix 2).

Amplifications were performed with Phusion Hi-Fi polymerase (New England Biolabs, Ipswich, Massachusettes, U.S.A.) using ±10 ng genomic DNA as template. An annealing temperature of 58°C was employed for all reactions. PCR buffer and cycling conditions followed the manufacturer's recommendations (New England Biolabs). Amplification products were purified using ExcelaPure 96-Well UF PCR purification plates (Edge Biosystems, Gaithersburg, Maryland, U.S.A.), and 25 ng of PCR product was used as template for direct sequencing using BigDye ver 1.0 (Applied Biosystems, Foster City, California, U.S.A.). Capillary sequencing was performed using an ABI3130 Genetic Analyzer (Applied Biosystems). Sequences were assembled using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.) and aligned using MAFFT ver. 7.52 (Katoh et al. 2002; Katoh & Standly 2013). Ambiguous nucleotides that were called by Sequencher 4.8 as heterozygous were scored as polymorphisms. Sequence positions containing indel polymorphisms were excluded from analyses of alignments. Selection of nucleotide substitution models was performed by the ModelTest method of Posada and Buckley (2004) as implemented by MEGA11 (Kumar et al. 2018). Maximum Likelihood (ML) analysis (Yang 1994) was performed by a heuristic tree search using nearest-neighbor interchange (NNI), with an initial tree obtained by distance analysis (NJ/BIONJ) as implemented by MEGA11. Distance analyses were performed with the neighbor-joining algorithm (Saitou & Nei, 1987) with a minimum-evolution objective function. Relative support for various branches was determined by bootstrap analyses (Felsenstein 1985).

To estimate divergence times, intron-spanning regions of the *COP1*, *DET1* and *MYB4* genes from populations of *P. thamnophila*, along with *Arabidopsis thaliana* ecotype Col-0, were aligned (as single genes and as concatenated) using MAFFT ver. 7.52 (Larkin et al. 2007). *Brassica rapa* ssp. *pekininsis* was included as an outgroup. A tree file was generated using the Maximum Likelihood method and a general time reversible model with discrete gamma distribution and invariant sites (GTR + Γ + I) selected using MEGA11. Divergence times were estimated using the Rel-Time method (Tamura et al. 2012; Tamura et al. 2018) as implemented by the MEGA11 software package (Kumar et al. 2018). We employed a uniform prior distribution and a general time reversible (GTR + Γ + I) model. For calibration of the molecular clock, we used the estimated times of divergence of *Arabidopsis* and *Physaria* of 15.7 MYA and 15.93 MYA (Huang et al. 2016; Walden et al. 2020).

RESULTS

Nuclear microsatellite analyses.—Using four microsatellite markers, population-pairwise genetic distances were estimated using a matrix of data from 284 individual plants representing the 14 U.S.A. populations (US) and three individual samples from the Rancho Loreto (LOR) population. In PCoA analysis, the LOR population was an extreme outlier, particularly in the first principal coordinate, which captured 86.1% of the variation in the dataset (Fig. 2). The mean pairwise Nei's unbiased genetic distance (*D*) among U.S.A. populations was 0.20 ± 0.24 (range 0.10–0.30), whereas the mean pairwise *D* between the LOR populations and each of the U.S.A. populations was 1.12 ± 0.43 (range 0.58–1.66) (Appendix 3). Similarly, the mean pairwise Nei's unbiased genetic identity (*I*) among U.S.A. populations was 0.83 ± 0.24 (range 0.75–0.91), whereas the mean pairwise *I* between the LOR populations and each of the U.S.A. populations was 0.36 ± 0.43 (range 0.19–0.52) (Appendix 4). These findings indicated that the LOR population was highly differentiated from all U.S.A. populations.

Nuclear gene sequence analysis.—To determine the phylogenetic placement of the LOR samples, Maximum Likelihood (ML) trees were constructed using intron-containing fragments from three nuclear genes, *COP1*, *DET1* and *MYB4* from *P. thamnophila* using the Hasegawa, Kishino and Yano substitution model (Hasegawa et al. 1985) with discrete gamma distribution and invariant sites (HKY + Γ + I). Additional *Physaria* species with overlapping or adjacent distributions in the southwestern U.S. and northern Mexico were included in our analysis to test the possibility that the LOR population might be more closely related to one of these other taxa. *Paysonia lasiocarpa* (Hook. ex A. Gray) S. Watson was included as an outgroup as *Paysonia* is considered to be a sister group to *Physaria* (O'Kane & Al-Shebaz 2002).

In each ML tree, samples from the LOR and U.S.A. populations formed a single monophyletic clade with substantial bootstrap support (99% for *COP1*, 100% for *DET1*, and 98% for *MYB4*). In none of the trees did the LOR samples show any phylogenetic relationship to *P. argyraea* (as originally identified) or to any of the other taxa examined. Several additional (shallow) nodes showed high bootstrap support, while deeper nodes were largely unsupported in these analyses. Importantly, the ML trees obtained from the three individual gene fragments (Figs. 3A–3C) were highly concordant, with only one case in which there was a disagreement among bootstrap supported clades (>50%). In this case, *P. lindheimeri* (A. Gray) O'Kane & Al-Shehbaz formed a clade with *P. sessilis* (S. Wats.) O'Kane & Al-Shehbaz in the *DET1* (96%) and *MYB4* (67%) trees, respectively, but grouped in a distinct clade with *P. argyraea* in the *COP1* tree (100%). As the ML trees from the three genes were largely concordant, we were confident that the *COP1*, *DET1* and *MYB4* sequences could be concatenated to produce a combined ML tree. This concatenated tree (Fig. 3D), obtained using the optimal GTR + Γ + I substitution model, retained all 100% bootstrap supported nodes from the individual gene trees, and placed *P. lindheimerii* as sister to *P. sessilis* with a bootstrap support of 58% (Fig. 3D). Considered together, these data indicate that the LOR sample is sister to the U.S.A. *Physaria thamnophila*, and we find no evidence for recent hybridization in the history of either of these two taxa.

To estimate the genetic distances among *Physaria* taxa, including the distance between the LOR sample and *P. thamnophila* from the U.S.A., we used neighbor-joining algorithm, employing a more conservative (less parameterized) Kimura 2-paramter substitution model (Kimura 1980), applied to the concatenated alignment (Fig. 4). The resulting nucleotide distances between the LOR and *P. thamnophila* (US) sample (0.019

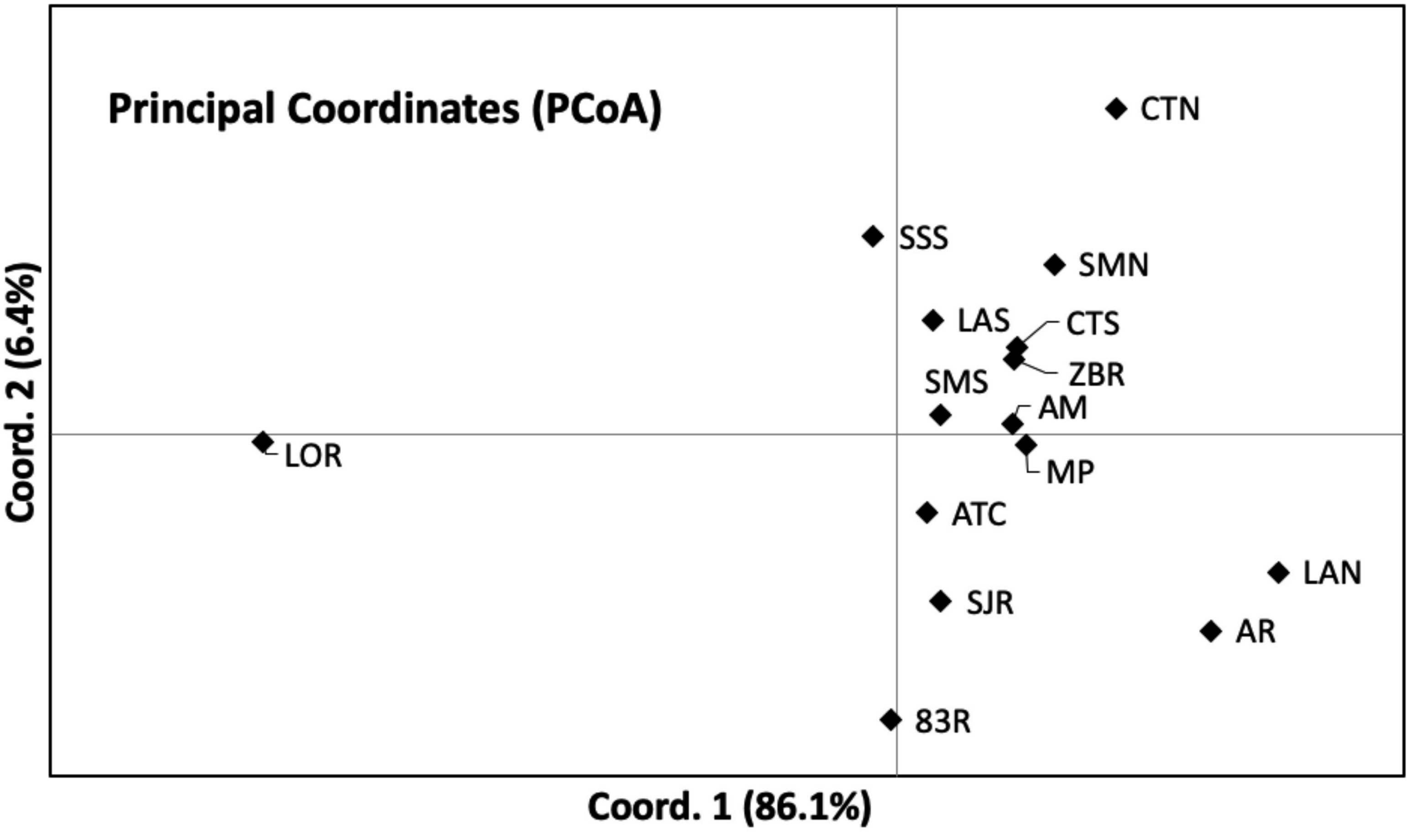

Fig. 2. Principle coordinates analysis (PCoA) of 15 populations of *P. thamnophila* based on microsatellite data. The first two coordinates capture 92.5% of the variation. Locations for individual US populations will be made available for research and conservation purposes upon request to US Fish and Wildlife Service, Austin TX.

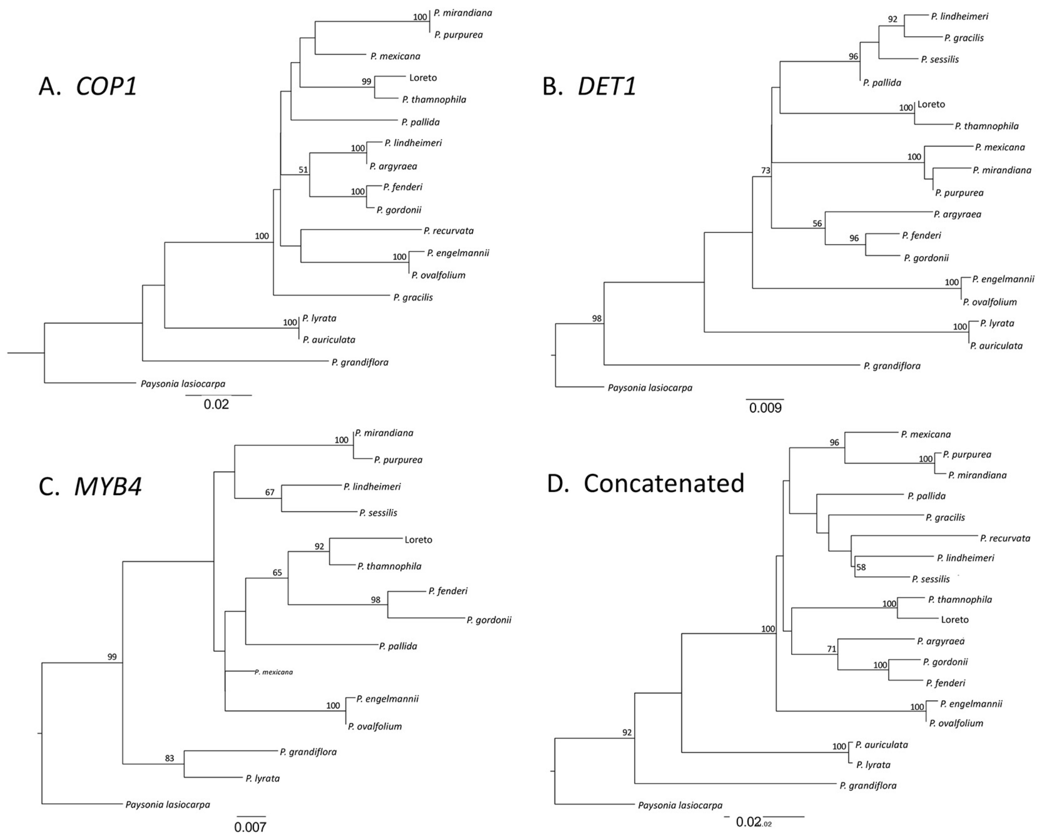

Fig. 3. Maximum Likelihood phylogenetic trees of selected *Physaria* taxa from the southwestern US and northern Mexico, based on nuclear gene intron-spanning sequences. A. *COP1*, B. *DET1*, C *MYB4*, D. Concatenated (three-gene) tree. Distance is indicated as nucleotide substitutions/site. Bootstrap values are indicated for all nodes with > 50% bootstrap support (500 replicates). *Paysonia lasiocarpa* (Hook. ex A. Gray) S. Watson was included as an outgroup.

substitutions/site) were on a scale similar to or exceeding those of several distinct species pairs with 100% bootstrap support in the NJ tree, including *P. lyrata* (Rollins) O'Kane & Al-Shehbaz and *P. auricula* (Englm. & A. Gray ex. S. Wats) O'Kane & Al-Shehbaz (0.07 substitutions/site), *P. purpurea* (A. Gray) O'Kane & Al-Shehbaz and *P. mirandiana* (Rollins) O'Kane & Al-Shehbaz (0.06 substitutions/site), *P. gordonii* (A. Gray) O'Kane & Al-Shehbaz and *P. fendlerii* (A. Gray) O'Kane & Al-Shehbaz (0.013 substitutions/site), and *P. ovifolium* (Rydb. ex Britton) O'Kane & Al-Shehbaz and *P. engelmanii* (A. Gray) O'Kane & Al-Shehbaz (0.003 substitutions/site).

To expand upon these findings, an additional NJ tree was constructed using all three available samples from the LOR population along with single representatives of each of the 14 sampled U.S.A. populations (Fig. 5A). This tree showed that the LOR and U.S.A. populations were mutually monophyletic and confirmed that they are highly differentiated, with minimum a genetic distance of 0.019 substitutions/site. A visual comparison of these intraspecific nucleotide distances to those between distinct *Physaria* species pairs is shown in Fig. 5B.

Time of divergence estimates.—Molecular-clock estimates for the time of divergence between the U.S.A. and LOR populations, based on a concatenated alignment of the three gene fragments, was 1.95 MYA, with a 95% confidence interval of 0.89 to 3.0 MYA. Time of divergence estimates from the individual *COP1*, *DET1* and *MYB4* markers were 1.2 MYA, 2.3 MYA, and 2.34 MYA, respectively. The ancient Rio Grande River originally fed internal basins in present day Colorado and New Mexico rather than flowing to the sea. By 2.06 MYA the river had cut into western Texas, and by 0.6–1.6 MYA had joined with the Pecos River to reach the Gulf of

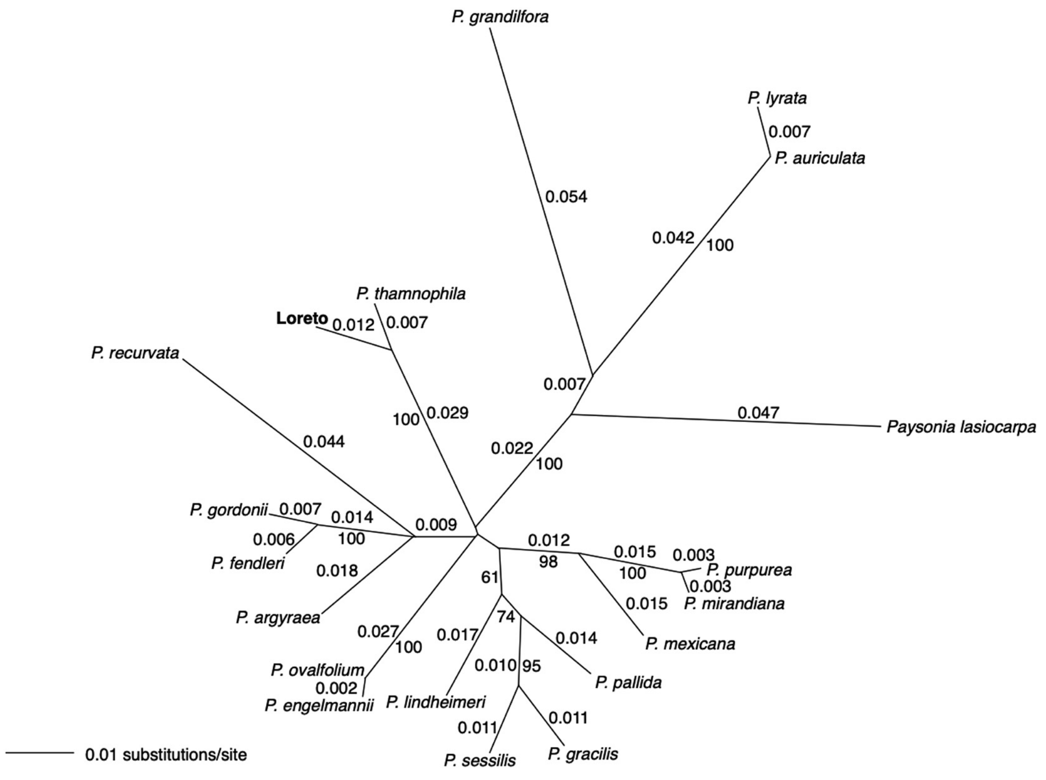

Fig. 4. Unrooted distance-based tree (NJ algorithm) of selected *Physaria* taxa from the southwestern U.S.A. and northern Mexico based on nuclear gene (*COP1*, *DET1* and *MYB4*) intron-spanning sequences. Distance is indicated as nucleotide substitutions/site. Bootstrap support (1,000 replicates) is indicated for all nodes with > 50% bootstrap support.

Mexico (Machette et al. 2007; Repasch et al. 2017). This event increased the watershed that drained into the present-day lower Rio Grande valley from ~100,000 km^2 to ~500,000 km^2, changing the river's impact on the substrate from aggradation to bedrock incision (Rapach et al. 2017), thus greatly altering the landscape, and potentially creating a barrier separating plant populations. Further, several episodes of catastrophic flooding, such as the release of a 4,000 km^2 lake in the San Luis Basin due to volcanic activity 0.44–0.69 MYA (Rapach et al. 2017) may have further contributed to geographic isolation. Evidence from both molecular dating and the geological record suggests a lengthy period of both genetic and geographic isolation of the U.S.A. and LOR populations, in the approximate range of 1–2.5 million years.

DISCUSSION

The genetic analyses described here provide a clear rationale for the consideration of the LOR population as an evolutionarily significant unit (ESU), and thus a priority for monitoring and protection—but does it warrant reclassification as a new species? Frankham et al. (2004) suggested that, in practice, two populations should be considered to be different species if they are as genetically differentiated as are two other well-recognized (e.g , morphologically distinct) species in a related group. The LOR and U.S. populations clearly meet this criterion (Fig. 5a, 5b). In addition, the U.S.A. and LOR populations also exhibited reciprocal monophyly (Fig. 5a), long considered a key defining feature of sister species (Kizirian et al. 2004). These findings suggest that the Rancho Loreto accession could be considered a cryptic species that is sister to the U.S.A. *P. thamnophila*.

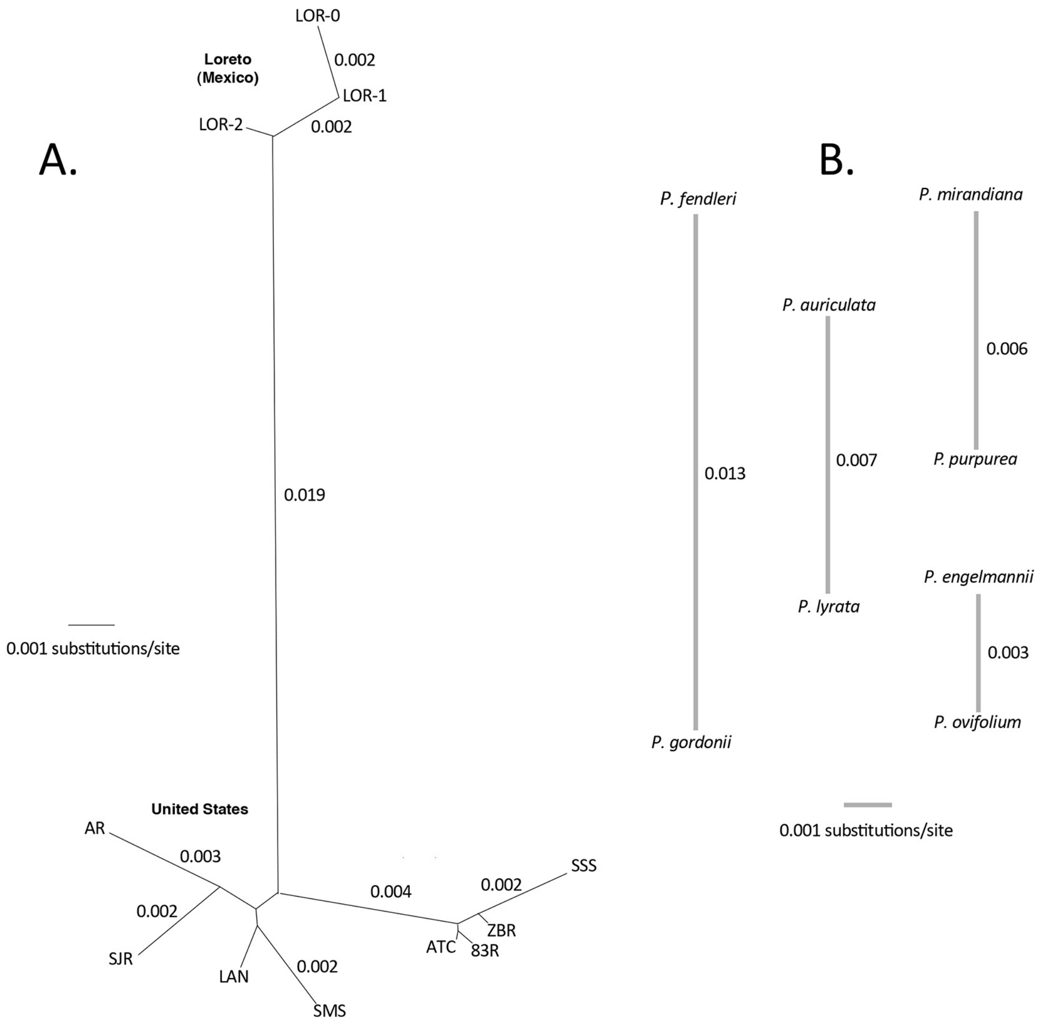

Fig. 5. Genetic distances of among *P. thamnophila* populations from a concatenated alignment of *COP1*, *DET1* and *MYB4* intron-spanning sequences. A. Unrooted phylogram based on NJ analysis of genetic distances of U.S.A. and Mexican populations. Each node represents a single representative sample from each population, except for Loreto, in which all three available individuals were included: LOR-0 is TAMU03549, A. Contreras-Arquieta et al., LOR-1 is TEX00138546, M. Johnson & J. Crutchfield, and LOR-2 was collected at the same location by Christopher Best. For populations with identical sequences, only one representative is shown. B. Genetic distances between selected pairs of sister *Physaria* species that formed monophyletic clades with 100% bootstrap support (see Fig. 4). An identical distance scale bar (0.01 nucleotide substitutions/site) is used for both panels A. and B. to allow for direct comparison.

However, previous examinations of field and herbarium specimens from the Rancho Loreto population did not show morphological distinctiveness that would prompt designation as a separate species. We therefore propose the taxonomic designation of subspecies to describe "geographically isolated variants" (NRCS 2010) or entities that are "both locally and regionally allopatric" (Christiansen 1987). Importantly, this designation also best meets the recommendations of Frankham et al. (2012) to employ taxonomic descriptions that are, ultimately, most beneficial for conserving global biodiversity. The designation *Physaria thamnophila* (Rollins & Shaw) O'Kane & Al-Shehbaz ssp. *loretensis*, is named for the sub-type location, and highlights the importance of this population, while at the same time encourages—and in fact compels—continued cooperation

among conservation entities across the international border. In particular, the LOR and the U.S.A. populations each serve as an ideal reciprocal outgroup for future ecological and evolutionary studies, and may provide a critical source of germplasm, if needed, for genetic augmentation and/or translocation.

TAXONOMIC TREATMENT

Physaria thamnophila (Rollins & Shaw) O'Kane & Al-Shehbaz subsp. **loretensis** Sedio, R.J. Williams, D.M. Price, C.F. Best, A. Contr , Manhart, & Pepper, **subsp. nov.** Type: ESTADOS UNIDOS DE MEXICO. Tamaulipas: Rancho Loreto, 1960, *M. Johnson & J. Crutchfield s.n.* (holotype: TEX00138546). Paratype: *A. Contreras-Arquieta et al. s.n.* (TAMU03549)

Strongly resembles *Physaria thamnophila* and should thus be considered as a cryptic taxon on the basis of gross morphology. An examination of morphological variation across the population is warranted. The known distribution of *Physaria thamnophila* subsp. *loretensis* is limited to a single population in northeastern Tamaulipas, Mexico, that is highly disjunct (>260 km) from the cluster of populations that make up *Physaria thamnophila*, located near the northern bank of the Rio Grande River (Rio Bravo del Norte) in Zapata and Starr Counties, Texas, U.S.A. While the distribution of *Physaria thamnophila* subsp. *loretensis,* is known from only one population, other populations may exist. The epithet *loretensis* is derived from the place name of the singular population, Loreto or Rancho Loreto, Tamaulipas, Mexico.

Physaria thamnophila subsp. *loretensis* differs from *P. thamnophila* by substantial genetic divergence as evidenced by differences in nuclear DNA sequences and high levels of differentiation in DNA microsatellite allele sizes as described above. This treatment as a subspecies is supported by the habitat distinctiveness of *P. thamnophila* subsp. *loretensis,* which is endemic to a prairie community in deep sandy soils on an unusual coastal caliche-sand plain. In contrast, *P. thamnophila* is found on shallow soils overlaying a calcareous layer (including oyster shells and gypsum) that is itself overlaying thick, low-permeability sandstone.

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DATA AVAILABILITY

All DNA sequences generated in this project (will be) submitted to the appropriate NCBI GenBank repository (OR651301-OR651351). Microsatellite marker data matrix is available through Dryad (https://doi.org/10.5061/dryad.4b8gththr). Locations for individual US populations will be made available for research and conservation purposes upon request to US Fish and Wildlife Service, Austin TX.

APPENDIX 1
Plant materials used in this study.

Taxon	Accession No.	Collector
Paysonia lasiocarpa (Hook. ex A. Gray) O'Kane & Al Shehbaz	TEX00211334	A. Richardson & B. King
Physaria argyraea (A. Gray) O'Kane & Al-Shehbaz	TAMU025574	M. Reed
Physaria auriculata (Engelm. & A. Gray ex. S. Wats.) O'Kane & Al-Shehbaz	USWCL 3011 (seed)	D. Dierig
Physaria engelmannii (A. Gray) O'Kane & Al-Shehbaz	TEX00441060	W.R. Carr
Physaria fendleri (A. Gray) O'Kane & Al-Shehbaz	USWCL 4042 (seed)	D. Dierig
Physaria gordonii (A. Gray) O'Kane & Al-Shehbaz	USWCL 2914 (seed)	D. Dierig
Physaria gracilis (Hook.) O'Kane & Al-Shehbaz	TAMU031303	M. Reed
Physaria grandiflora (S. Wats. ex. Hook.) O'Kane & Al-Shehbaz	TAMU027894	M. Reed
Physaria lindheimeri (A. Gray) O'Kane & Al-Shehbaz	TEX00442579	W.R. Carr et al.
Physaria lyrata (Rollins) O'Kane & Al-Shehbaz	USWCL 3000 (seed)	D. Dierig
Physaria mexicana (Rollins) O'Kane & Al-Shehbaz	TEX00472025	M.H. Mayfeld et al.
Physaria mirandiana (Rollins) O'Kane & Al-Shehbaz	TEX00148527	NA
Physaria ovifolium (Rydb. ex Britton) O'Kane & Al-Shehbaz	TAMU035994	M. Reed
Physaria pallida (Torr. & A. Gray) O'Kane & Al-Shehbaz	(seed)	Houston Arboretum
Physaria purpurea (A. Gray) O'Kane & Al-Shehbaz	TEX00440520	T. Gray & B.L. Turner
Physaria recurvata (Engelm. ex. A. Gray) O'Kane & Al-Shehbaz	TAMU027369	C. Barnett
Physaria sessilis (S. Wats.) O'Kane & Al-Shehbaz	TEX00353747	G.L. Webster & B.L. Westlund
Physaria thamnophila (Rollins & Shaw) O'Kane & Al-Shehbaz	TAMU035459	A. Contreras-Arquieta et al.
Physaria thamnophila (Rollins & Shaw) O'Kane & Al-Shehbaz	TEX00148546	M. Johnson & J. Crutchfield
Physaria thamnophila (Rollins & Shaw) O'Kane & Al-Shehbaz	Field-collected tissue samples as described in materials and methods	

APPENDIX 2
PCR Primers used in this study.

Microsatellites

Locus	Forward	Reverse
Pt8	TTGACAGGGTGAGATCATACATTC	GGGGAGCACTGTTTATCTGGAC
Pt10	GTCTGAAACCACCACCCATAGC	GTTGCGGGTGAGGATAGACC
Pt11	CATTCTCTATCTGTAAGCTCCATCG	GAGAAAAGAGATAACCGCTCGTGC
Pt14	CAGATGTATCCAATCACATAATTCGAC	TTCTACACTTCTTGTTATCCAACATGAC

Nuclear Genes (EPIC)

Locus	Forward	Reverse
COP1	ACGAGGCAGGAAGCAAGTGT	CACTGTGAGACCCACAAAGTTCTT
DET1	GGTTCAGTTTTTGGATCGACA	GGAGGGACTTTGTGACTGACA
MYB4	CAACTATCTCCGGCCTGACCT	AGGAAGACTGATTCTGAGCTCAAG

APPENDIX 3

Pairwise Population Matrix of Nei's Unbiased Genetic Distance (*D*)

AR	CTS	CTN	LAN	SMN	AM	ATC	83R	ZBR	SSS	SMS	LAS	MP	SJR	LOR	
0.000															AR
0.193	0.000														CTS
0.447	0.228	0.000													CTN
0.196	0.198	0.137	0.000												LAN
0.203	0.203	0.386	0.128	0.000											SMN
0.237	0.203	0.167	0.084	0.234	0.000										AM
0.138	0.356	0.698	0.357	0.321	0.199	0.000									ATC
0.244	0.393	0.264	0.284	0.383	0.162	0.381	0.000								83R
0.187	0.333	0.361	0.247	0.360	0.205	0.326	0.123	0.000							ZBR
0.579	0.328	0.309	0.387	0.382	0.135	0.311	0.264	0.269	0.000						SSS
0.232	0.298	0.290	0.140	0.310	0.151	0.274	0.102	0.173	0.205	0.000					SMS
0.210	0.371	0.566	0.236	0.366	0.199	0.272	0.361	0.102	0.380	0.207	0.000				LAS
0.166	0.348	0.594	0.243	0.331	0.267	0.125	0.406	0.237	0.478	0.215	0.197	0.000			MP
0.227	0.217	0.440	0.081	0.307	0.160	0.219	0.292	0.259	0.216	0.116	0.307	0.155	0.000		SJR
1.249	1.370	1.657	0.578	0.662	0.957	1.448	1.317	0.972	1.459	1.300	0.877	1.342	1.556	0.000	LOR

APPENDIX 4

Pairwise Population Matrix of Nei's Unbiased Genetic Identity (*I*)

AR	CTS	CTN	LAN	SMN	AM	ATC	83R	ZBR	SSS	SMS	LAS	MP	SJR	LOR	
1.000															AR
0.824	1.000														CTS
0.640	0.796	1.000													CTN
0.822	0.820	0.872	1.000												LAN
0.816	0.816	0.680	0.880	1.000											SMN
0.789	0.816	0.846	0.919	0.791	1.000										AM
0.871	0.700	0.498	0.700	0.725	0.819	1.000									ATC
0.783	0.675	0.768	0.753	0.682	0.851	0.683	1.000								83R
0.829	0.717	0.697	0.781	0.698	0.815	0.722	0.884	1.000							ZBR
0.560	0.720	0.734	0.679	0.683	0.873	0.733	0.768	0.764	1.000						SSS
0.793	0.743	0.748	0.870	0.733	0.860	0.761	0.903	0.841	0.815	1.000					SMS
0.810	0.690	0.568	0.789	0.693	0.819	0.762	0.697	0.903	0.684	0.813	1.000				LAS
0.847	0.706	0.552	0.784	0.719	0.765	0.882	0.667	0.789	0.620	0.807	0.822	1.000			MP
0.797	0.805	0.644	0.922	0.735	0.852	0.803	0.747	0.772	0.806	0.891	0.736	0.856	1.000		SJR
0.287	0.254	0.191	0.561	0.516	0.384	0.235	0.268	0.378	0.233	0.272	0.416	0.261	0.211	1.000	LOR

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