Systematic Botany (2021), 46(3): pp. 611–627

DOI 10.1600/036364421X16312067913318
Date of publication October 25, 2021

Molecular Systematics of Tribe Physarieae (Brassicaceae) Based on Nuclear ITS, *LUMINIDEPENDENS*, and Chloroplast *ndhF*

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Communicating Editor: Adriano Stinca

Abstract—Physarieae is a small tribe of herbaceous annual and woody perennial mustards that are mostly endemic to North America, with its members including a large amount of variation in floral, fruit, and chromosomal variation. Building on a previous study of Physarieae based on morphology and *ndhF* plastid DNA, we reconstructed the evolutionary history of the tribe using new sequence data from two nuclear markers, and compared the new topologies against previously published cpDNA-based phylogenetic hypotheses. The novel analyses included ca. 420 new sequences of ITS and *LUMINIDEPENDENS* (*LD*) markers for 39 and 47 species, respectively, with sampling accounting for all seven genera of Physarieae, including nomenclatural type species, and 11 outgroup taxa. Maximum parsimony, maximum likelihood, and Bayesian analyses showed that these additional markers were largely consistent with the previous *ndh*F data that supported the monophyly of Physarieae and resolved two major clades within the tribe, i.e., DDNLS (*Dithyrea*, *Dimorphocarpa*, *Nerisyrenia*, *Lyrocarpa*, and *Synthlipsis*) and PP (*Paysonia* and *Physaria*). New analyses also increased internal resolution for some closely related species and lineages within both clades. The monophyly of *Dithyrea* and the sister relationship of *Paysonia* to *Physaria* was consistent in all trees, with the sister relationship of *Nerisyrenia* to *Lyrocarpa* supported by *ndhF* and ITS, and the positions of *Dimorphocarpa* and *Synthlipsis* shifted within the DDNLS Clade depending on the employed data set. Finally, using the strong, new phylogenetic framework of combined cpDNA + nDNA data, we discussed standing hypotheses of trichome evolution in the tribe suggested by *ndhF*.

Keywords—Cruciferae, multiaperturate pollen, mustards, trichomes.

Mustards in the Physarieae B.L.Rob. are easily distinguished from other members of the Brassicaceae by their multicolpate (vs. tricolpate) pollen. Physarieae is a predominately North American cruciferous tribe that exhibits extensive variation in vegetative and reproductive morphological characters within its seven genera. Members of the tribe grow in temperate and arid regions and frequently occur as narrow edaphic endemics (Rollins and Shaw 1973; Rollins 1993; Fuentes-Soriano 1994; Al-Shehbaz 2010; O'Kane 2010). Several *Physaria* (Nutt. ex Torr. & A.Gray) A.Gray species are restricted to limestone, gypsum, or serpentine substrates (Rollins and Shaw 1973). *Dithyrea californica* in the southwestern part of the Sonoran Desert and *D. maritima* in Baja California Norte, Mexico are limited to sandy soils, while *Nerisyrenia* Greene and *Synthlipsis* A. Gray, both found in the Chihuahuan Desert, grow mostly in gypsum and sodium chloride rich soils (Bacon 1978; Rollins 1993).

The largest genus, *Physaria*, comprises ca. 80% of the tribe's species diversity (108/136 species). This speciose genus (sensu Al-Shehbaz and O'Kane 2002) includes a great deal of morphological and cytological diversity, and occurs mainly in the western and southwestern United States and in arid regions of Mexico, but few species are found in Canada and in the Russian Arctic. Six species of *Physaria* also show the typical antitropical American distribution (O'Kane and Al-Shehbaz 2004; Al-Shehbaz and Prina 2009) often seen in other Brassicaceae and more distantly related vascular plant groups (Brassicaceae: *Exhalimolobos* Al-Shehbaz & C.D.Bailey, Bailey et al. 2007; *Pennellia* Nieuwl., Fuentes-Soriano 2004, Salariato et al. 2019; *Cardamine* L., Carlsen et al. 2009; other vascular plant groups: Simpson et al. 2017 and references therein). Narrow endemics of the genus (ca. 20%) are commonly found in Mexico (*P. argentea*, *P. inflata*, *P. mexicana*, *P. mirandiana*, *P. rosei*, *P. wyndii*). One of the most well-known species, *P. fendleri*, is a valuable alternative commodity crop that has been introduced into the food production industry for its seed oils and in the industrial manufacture of soaps, lubricants, hydraulic fluids, paints, dyes, coatings, inks, cold resistant plastics, waxes, polishes, and pharmaceuticals (Salywon et al. 2005).

Family-level phylogenetic studies based on chloroplast and nuclear data suggest that multicolpate pollen is a synapomorphy of the monophyletic Physarieae, also known as the Polycolpate lineage (Al-Shehbaz et al. 2006; Bailey et al. 2006; Beilstein et al. 2006, 2008). The plastid *ndhF* phylogenetic analysis of Fuentes-Soriano and Al-Shehbaz (2013) strongly supported Physarieae as monophyletic and identified two major well-supported clades within it. The first clade included the genera *Dithyrea*, *Dimorphocarpa* Rollins, *Nerisyrenia*, *Lyrocarpa* Hook. & Harv. and *Synthlipsis* (the DDNLS Clade), while the second comprised *Physaria* and *Paysonia* O'Kane & Al-Shehbaz (the PP Clade), but internal resolution within these clades was poor. Because the cpDNA represent only the typically maternal history of the group, it was critical to generate additional phylogenetic analyses based on nuclear markers (nDNA). Until now, the most comprehensive nuclear phylogeny of Physarieae used the Internal Transcribed Spacer (ITS) and included no more than four representatives for each genus (Bailey et al. 2006).

The previous *ndhF* study (Fuentes-Soriano 2010; Fuentes-Soriano and Al-Shehbaz 2013) found that the combination of multiple characters such as larger fruit width/length ratios, longer radicles relative to cotyledon length, and longer fruiting styles are characteristics of the tribe, especially when compared to those found in sister tribes such as Halimolobeae and Camelineae. These patterns of trait evolution suggest that traditional classifications based on single characters from seeds or fruits led to erroneous circumscriptions and to equivocal placements of closely-related genera in distantly-related tribes (Gray 1850; Bentham and Hooker 1862; Prantl

1891; Robinson 1895; Hayek 1911; Schulz 1936; Janchen 1942). It is unclear if ploidy variation could potentially interfere with the concordance of chloroplast- and nuclear-based phylogenies of Physarieae. Al-Shehbaz et al. (2006) predicted a basic chromosome number of $x = 8$, but several studies have reported widespread variation in chromosome numbers for the tribe (Rollins, 1939, 1941, 1993; Appel and Al-Shehbaz 2003), with an especially high incidence of polyploidy in members of the DDNLS Clade (Bacon 1975, 1978; Rollins 1979; Rollins and Rüdenberg 1979). Additional sampling and sequence data from single- or low-copy nuclear genes were needed to further test the monophyly of Physarieae and to provide a strong framework to study global and local patterns of trait evolution within this small but extremely morphologically and chromosomally diverse tribe (Fuentes-Soriano in prep.).

Here the evolutionary history of Physarieae was reconstructed using new sequence data from two nuclear markers and then compared against previously published cpDNA-based phylogenetic hypotheses. Analyses include ca. 420 new sequences of ITS and *LUMINIDEPENDENS* (*LD*) markers for 39 and 47 species, respectively, with sampling representing all seven genera of Physarieae, including their nomenclatural type species.

Materials and Methods

Taxon Sampling—The sampling strategy included a total of 48 putatively diploid species of Physarieae (each represented by two different accessions) selected to capture a broad range of morphological, geographic, and cytogenetic variation. Total sampling consisted of 110 accessions for the tribe and 11 outgroup species, which were chosen based on previously published family-level analyses of tribes: Alysseae, Boechereae, Camelineae, Crucihimalayeae, Descurainieae, Halimolobeae, Lepidieae (Appendix 1; see also Bailey et al. 2006; Beilstein et al. 2006, 2008; Warwick et al. 2008; Couvreur et al. 2010; Warwick et al. 2010; Huang et al. 2015; Nikolov et al. 2019). Sequences of seven outgroup taxa were taken from GenBank: *Arabidopsis thaliana, Capsella bursa-pastoris, Descurainia sophia, Exhalimolobos berlandieri, Neslia paniculata, Pennellia longifolia*, and *Transberingia bursifolia*.

DNA Isolation—DNA was extracted from silica-gel dried leaves following the standard CTAB protocol (Doyle and Doyle 1987) and purified in cesium-chloride – ethidium-bromide gradients by ultracentrifugation or from herbarium material following Stefanovicì et al. (2002).

DNA Amplification—The ITS nuclear data set comprised the 5.8S gene flanked by the internal transcribed spacers ITS1 and ITS2, and was amplified with the primers ITS4 (White et al. 1990) and ITS18S (Howarth et al. 2003). The *LD* sequences extend from intron 4 to exon 7 and were amplified with LD-D1F and LD-XC4R primers (Slotte et al. 2006).

ITS and *LD* PCR reactions were performed in 25 μL total volume including 5 μL of 5 × reaction buffer, 2 μL of 2.5 mM $MgCl_2$, 2 μL (ITS) or 3 μL (*LD*) of 2.5 mM dNTPs, 3 μL of each primer 10 μM, 0.5 μL of Taq polymerase (5 units/μL) (Promega, Madison, Wisconsin), and 0.5 μL of DMSO. Cycling reactions for ITS and *LD* were 4 minutes at 95°C (*ITS*) or 2 minutes at 94°C (*LD*), 34 (ITS) or 35 (*LD*) cycles of 30 sec at 94°C, 1 minute at 55°C (ITS) or 1.5 minutes at 57°C (*LD*), 1.5 (ITS) or 2 (*LD*) minutes at 72°C, and finally 7 (ITS) or 9 (*LD*) minutes at 72°C. The PCR products of both nDNA regions were purified with a QIAquick gel extraction kit (Qiagen Inc., Redwood City, California).

Cloning and Sequencing—In a pilot study, 20 accessions, representing ten species of Physarieae exhibiting a wide range of morphological variation, were investigated to identify locus copy number, orthologous regions and variation in nuclear loci. After this initial assessment, a minimum of two clones were screened for all 103 accessions representing the remaining species. PCR fragments from at least two separate PCR reactions were cloned to eliminate labeling and pipetting errors, and then sequenced following Sambrook et al. (1989) and Mathews et al. (2000). Sequence reactions used the fluorescent ABI Prism Big Dye 3.1 (Applied Biosystems, Foster City, California) to label the DNA for analysis in an ABI 3100 (Applied Biosystems) sequencer at the University of Missouri-St. Louis or at the PennState University Nucleic Acid Facility (State College, Pennsylvania). Universal primers T7 and SP6 were used for sequencing both ITS and *LD*.

Sequence Editing and Alignment—SeqMan v. 4 (DNASTAR, Madison, Wisconsin) and GENEIOUS v. 4.0.2 (Drummond et al. 2009) were used for editing and contig assembly. Only double-stranded sequences with at least 85% overlap and Phred scores above 20 as estimated by PhredPhrap (Ewing and Green 1998; Ewing et al. 1998) and 4peaks v. 1.7 (Griekspoor and Groothuis 2005) were considered good quality sequences and thus accepted for further analysis. Base pairs with scores below 20 were eliminated from the analysis except when they matched the complementary strand with Phred scores greater than 20.

Sequence identities were confirmed by comparing sequences of available Brassicaceae accessions deposited in GenBank. Nucleotide sequences of both ITS and *LD* sequences were initially aligned in MUSCLE (Robert 2004), followed by manual alignment using MacClade v. 4 (Maddison and Maddison 2005). Alignment of *LD* exons was guided by identification of open reading frames, exon positions, and stop codons in MacClade and protein alignment using *Arabidopsis thaliana* as a reference species in MUSCLE and GENEIOUS.

Phylogenetic Analyses—The g_1 statistics were obtained from each data set to distinguish phylogenetic signal from random noise (Hillis and Huelsenbeck 1992), and the test was performed with 10,000 replicates as implemented in PAUP v. 4.04b. Data sets were examined individually and combined. Pairwise comparisons of data sets included only those taxa in common for the combined partitions. Conflict among data sets was evaluated before merging them using a partition homogeneity test or incongruence length difference (ILD) test (Farris et al. 1994). The ILD tests were conducted in PAUP v. 4.04b with all invariant characters removed (Cunningham 1997), simple addition sequence, TBR branch swapping, and MAXTREES set to 500 random partitions. For each of the pairwise data partitions, 500 random partitions were analyzed as recommended by Johnson and Soltis (1998).

Phylogenies were constructed using maximum parsimony (MP) with all characters equally weighted, maximum likelihood (ML), and Bayesian analysis (BI), and indels coded as missing data. Analyses were run on the Cyberinfrastructure for Phylogenetic Research (CIPRES) cluster computer housed at the San Diego Supercomputer Center, University of California (http://www.phylo.org) and on the Beowulf computer cluster at the University of Missouri–St. Louis. Parsimony ratchet searches (Nixon 1999) were conducted using PAUPMacRat (Sikes and Lewis 2001) implemented in PAUP v. 4.04 b10 for UNIX (Swofford 2002). Searches consisted of 20 independent replicates of 200 iterations, each with 15% of the characters re-weighted per iteration, and with the strict consensus of the resulting trees generated in PAUP. Bootstrap analysis was used to evaluate the support of specific branches and clades (Felsenstein 1985). Bootstrap values were calculated with 1000 full heuristic bootstrap replicates, one random sequence addition, tree-bisection-reconnection (TBR) branch swapping, and MULTREES = yes options.

For each individual gene data set ML analyses used the best-fitting evolutionary model selected by Modeltest v. 3.6 (Posada and Crandall 1998) according to the Akaike information criteria (AIC), TrN + G for ITS and HKY85 + I + G for *LD*. Likelihood replicates (1000) were run on CIPRES using RAxML bootstrapping (Stamatakis et al. 2008). The ML analyses of combined data sets were estimated as a single partition under the GTR + G model of evolution in RAxML, and as partitioned data sets following the method proposed by Meerow et al. (2009) using models of evolution and Treefinder scripts generated in KAKUSAN4 v. 2 (Tanabe 2007). Scripts were implemented in Treefinder to run ML analyses (Jobb 2008). The latter strategy allowed parameters to be optimized independently among different genes included in a combined data set.

In the BI analyses, MrModeltest 2.2 (Nylander 2004) selected models GTR + G for ITS and HKY + I + G for *LD*, and those algorithms were implemented for single gene and combined data sets in MrBayes v. 3.1 (Huelsenbeck and Ronquist 2001). The BI analyses were conducted with two independent runs of four chains for 5,000,000 generations per run (sampling every 1000 generations). Convergence across runs was evaluated by plotting log-likelihood against the number of generations. The data reached convergence within the first 100,000 generations, but the first 200,000 generations of each run were conservatively discarded as the burn-in. Bayesian posterior probabilities were obtained from the majority-rule consensus trees generated in PAUP.

Initial phylogenetic analyses included sequences of all clones (ITS: 224 clones and *LD*: 219 clones). To minimize computational effort and reduce redundancy, clones were pruned from the original data sets according to the following four rules: 1) a single sequence was chosen at random from a well–resolved, monophyletic species clade to represent the species if the

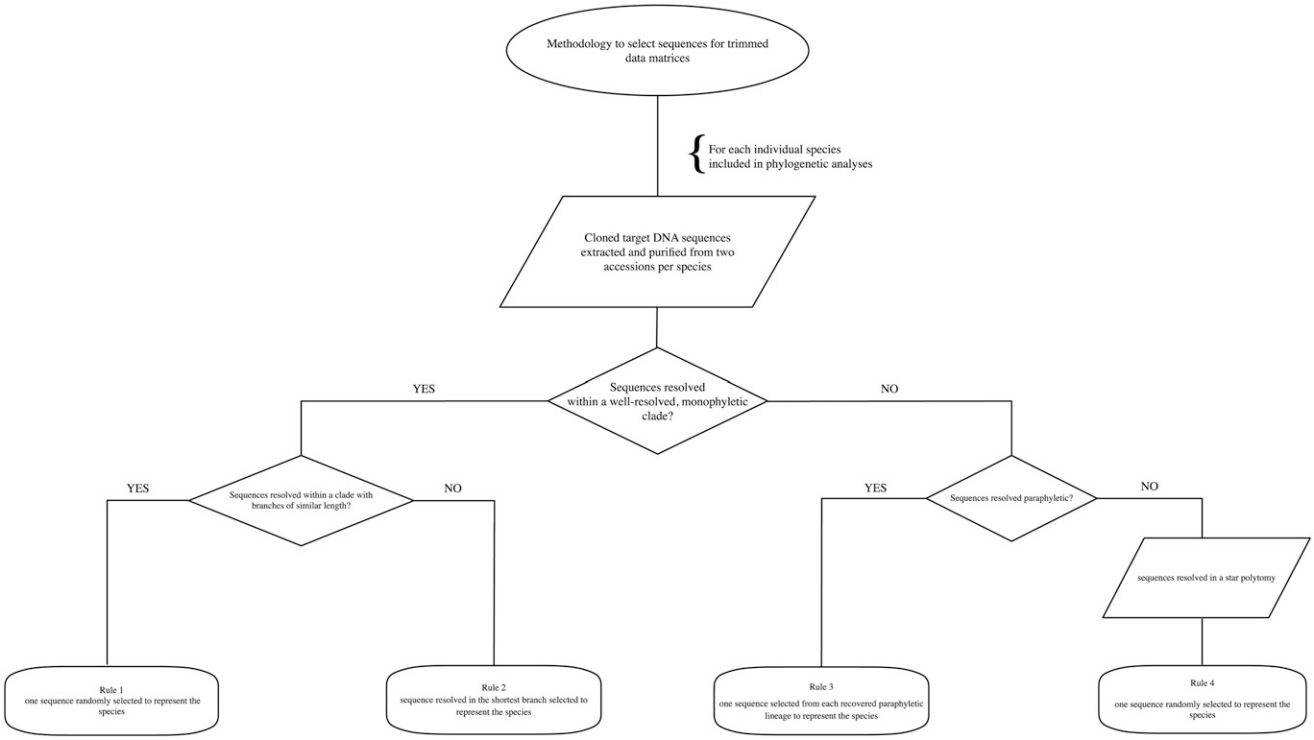

FIG. 1. Flow chart showing how sequences were selected to trim data matrices used in downstream phylogenetic analyses.

compared sequences differed by no more than three base pairs (bp) and resolved in branches of similar length; 2) the sequence resolved in the shortest branch length of a well-supported, resolved species clade was chosen to represent the species; 3) if accessions representing a single species were paraphyletic, then a sequence for each different descendent subgroup within the clade was chosen to represent the species; 4) if accessions representing a single species resolved in a start polytomy, then one sequence was chosen at random to represent the species (Fig. 1).

When clones from the same accession failed to form a monophyletic group, a careful check was made to identify potential errors due to contamination or labeling mistakes, and for the presence of conflicting phylogenetic signal using Splits graphs in the software SplitsTree v. 4.3 (Huson and Bryant 2006). Sequences with conflicting phylogenetic signals were included in the initial phylogenetic analyses to investigate their effects on resulting tree topologies.

Likelihood Topology Tests—Topological congruence and evolutionary hypotheses were evaluated using the Shimodaira-Hasegawa test (S-H test, Shimodaira and Hasegawa 1999) in PAUP v. 4.04b. To obtain full-taxon compatibility among the various tree topologies, we reduced the nuclear and chloroplast data sets to 48 taxa and re-ran the phylogenetic analyses. The S-H tests included comparisons between the optimal trees (unconstrained) from the maximum likelihood analyses of ITS, *LD*, combined ITS + *LD*, and chloroplast *ndhF*. We also created less-resolved constraint trees that included only well-supported nodes either with $> 50\%$ or $> 70\%$ bootstrap support. Poorly supported nodes were defined as having less than 50% bootstrap support and were considered ambiguous polytomies.

Additional S-H tests were run using the combined data sets to test support for particular relationships and estimates of character evolution suggested by previous studies. Putative sister relationships were tested as follows: *Dithyrea* + *Dimorphocarpa* as suggested by Rollins (1979) and supported by earlier *ndhF* data (Fuentes-Soriano and Al-Shehbaz 2013); *Synthlipsis* with all other members of the DDNLS Clade suggested by the *ndhF* data (Fuentes-Soriano and Al-Shehbaz 2013); the alliance of *Synthlipsis, Nerisyrenia*, and *Lyrocarpa* as suggested by Bacon (1978). Earlier hypotheses of trichome evolution in Physarieae as proposed by Fuentes-Soriano (2010) and Fuentes-Soriano and Al-Shehbaz (2013) were also tested. Constraint trees were created to force the tested group to be monophyletic, while the rest of the taxa were placed at the base of a completely unresolved tree using MacClade 4.08 (Maddison and Maddison, 2005). Constraint trees were compared to the ML unconstrained trees. If likelihood values for the topologies being compared were not significantly different, each topology was considered an equally likely phylogenetic hypothesis. The S-H tests were run with full optimization and 1000 bootstrap replicates.

Trichome type definitions were taken from Fuentes-Soriano and Al-Shehbaz (2013) and placed on a suitable tree to facilitate visualization of variation of trichome morphology in sampled taxa.

RESULTS

ITS Data—The ITS data set had an average sequence length of 553 bp, with sequences varying from 541 bp long in *Dithyrea californica* to 559 bp in *Physaria angustifolia*, *P. arizonica*, *P. gracilis*, and *P. kingii*. ITS sequences had the highest percentage of informative characters (slightly over 25%), the largest G + C content of any of the gene regions included in this study (53.07%), a significant nonrandom structure as determined by g_1 statistics, and uncorrected pairwise divergences of up to 14.8% among the most distantly related Physarieae taxa. Summary statistics of all sequences are provided in Table 1.

Sequences of *Dithyrea*, *Lyrocarpa*, *Nerisyrenia*, and *Synthlipsis* shared a nine bp indel at positions 73–81 in the alignment, whereas the two *Dimorphocarpa* species analyzed had a six bp sequence in common at positions 73–78. *Physaria filiformis* was the only analyzed species with an eight bp indel at positions 26–33.

LD Data—The *LD* sequences had an average length of 638 bp, and varied from 619 bp long in *Physaria rectipes* to 651 bp in *Dimorphocarpa wislizeni*. This marker showed 136 potentially parsimony informative characters, a significant nonrandom structure as determined by g_1 statistics, a lower G + C content (35.64%) than ITS sequences, and an 8.6% sequence divergence within the most distantly related taxa of Physarieae (Table 1). In the sequence alignments *D. wislizeni* was

TABLE 1. Alignment statistics including the significance of nonrandom structure (g_1) and the maximum parsimony analyses for the nDNA, *ndhF*, and combined nuclear data sets. The *ndhF* values taken from Fuentes-Soriano and Al-Shehbaz (2013). Superscript 1 indicates total amount including all sequences. Superscript 2 indicates total amount excluding all redundant sequences. CI = Consistency index; RI = Retention index, PI = phylogenetically informative sites. Note g_1 calculated from 10,000 random trees.

		Genomic regions				
		ITS	*LD*	ITS + *LD*	*ndhF*	*ndhF* + ITS + *LD*
Ingroup + Outgroup	Physarieae species	48	48	48	44	44
	Aligned length	595	691	1286	2063	3350
	Average sequence length	553	638	1191	2049	3239
	Total sequences[1]	224	219	58	53	48
	Total sequences[2]	62	64	48	48	48
Ingroup[2]	Variable sites (%)	222 (37.31)	239 (34.59)	463 (36)	183 (8.87)	615 (18.36)
	PI (%)	149 (25.04)	136 (19.68)	288 (22.4)	78 (3.78)	333 (9.94)
	Ratio of PI: variable sites	0.67	0.57	0.62	0.43	0.54
	Uncorrected pairwise divergence (%)	14.8	8.6	45.63	2.8	41
	GC base pair content (%)	53.07	35.64	43.94	30.56	42.75
Maximum Parsimony Analysis	Number of trees retained	1921	202	165	213	96
	Tree length	1131	701	1476	489	1830
	CI	0.49	0.63	0.56	0.71	0.61
	RI	0.88	0.89	0.78	0.83	0.77
	g_1	−0.435	−0.443	−0.383	−0.333	−0.554

exceptional for having a large indel of 23 bp at positions 81–104, whereas *Synthlipsis* and *Lyrocarpa* species shared two small indels of five and four bp each at positions 81–85 and 88–91, respectively. *Paysonia* species showed a indel of eight bp at positions 138–145 in the alignment.

ITS Phylogenetic Analyses—The ML and BI analyses provided support for the greatest number of clades (see Fig. S1 in Fuentes-Soriano and Kellogg 2021). The phylogenetic hypotheses generated from the 224-sequence data set (representing 103 accessions) using MP, ML, and BI analyses were mostly congruent as shown by the S-H test (Table 2), and resolved the majority of sequences from single accessions as monophyletic (Fig. S1). However, for several species the biological or technical replicates were closely related, but were either unresolved or placed in different positions (e.g., some clones of *Lyrocarpa coulteri*, *L. xantii, Nerisyrenia incana, N. johnstonii, Paysonia perforata, Physaria acutifolia*, *Ph. angustifolia*, *Ph. arizonica*, *Ph. bellii*, *Ph. eburniflora*, *Ph. floribunda*, and *Ph. gracilis*). Furthermore, sequences from a few accessions (*Dimorphocarpa wislizeni*, *Nerisyrenia incana*, *N. johnstonii*) showed conflicting phylogenetic signal as identified in the SplitsTree software, leading to the loss of resolution within the *Nerisyrenia* and *Dimorphocarpa* clades (data not shown). The exclusion of these sequences, however, did not affect results observed in the general tree topology (Fig. 2).

To minimize data redundancy, reduce sequence biases, and incorrect paralog assignment potentially misleading signal, the full ITS data set was trimmed to 62 sequences (Fig. 2). Doing so lowered the number of potentially parsimony informative characters, and consequently the percentage of sequence variation used in subsequent analyses (Table 1). The phylogenetic analyses of the reduced set yielded trees with topologies similar to those resolved with the full data set, confirming that the trimmed sequences were mostly redundant.

ITS supported the monophyly of Physarieae (MP 100; ML 100; BI 100; Fig. 2), while identifying Camelineae as sister to Physarieae (MP 80; ML 91; BI 89) and resolving the five-tribe lineage [[Physarieae + Camelineae] + [Halimolobeae + Boechereae]] + Lepidieae as sister to Descurainieae. ITS also recognized the previously identified DDNLS and PP clades (Fuentes-Soriano and Al-Shehbaz 2013; Mazie and Baum 2016), and supported the monophyly of six of the seven genera of the tribe (*Dithyrea*, *Lyrocarpa*, *Nerisyrenia*, *Paysonia*, *Physaria*, *Synthlipsis*). However, ITS analyses revealed new well-supported incongruencies between the *ndhF*-based phylogeny, including the sister relationship of *Dithyrea* to *Dim. membranacea* (MP 80; ML 83; BI 100), the sister position of *Dimorphocarpa wislizeni* to the rest of DDNLS members, a paraphyletic *Dimorphocarpa*, and the sister relationship of *Lyrocarpa* to *Nerisyrenia* (MP 90; ML 90; BI 100). ITS resolved within *Physaria*, several species pairs as sisters (bootstrap support > 70%; e.g. *Ph. filiformis* + *Ph. globosa*, *Ph. angustifolia* + *Ph. gracilis*, *Ph. rosei* + *Ph. argentea*), but other relationships in the genus remain uncertain. ITS resolved a successive sister relationship among *Pa. auriculata*, *Pa. grandiflora*, *Pa. lasiocarpa*, and the unresolved [*Paysonia lescurii-Pa. lyrata-Pa. densipila-Pa. stonensis*] Clade.

LD Phylogenetic Analyses—All methods of phylogenetic reconstruction gave trees with congruent topologies as indicated by the S-H tests (Table 2). The complete *LD* data set (219 sequences representing 98 accessions) resolved most of the sequences from the same species as monophyletic, with most of the sequences of individual accessions coalescing within the same clade (see Fig. S2 in Fuentes-Soriano and Kellogg 2021). Based on the findings, the full *LD* data set was trimmed down to 64 sequences, thereby reducing the number of potentially parsimonious informative characters from 190 to 119. The resulting 64-sequence *LD* data set generated trees with topologies similar to those recovered using the full data set, a finding that suggests that the 155 trimmed sequences were redundant (Fig. 3).

The *LD*-based tree provided more phylogenetic structure than the ITS tree, and in contrast to ITS, it was consistent with morphologically-defined species limits. *LD* differed from ITS in three fundamental ways: 1) Physarieae resolved successively sister to Descurainieae (vs. Camelineae in ITS; MP 98; ML 100; BI 100; Fig. 3) and Lepideae, while this three-tribe lineage was placed sister to the [[Halimolobeae + Boechereae] + Crucihimalayeae] + *Capsella-Neslia* Clade, 2) *Synthlipsis* resolved sister to *Lyrocarpa* + *Dimorphocarpa wislizeni*, and 3) *Nerisyrenia* resolved sister to *Dithyrea*.

The *LD* analyses recovered two novel clades within *Physaria*, herein designated Clade 1, albeit poorly supported, and a

TABLE 2. Results of the Shimodaira-Hasegawa tests showing topological differences among maximum likelihood, Bayesian and parsimony phylogenetic estimations. Constraint trees are listed in order of appearance in text. Significantly different values are denoted with asterisks in the *p* value column.

	Phylogenetic estimates	-Ln Likelihood	Difference from the best tree	*p* value
ITS	Maximum parsimony	7421.13893	0.66056	0.492
	Bayesian analysis	7140.33488	7.4999	0.313
	Maximum likelihood	7132.83498	best	
	ITS tree, > 50% supported nodes			
	LD tree, > 50% supported nodes	5358.4408	51.0452	0.066
	ndhF tree, > 50% supported nodes	4714.0891	16.37829	0.313
	ITS tree, > 70% supported nodes			
	LD tree, > 70% supported nodes	5335.13891	27.74331	0.24
	ndhF tree, > 70% supported nodes	4714.0891	25.50914	0.211
LD	Maximum parsimony	5745.16856	0	0.718
	Bayesian analysis	5746.6939	1.52535	0.257
	Maximum likelihood	5745.16855	best	
	***LD* tree, > 50% supported nodes**			
	ITS tree, > 50% supported nodes	6032.2382	427.34567	0.000*
	ndhF tree, > 50% supported nodes	4330.81342	201.56906	0.000*
	***LD* tree, > 70% supported nodes**			
	ITS tree, > 70% supported nodes	6041.06092	535.03088	0.000*
	ndhF tree, > 70% supported nodes	4283.99617	154.7518	0.000*
ITS + *LD*	Maximum parsimony	9867.64158	3.83484	0.244
	Bayesian analysis	9884.34696	20.54023	0.08
	Maximum likelihood	9863.80674	best	
	Combined, > 50% supported nodes			
	ITS tree, > 50% supported nodes	9959.15946	76.61843	0.089
	LD tree, > 50% supported nodes	10028.3204	20353080	0.343
	ndhF tree, > 50% supported nodes	9301.97107	127.38371	0.000*
	Combined, > 70% supported nodes			
	ITS tree, > 70% supported nodes	10120.38824	203.42873	0.002*
	LD tree, > 70% supported nodes	10120.38824	111.90026	0.013*
	ndhF tree, > 70% supported nodes	9301.97107	127.38371	0.000*
ndhF	***ndhF* tree, > 50% supported nodes**			
	ITS tree, > 50% supported nodes	5901.5996	98.22112	0.05
	LD tree, > 50% supported nodes	5987.27399	85.67439	0.021*
	Combined, > 50% supported nodes	5908.94217	56.16417	0.142
	***ndhF* tree, > 70% supported nodes**			
	ITS tree, > 70% supported nodes	5908.94271	105.56422	0.041*
	LD tree, > 70% supported nodes	5938.2185	29.27578	0.283
	Combined, > 70% supported nodes	5908.9427	56.16417	0.142
nDNA + cpDNA	Maximum parsimony	15126.03783	0.67484	0.533
	Bayesian analysis	15478.3498	12.6672	0.089
	Maximum likelihood	15346.03287	best	
Evolutionary hypothesis	*Dithyrea* + *Dimorphocarpa*	15346.1064	0.07353	0.505
	Synthlipsis + [DDNL Clade]	15347.01652	0.98365	0.462
	Lyrocarpa + *Nerisyrenia* + *Synthlipsis*	15347.86594	1.83308	0.472
	Highly-branched trichomes (DDNLS Clade)	15346.81049	0.77762	0.491
	Moderately-branched trichomes (*Paysonia*)	15346.42307	0.3902	0.49
	Stellate trichomes (*Physaria*)	15346.42307	0.42033	0.59

better-supported Clade 2 (neither clade recovered in the ITS analyses). Within Clade 1, three subclades were variously supported, from weakly to strongly, and designated here as Subclades A–C (Fig. 3). *LD* data provided little resolution within these subclades. Relationships within *Paysonia* were mostly consistent with those observed for ITS.

cpDNA Phylogenetic Analysis—The *ndhF* topology differed from the combined and individual nuclear-derived topologies in three primary respects: 1) placement of *Synthlipsis* as sister to all the other members of the DDNLS Clade, 2) resolution and strong support of the monophyly of *Dimorphocarpa* and its sister relationship to *Dithyrea*, and 3) relationship of *Paysonia grandiflora* and *Pa. lasiocarpa* as sister species (recovered in Fuentes-Soriano 2010; Fuentes-Soriano and Al-Shehbaz 2013).

Combined ITS + LD Phylogenetic Analyses—Analysis of the two nuclear regions yielded an average of 1286 aligned positions, of which 343 were from exons and 944 were from introns and spacers. Slightly over 18.3% of the characters were variable and 9.9% phylogenetically informative (Table 1). The combined nuclear data set had significant nonrandom structure as defined by the g_1 statistic (Table 1). MP, ML, and BI phylogenetic reconstructions showed similar topologies (Table 2).

The combined nDNA data set yielded a different tree topology than those inferred from individual nuclear genes. However, most of the conflict affected relationships that were only weakly supported by one of the individual nuclear data sets. The ITS + *LD*-based tree supported the sister relationship of Physarieae to Camelineae inferred by the ITS loci (Fig. 4), not to Descurainieae as suggested by the *LD* nuclear region analysis alone. In all analyses there was phylogenetic instability within the DDNLS Clade in those relationships showing weak support (Figs. 2–4). In the ITS and combined nDNA analyses and with variable support, *Nerisyrenia* was sister to *Lyrocarpa*, whereas in *LD Nerisyrenia* was sister to

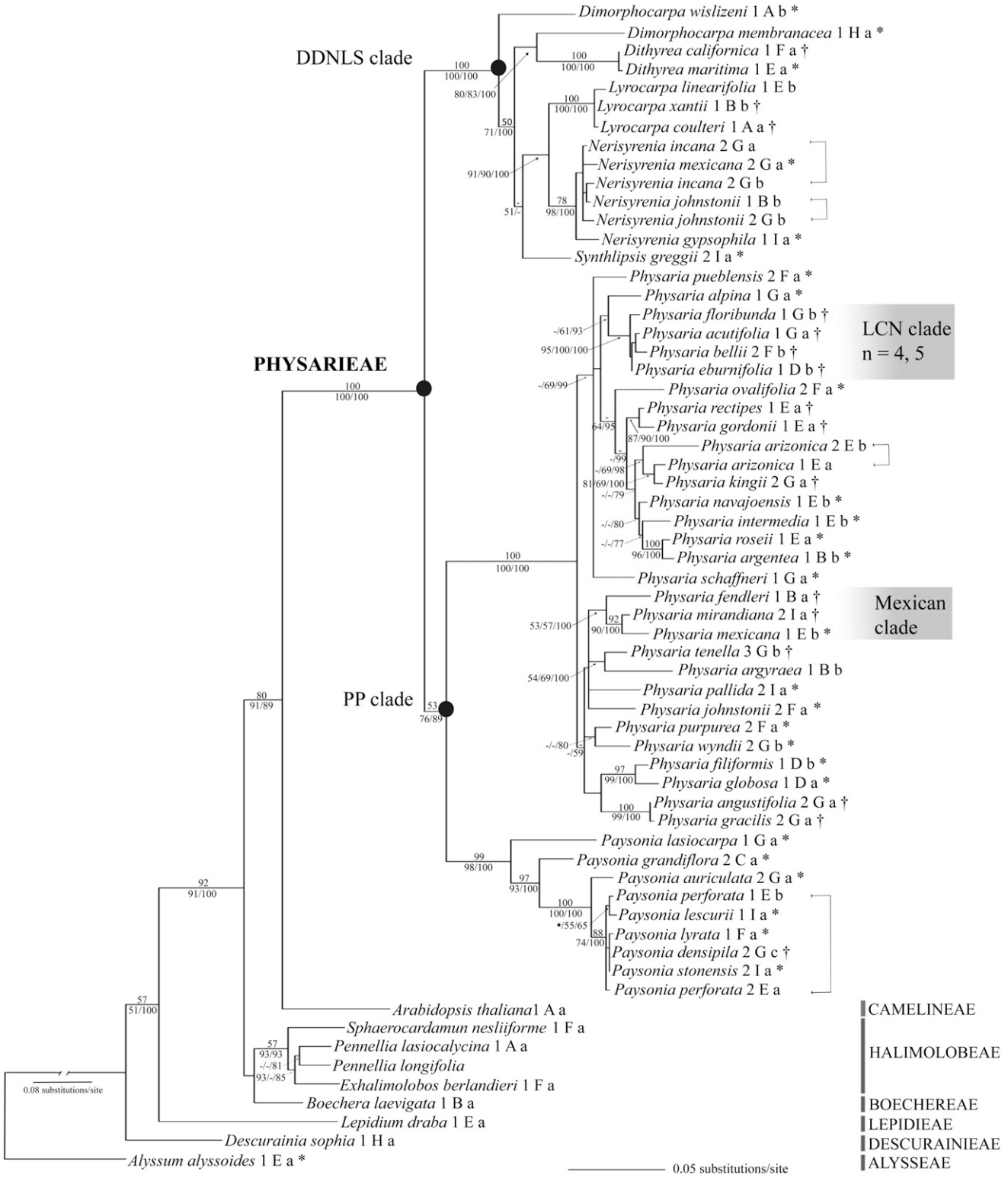

FIG. 2. Maximum likelihood (ML) phylogram for the reduced 62-sequence ITS data set of Physarieae and outgroup taxa. Maximum parsimony bootstrap values are placed above branches or listed first in a series of numbers separated by forward slashes; ML bootstrap values are below branches or listed after MP support values; Bayesian posterior probabilities are listed after ML support values. A dash along a branch indicates no support (< 50% support value), and a solid star indicates alternative resolutions. After each taxon name label, the number indicates the specific accession, followed by the capitalized letter indicating the PCR reaction, and then the lower-case letter denoting the clone from a single PCR reaction. An asterisk at the end of the taxon label indicates that the sequence represents two accessions, whereas the † symbol shows that the species accessions and clones from individual accessions were unresolved in a partially resolved clade or soft polytomy. Double-headed arrows indicate situations where individual accessions or individual sequences representing the same species were resolved polyphyletic. Clades without values have very low support.

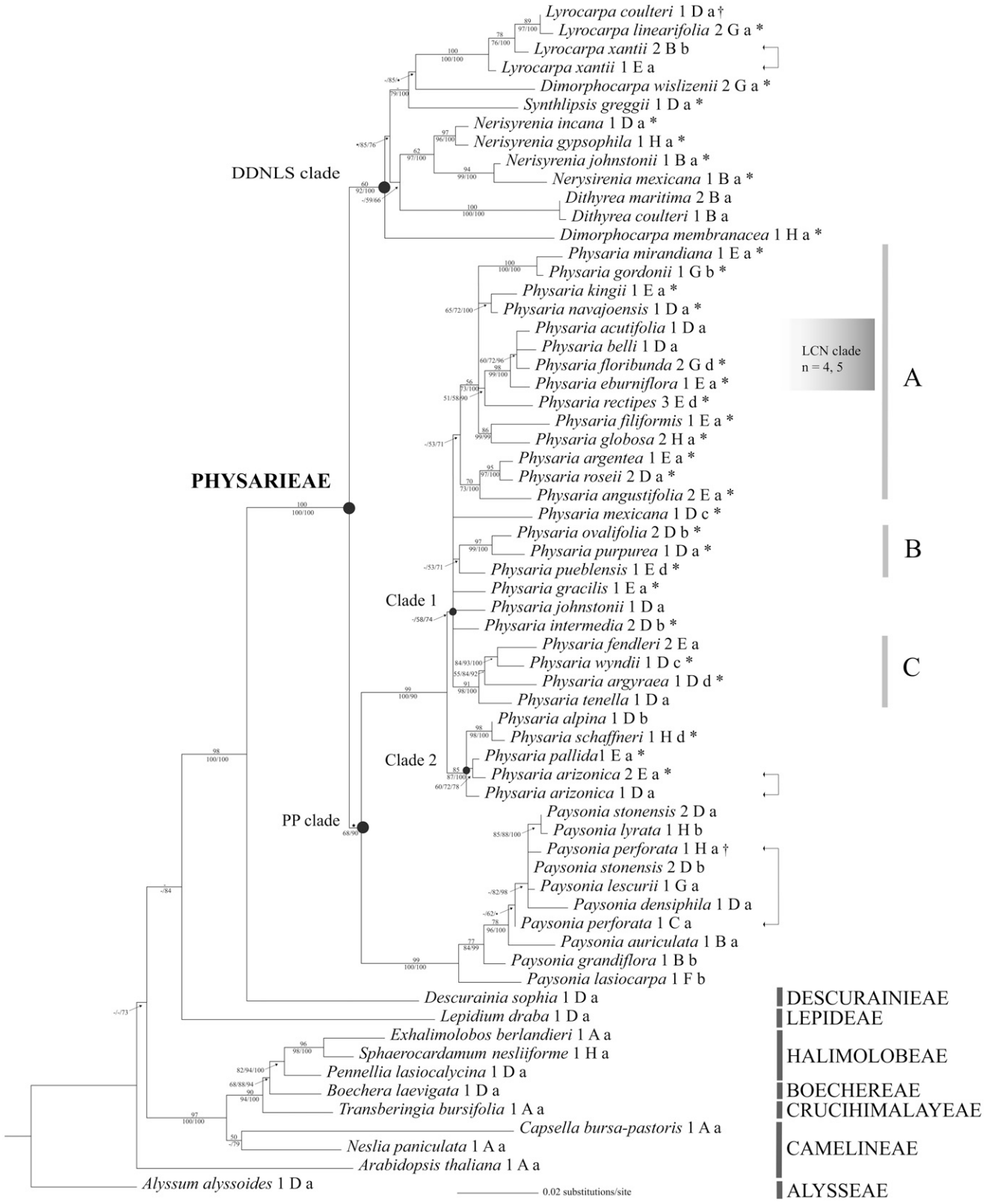

FIG. 3. Maximum likelihood (ML) phylogram for the reduced 64-sequence *LD* data set of Physarieae and outgroup taxa. Maximum parsimony bootstrap values are placed above branches or listed first in a series of numbers separated by forward slashes; ML bootstrap values are below branches or listed after MP support values; Bayesian posterior probabilities are listed after ML support values. A dash along a branch indicates no support (< 50% support value), and a solid star indicates alternative resolutions. After each taxon name label, the number indicates the specific accession, followed by the capitalized letter indicating the PCR reaction, and then the lower-case letter denoting the clone from a single PCR reaction. An asterisk at the end of the taxon label indicates that the sequence represents two accessions, whereas the † symbol shows that the species accessions and clones from individual accessions were unresolved in a partially resolved clade or soft polytomy. Double-headed arrows indicate situations where individual accessions or individual sequences representing the same species were resolved polyphyletic. Clades without values have very low support.

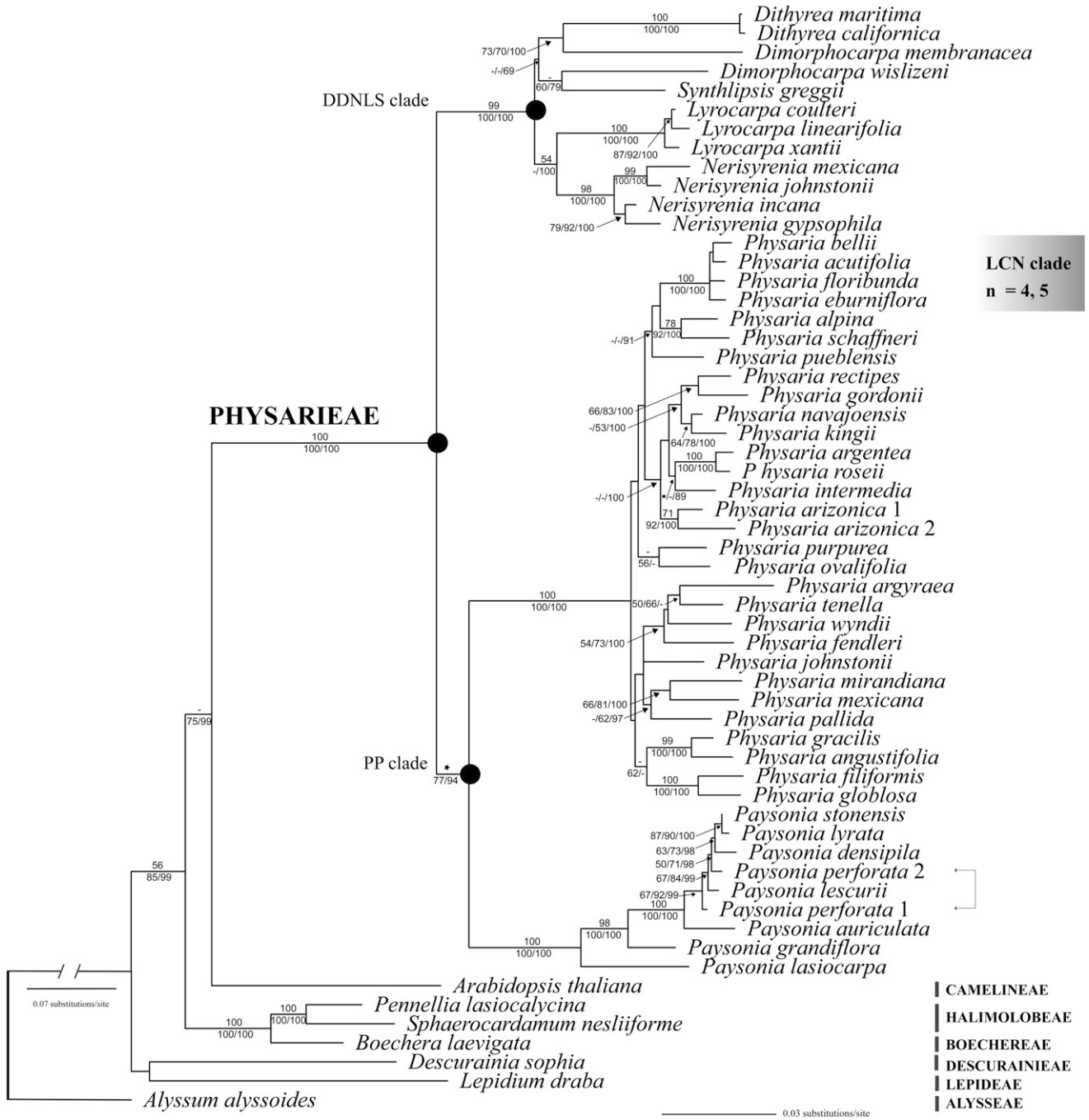

FIG. 4. Maximum likelihood (ML) phylogram of the combined analyses of ITS and *LD* of Physarieae and outgroup taxa. Maximum parsimony bootstrap values are placed above branches or listed first in a series of numbers separated by forward slashes; ML bootstrap values are below branches or listed after MP support values; Bayesian posterior probabilities are listed after ML support values. A dash along a branch indicates support values below < 50% or no support, and a solid star indicates alternative resolutions. Double-headed arrow indicates a situation where individual accessions or individual sequences representing the same species were resolved polyphyletic and more distantly resolved in the tree. Clades without values have very low support.

Dithyrea. While the combined nDNA analysis resolved *Synthlipsis* as sister to *Dimorphocarpa wislizeni* with moderate support (MP 50; ML 60, BI 79), the genus shifted to different positions in the individual gene analyses. ITS supported the relationship of *Synthlipsis* to the *Nerisyrenia* + *Lyrocarpa* lineage (ML 51; Fig. 2), yet *LD* placed *Synthlipsis* sister to the *Lyrocarpa* + *Dimorphocarpa wislizeni* lineage (MP 43; ML 79; BI 100).

Results obtained from the combined nuclear sets and individual ITS analyses agreed on the placement of *Dithyrea* as sister to *Dimorphocarpa membranacea*, in contrast with *LD* data that resolved *Dithyrea* as sister to *Nerisyrenia*, but only with low bootstrap values. Although all analyses suggested the paraphyly of *Dimorphocarpa*, only the combined nuclear data set placed the non-reciprocally monophyletic species of *Dimorphocarpa* together within a clade including *Dithyrea* (albeit with very low bootstrap support values and low posterior probabilities).

The combined and individual nuclear gene analyses moderately supported the sister relationship of *Physaria* and

Paysonia (MP no support; ML 77; BI 94), and within *Physaria* resolved a clade comprising four species (*Ph. acutifolia*, *Ph. bellii*, *Ph. eburniflora*, and *Ph. floribunda*), which have always been treated as *Physaria* s. s. (i.e. excluding *Lesquerella* S. Watson taxa). This four-species clade possesses the lowest chromosome numbers ($n = 4, 5$) in Physarieae and other closely related tribes (new clade named the LCN Clade hereafter for its low chromosome numbers; Fig. 4). Relationships within the LCN Clade were unresolved.

All nDNA-based analyses recognized the sister taxa relationships of the Midwestern species *Physaria filiformis* + *Ph. globosa*, and also the Mexican species *Ph. argentea* + *Ph. rosei*. The combined analyses supported the monophyly of the 4-species Subclade C of *Physaria* recovered in the *LD* topology (Figs. 3–4).

In the combined analysis *Physaria fendleri* is sister to the successively sister species *Ph. wyndii*, *Ph. argyraea*, and *Ph. tenella* (Fig. 4). This finding contrasted with results recovered by the ITS and *LD* analyses. In the ITS tree, *Physaria fendleri* was sister to the *Ph. mirandiana* + *Ph. mexicana* clade, whereas in the *LD*-based topology, *Physaria fendleri* + *Ph. wyndii* were successively sisters to the North American *Ph. argyrea* and *Ph. tenella*. These conflicting results may indicate a complex evolutionary history among *Ph. fendleri* and closely related species. All accessions of *Ph. arizonica*, an apparent paraphyletic species in the separate ITS and *LD* analyses, were monophyletic in the combined analysis.

The combined and individual nuclear data sets agreed in the successive sister relationship of the westernmost-distributed species of *Paysonia* (*Pa. lasiocarpa* and *Pa. grandiflora*) with a mostly unresolved group comprising the remaining eastern species of the genus (6 spp.) (Figs. 2–4).

Combined cpDNA + nDNA Phylogenetic Analyses—Analysis of the combined nDNA and cpDNA data sets yielded an average of 3350 aligned positions. Nearly 19% of the characters were variable, whereas 14.83% of the positions were phylogenetically informative (Table 1). The g_1 statistic showed that the data set had significant nonrandom structure (Table 1).

The combined nDNA + cpDNA data set analyses supported the DDNLS and PP clades (Fig. 5). Within the DDNLS Clade, the sister relationship of *Nerisyrenia* to *Lyrocarpa* was strongly supported (MP 100; ML 71; BI 99) as in the ITS and combined nuclear analyses (Fig. 4), *Dithyrea* was sister to *Dim. membranacea*, and the phylogenetic resolution of *Synthlipsis* remained ambiguous. In the PP Clade, the combined nDNA + cpDNA data set grouped species of *Physaria* into three major Subclades (A–C).

Subclade A, comprised mostly of the North American species sampled in this study, was further subdivided into Clades 1 and 2 (Fig. 5). The weakly supported Clade 1 (MP 58; ML 100; BI 89) was generally a polytomy containing the monophyletic aforementioned LCN Clade and Clade 2, a lineage composed of the narrowly distributed American Midwestern annual species *Physaria globosa, Ph. filiformis,* and *Ph. angustifolia* (MP 100; ML 72; BI 100). Subclade B included, as in the *ndhF* data analyses, all Mexican species of the genus sampled in this study (MP 100; ML 61; BI 89). Subclade C was formed by *Physaria argyraea* and *Ph. tenella* (MP 100; ML 100; BI 100). The combined nDNA + cpDNA data set supported the phylogenetic relationships of *Paysonia* as inferred by the nDNA data sets.

All four-gene data sets (ITS, *LD*, ITS + *LD*, *ndhF*) independently supported the monophyly of Physarieae, the two main intratribal clades, and six of seven genera of the tribe. Within *Physaria* and *Paysonia*, the four data sets resolved the sister relationships of *Physaria tenella* to *Ph. argyraea, Ph. argentea* to *Ph. rosei*, supported the successively sister relationship of the Southwestern species of *Paysonia* (i.e. *Paysonia grandiflora*, *Pa. lasiocarpa* and *Pa. auriculata*), and the closest relationship of *Pa. auriculata* to the more easterly distributed species of the genus (i.e. *Pa. densipila*, *Pa. lescurii*, *Pa. lyrata*, *Pa. perforata,* and *Pa. stonensis*). Data matrices and trees are archived in the Dryad Digital Repository (Fuentes-Soriano and Kellogg 2021).

Incongruence Tests Among and Between Nuclear and Chloroplast Data Sets—ILD tests indicated that ITS and *LD* ($p = 0.008$) and combined nuclear and *ndhF* data partitions ($p = 0.004$) failed to reject the null hypothesis of congruence based on $p < 0.05$. Although Farris et al. (1994) recommended using a threshold of $p < 0.05$ for accepting data combinability, several studies indicated problems of accuracy in ILD and inferred that p values < 0.05, and even those as low as 0.001, should not preclude data set combination (Sullivan 1996; Cunningham 1997; Davis et al. 1998; Flynn and Nedbal 1998; Messenger and Meguire 1998; Yoder et al. 2001; Dowton and Austin 2002; Meerow et al. 2009).

The S-H tests identified significant differences among ITS, *LD*, ITS + *LD*, and *ndhF* unconstrained tree topologies. However, the tests yielded different results when using constraint topologies, including only branches with either $> 50\%$ or $> 70\%$ bootstrap support values. In the constrained analyses, the *LD* topology was significantly different than the ITS topology, but the converse was untrue (Table 2). Similarly, pairwise comparisons of constrained topologies with nodes with $> 70\%$ bootstrap support showed that *ndhF* and individual or combined nuclear-derived tree topologies were not significantly different. However, comparisons of *LD* topology and combined nuclear-derived topologies to *ndhF* were significantly different even if topologies were constrained to include 50% or 70% bootstrap support. Generally speaking, the S-H tests confirmed that at least some of the differing relationships within the trees cannot be rejected or confirmed, especially among members of the DDNLS Clade (Table 2). Results further suggested that the lack of consistency among S-H tests may be related to weakly supported nodes seen in rival trees. Therefore, rather than indicating that the data sets should not be combined, the results apparently showed the sensitivity of the ILD and S-H tests to differences in character distribution between data sets and branch support. Consequently and since several monophyletic groups were consistently resolved independently by individual gene analysis here, we inferred that there were no compelling reasons to keep the data sets discrete and chose to use a single combined three-gene data set in all subsequent phylogenetic analyses.

Combining nDNA and cpDNA data sets revealed some novel relationships leading to a better overall estimate of phylogeny. Results suggested that evidence for unique relationships could be present in each individual data set but that this signal is confounded potentially due to rates of sequence divergence, weak character support, or high levels of homoplastic characters (Mason-Gamer and Kellogg 1996; Sullivan 1996; DeSalle and Brower 1997; Siddall 1997; Wendel and Doyle 1998; Smith 2000; Gontcharov et al. 2004; Stefanovicì and Olmstead 2004; Doust et al. 2007).

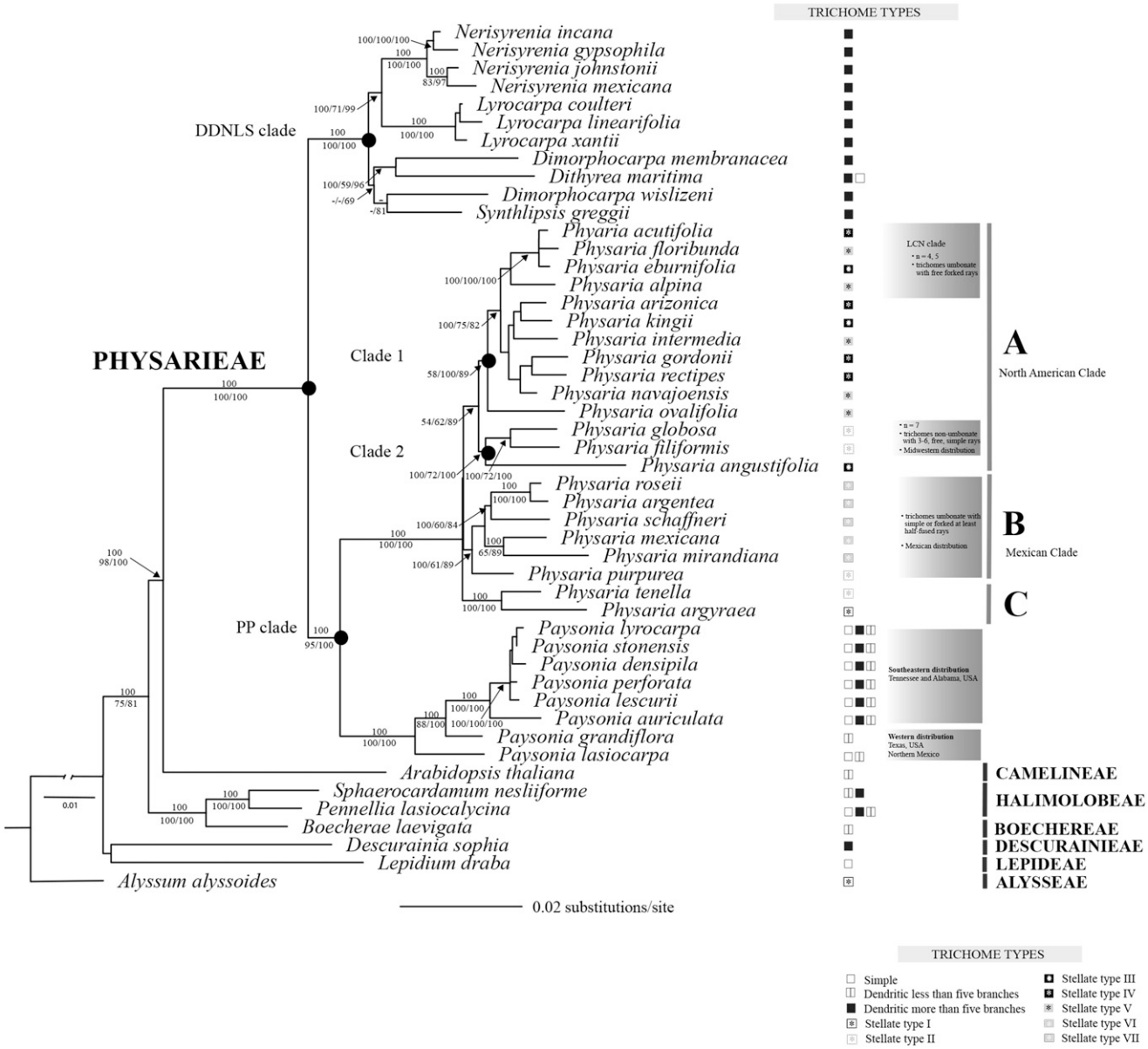

FIG. 5. Maximum likelihood (ML) phylogram of the combined analyses of nDNA (ITS and *LD*) and *ndhF* of Physarieae and outgroup taxa. Along the branches a dash indicates support values below < 50%. Maximum parsimony bootstrap values are placed above branches or reported first in a series of numbers separated by forward slashes; ML bootstrap values are below branches or listed after MP support values; Bayesian posterior probabilities are listed after ML support values. *Physaria* Clades A, B, and C are indicated by vertical bars, and clades sharing a combination of characters are denoted by shaded boxes. Trichome types defined by branching morphology are mapped onto the tree.

Test of Taxonomic and Evolutionary Hypotheses—In the combined analysis of nDNA and cpDNA, the data did not justify rejection of hypotheses previously presented in Fuentes-Soriano and Al-Shehbaz (2013) proposing that *Dimorphocarpa* is sister to *Dithyrea*, that there is a close alliance between *Synthlipsis* and *Nerisyrenia* and *Lyrocarpa*, and as suggested here that there is a close sister relationship of *Synthlipsis* to all of the other members of the DDNLS Clade. These findings must be treated with caution due to the effect of low phylogenetic support in the performance of the S-H test (Table 2). Trends of trichome evolution proposed for Physarieae in the prior study were similarly not rejected given the new *ndhF* data. Based on combined evidence and implementing trichome characters available in Fuentes-Soriano and Al-Shehbaz (2013), it appears that densely branched dendritic trichomes (single cells branching alternately from a main stalk) are ancestral for the tribe, stellate trichomes are derived in *Physaria*, and the reduction in the number of branches from a highly dendritic state to a moderately branched and forked condition are secondarily derived in *Paysonia.*

DISCUSSION

Comparative Utility of ITS and LD Sequence Data in Physarieae—Molecular analyses here permitted an exploration into the relative utility of ITS sequences for reconstructing phylogenetic relationships of Physarieae, while yielding an independent source of phylogenetic signal from the nuclear genome by simultaneously analyzing the single-copy

LD nuclear gene. This is relevant because some of the most prevalent confounding signals of ITS leading to erroneous phylogenetic inferences are a result of excessive sequence diversity, incomplete ribosomal lineage sorting, and misidentifications of orthologous and paralogous copies (Alvarez and Wendel 2003; Gontcharov et al. 2004; Stefanovicì and Olmstead 2004).

The parsimony statistical comparisons between ITS and *LD* data sets indicated that ITS exhibited almost twice as much variation, as reflected by the overall pairwise differences seen in the ITS and *LD* datasets, 8.64% compared to 4.87%, respectively, and in the ratio between potentially phylogenetic informative characters and variable sites (Table 2). Consistency Index (CI) values suggested that ITS sequences have more homoplastic sites than *LD* sequences (ITS: 0.49 vs. *LD*: 0.63), however homoplasy levels may be inherently biased by rates of gene sequence divergence (Sanderson and Donoghue 1989; Hauser and Boyajian 2005), or high A + T or G + C contents (Muse 2000; Cronn et al. 2002). Since the two nuclear markers did not exhibit a remarkably different degree of AT-richness (ITS: 56.93% vs. *LD*: 64.36%) while showing very similar retention indexes (RI) in both the ITS (0.88) and *LD* (0.89) trees, it is likely that the observed CI values were affected by differences in gene sequence divergence (Table 1). All together, data here indicate that despite ITS changes (noise or homoplasy), the gene only had a weaker phylogenetic signal than *LD*.

Phylogenetic Relationships—The combined analysis of chloroplast and nuclear markers employed in this study along with a more complete taxon sampling provided additional evidence for the monophyly of Physarieae (Couvreur et al. 2010; Huang et al. 2015; Nikolov et al. 2019) and confirmed the presence of two major lineages, i.e. the DDNLS and PP clades identified in previous studies (Fuentes-Soriano and Al-Shehbaz 2013; Mazie and Baum 2016). Analyses indicated that apparent discrepancies among nuclear and chloroplast DNA, as shown by the differences in placement of genera in the DDNLS Clade and in the species of *Physaria* and *Paysonia*, may be spurious, and hence favored our decision to combine the chloroplast and nuclear data sets together.

The sister relationship of *Nerisyrenia* and *Lyrocarpa* recovered in the combined analyses of nDNA and cpDNA data indicate that the disparate historical placements of these two genera in tribes Thlaspideae and Sisymbrieae by Bentham and Hooker (1862), in Schizopetaleae and Hesperideae by Prantl (1891), and in Schizopetaleae and Arabideae by Hayek (1911), respectively, were erroneous because of the reliance on single distinguishing morphological characters (e.g. seed anatomy, fruit shape), which are prone to parallel or convergent evolution within Brassicaceae (Al-Shehbaz et al. 2006; Franzke et al. 2011). Morphologically, *Nerisyrenia* and *Lyrocarpa* share a perennial life cycle, highly dendritic branched trichomes, strongly 2-lobed stigmas, and possess the longest fruits found in the tribe (Fuentes-Soriano 2010).

Relationships uncovered using the combined molecular dataset supported the monophyletic and almost entirely gypsum-restricted, endemic genus, *Nerisyrenia*, as well as the two evolutionary lines within it as previously defined on the basis of morphology, chemistry, geographic distribution, and geological history (Bacon 1975, 1978). However, earlier inferences of the ancestral basic chromosome number of the genus remain ambiguous. Bacon (1975), based on 150 chromosome counts for 11 of the 12 species of *Nerisyrenia*, hypothesized that the basic chromosome number was $x = 10$. Overlapping chromosome number information in this section of the tree showed that within each of the main *Nerisyrenia* lineages there were two different basic chromosome numbers, *Nerisyrenia incana* (Lineage 1, sensu Bacon 1975) and *N. johnstonii* (Lineage 2, sensu Bacon 1975) with $x = 10$, and *N. gypsophila* (Lineage 1) and *N. mexicana* (Lineage 2) with $x = 9$. Moreover, problems with sequence resolution of *Nerisyrenia incana* and *N. johnstonii*, especially when compared with ITS data (Figs. 2, S1), is likely due to hybridization or events of incomplete lineage sorting at least among populations of sympatric species. Personal observations in the field indicate that this is especially true in hybrid swarms of *N. castillonii* Rollins and *N. incana*, and *N. camporum* (A.Gray) Greene and *N. linearifolia*. Expanding sampling to include species and cytotypes of highly polyploid species (ca. 6–8 total species) and also species complexes with clearly overlapping morphological and phytochemical patterns of variation (Bacon 1975; Fuentes-Soriano 1994) to address significant evolutionary phenomena such as polyploidy, hybridization, trait evolution, and edaphic endemism.

The nuclear molecular data lacked conclusive support for the segregation of *Dimorphocarpa* from *Dithyrea* as suggested by Rollins (1979). In ITS, ITS + *LD,* and *ndhF* analyses, *Dimorphocarpa membranacea*, the narrowly endemic Mexican species of the genus, appears to be more closely related to *Dithyrea* (Sonoran Desert), whereas the widespread *Dimorphocarpa wislizeni* (Southwestern USA) is phylogenetically unstable. It is possible that this lack of resolution reflects significant evolutionary processes such as lineage sorting, introgression, or perhaps is due to insufficient variation in the studied markers. Interestingly, several morphological, cytogenetic, ecological, and geographic differences can be found within these closely related taxa. *Dithyrea* and *Dimorphocarpa* share indehiscent, didymous, two-seeded fruits that are strongly compressed perpendicularly to the septum, but the two differ substantially in floral features, pollen, and extrafloral nectaries. Pollen size and ornamentation in *Dithyrea* is unusual in the whole Physarieae, with the grains being 4-aperturate, coarsely reticulate, and baculate in the lumen. Chromosomal evidence also suggests the distinctiveness of *Dithyrea* and *Dimorphocarpa*. Rollins (1979) proposed a basic chromosome number of $x = 9$ for the monobasic *Dimorphocarpa*, while reporting $x = 10$ for the $2n = 20$, $2n = 6x = 60$, and $2n = 8x = 80$ in *Dithyrea*. Moreover, *Dithyrea* and *Dimorphocarpa* are isolated from each other by ecological differences (e.g. climate variation, soil types) and geological barriers. *Dithyrea* grows on the northwest side of the Sierra Madre Occidental (Sonoran Desert), whereas *Dimorphocarpa* ranges from the eastern side of the Sierra Madre into the eastern desert areas of North America.

Combined analyses of nDNA and cpDNA provide support for the monophyly of *Physaria* and the grouping of the North American species of *Physaria* into Clades A, B, and C, which are each further supported by distinct morphological and geographic distribution differences. The North American *Physaria* Clade A, including ca. 64% of the species representing *Physaria*, consistently appeared in all data sets, including the four species forming the well-supported LCN Clade and the sister group, formed by the narrowly distributed Mideastern USA species *Physaria filiformis-Ph. globosa*. Members of the LCN Clade, named for their low chromosome numbers, share a perennial life cycle, globose didymous capsules with 2–4

ovules per locule, and non-umbonate stellate trichomes with free forked rays. No other member of Clade A shows this unique suite of characters. The LCN species are mainly distributed in temperate habitats of the USA, i.e. Colorado, Montana, northern New Mexico, Utah, and Wyoming, and typically occur at high elevations ranging from 1500–3500 m. The corresponding diversification of *Physaria* s. s. (ca. 22 species, excluding *Lesquerella* taxa) in montane and higher elevation regions have a vast and complex genomic history, reflected by polyploidy (Rollins 1993 and references therein), neopolyploidization events, and variable genome sizes (Fuentes-Soriano 2010; Hohmann et al. 2015). Perhaps the genomic events may have conferred higher adaptability and increased tolerance to these environments, thus facilitating species radiation (Jordon-Thaden and Koch 2008; Sklenár et al. 2011; Karl and Koch 2013). Further molecular data and more complete sampling of species of *Physaria* s. s. with $n = 4, 5$ (ca. 8 out of 22 species) are needed to test the extent of secondary diploidization events after ancient whole genome duplications and the evolutionary implications suggested for Physarieae (albeit with poor sampling) and other members within the major Brassicaceae Lineage 1 (Hohmann et al. 2015; Mandáková et al. 2017a, 2017b). The sister relationship of *Physaria filiformis* and *Ph. globosa* inferred in Clade A is supported by a shared chromosome number of $n = 7$, an annual life cycle (Rollins 1993), and by a reduction in the number of rays (3 to 6 vs. 6 or more) in the stellate trichomes (Fuentes-Soriano and Al-Shehbaz 2013). This species pair has a very narrow distribution in cedar glades and limestone outcrops in open woods of Midwestern USA states (Missouri, Kentucky, Arkansas, and Tennessee).

Results of the combined analyses of nDNA + cpDNA support the alliance of the endemic, Mexican species of *Physaria* (Clade B), which are characterized by their umbonate trichomes with simple or forked rays that are fused at the base for at least half of their total length (Fuentes-Soriano and Al-Shehbaz 2013). Although most of the members in this clade are found in the Chihuahuan Desert, the sister species *Physaria rosei* and *Ph. argentea* are narrow edaphic endemics occurring in central arid regions of Mexico. These two species share a unique combination of characters: a prostrate perennial life cycle and oblong, compressed (angustiseptate) fruits with short styles (Fuentes-Soriano 2010).

The combined nDNA and cpDNA analyses support Rollins' (1952) hypotheses of *Paysonia* evolution in Western USA and Northwestern Mexico (i.e. *Pa. lasiocarpa* and *Pa. grandiflora*), with a radiation into the Midwestern (Oklahoma, *Pa. auriculata*) and Southeastern USA by the closely-related species *Paysonia auriculata* (Alabama), and *Pa. densipila*, *Pa. stonensis*, and *Pa. perforata* (all three species in Tennessee), where populations are reportedly hybridizing (Fig. 5; Rollins 1952, 1954, 1955, 1957, 1988; Rollins and Shaw 1973; Rollins and Solbrig 1973). Moreover, these data suggest that the hypothetical *Paysonia* ancestor likely had a basic chromosome number of $x = 9$, which was later reduced to $n = 8$ (Rollins 1955; Fuentes-Soriano and Al-Shehbaz 2013). While the lack of phylogenetic resolution among Southeastern species may reflect a complex evolutionary history, neither chromosome counts nor the morphology of the samples studied (Fuentes-Soriano in prep.) showed any signs of hybridization. Studies of the population genetics of these particular species and related taxa are needed to clarify taxonomic limits.

Morphological Implications—Although our results strongly suggest that multiaperturate pollen grains are a synapomophy unifying Physarieae, interesting differences are found in the patterns of macromorphological variation and species diversity between the two main clades of the tribe.

The relatively small DDNLS Clade (21 species in five genera) has historically experienced major changes in many reproductive morphological features such as flowers, fruits, and seeds, while the much larger PP Clade (115 species in two genera) has undergone sizeable modifications to trichome morphology (Fig. 5) but only exhibits subtle variation with respect to fruit structure. The greater diversity found within the DDNLS Clade supports Stebbins' (1952) hypothesis suggesting that aridity, geographic isolation, and edaphic and regional climatic fluctuations precede rapid rates of divergence and concomitant morphological changes.

Although understanding many patterns of morphological evolution within Physarieae remains elusive, some insights are gained regarding the evolution of trichomes in the tribe. The new data presented here further support the hypotheses of Fuentes Soriano and Al-Shehbaz (2013) that the: 1) dendritic trichomes were ancestral, 2) trichome branch reduction to 2 or 3 branches was derived in *Paysonia,* 3) stellate trichomes (single cells with radially arranged branches) evolved in the *Physaria* stem lineage, and 4) branch webbing in stellate trichomes of *Physaria* was derived (Table 2; Fuentes-Soriano and Al-Shehbaz 2013; Mazie and Baum 2016).

Simple trichomes appear to have evolved twice within the tribe independently, i.e. in the DDNLS Clade in *Dithyrea* and again in the *Paysonia* Clade. However, simple trichomes in *Dithyrea* have rounded tips and are not tapered, whereas in *Paysonia* the tips are acute and the trichomes are tapered from the bases to the apices (trichomes of *P. densipila* are exceptionally "bulbous" at the base).

Based on our sampling within *Physaria*, the most common kinds of stellate trichome are those belonging to Types I–III (Fig. 5) as previously described by Fuentes-Soriano (2010) and Fuentes-Soriano and Al-Shehbaz (2013). For reference, the three trichome types are defined as: Type I, stellate, umbonate with simple rays fused for $< 1/2$ of their length; Type II, stellate, umbonate and with forked and free rays; Type III, stellate, lacking an umbo and with simple and forked rays, fused for $< 1/2$ of their length.

Given the large number of constituent species (ca. 108), long tree branches separating *Physaria* from its sister group *Paysonia* and the DDNLS Clade, and the unique trichome diversity, stellate trichomes may possibly represent a key innovation for *Physaria*. Molecular evolutionary studies of the candidate gene for trichome branching patterns *BRANCHLESS TRICHOME* (*BLT*) support this hypothesis by revealing a shared positive selection for certain codons of *BLT* across the *Physaria* Clade (Mazie and Baum 2016). Further, evolutionary developmental studies, research about gene interactions, gene dosages, and DNA-contents (endoreduplication), among other factors known to influence the morphology of trichomes in the closely related *Arabidopsis thaliana*, are needed to shed additional light on this subject (Hülskamp et al. 1994; Larkin et al. 1996; Hauser et al. 2001; Bouyer 2004; review in Beilstein and Szymanski 2004). This study additionally identified a sister relationship of *Nerisyrenia* with *Lyrocarpa* and supports the previously identified relationships between *Paysonia* with *Physaria*. Although the monophyly of

Dithyrea as currently circumscribed is indisputable, the position of *Dimorphocarpa* relative to *Dithyrea*, and *Synthlipsis* relative to the other members of the DDNLS Clade remains uncertain.

The lack of local resolution within the tribe could be interpreted as evidence of slow molecular evolution by the markers used in this study and rapid rates of morphological diversification. The lower number of species in the DDNLS Clade contradicts the presence of rapid evolution; it is possible that the present-day clade only shows evolutionary branch tips of a formerly richer lineage as suggested for other Brassicaceae (Rollins and Shaw 1973). This is plausible since extreme aridity, edaphic, and climatic conditions in which the Physarieae occurs are credited with stimulating relatively rapid evolution (Stebbins 1952; Raven 1964; Axelrod 1967). Future phylogenetic studies including more extensive sampling of *Physaria*, additional population-level studies of specific groups, and developmental data will provide evidence when testing the effect of among-lineage variation in diversification rates and explain morphological disparities among and within extant major groups of Physarieae.

Enough data have accumulated to provide an alternative phylogenetic framework of Physarieae permitting re-evaluation of patterns of morphological evolution within the tribe (Fuentes-Soriano in prep.). Furthermore, this framework can guide sampling when studying local regions of the phylogeny of Physarieae still in need of better phylogenetic resolution.

Acknowledgments

The authors thank P. F. Stevens, J. C. Pires, Z. S. Rogers, I. A. Al-Shehbaz, and three anonymous reviewers for providing valuable suggestions and discussions that greatly helped to improve the manuscript. SFS is very thankful to the senior members of the Kellogg lab for providing valuable training and feedback regarding wet and dry lab analyses. Both authors are grateful to the curators of B, ENCB, F, GA, GH, MEXU, MO, NMC, OSUB, TEX, and US for loaning herbarium material. L. O. Alvarado-Cárdenas, H. Flores-Olvera, G. Gust, C. Hinostrosa, Z. S. Rogers, and R. Torres-Colín assisted SFS with field work. Funding was provided to SFS by the Consejo Nacional de Ciencia y Tecnología (Mexican government), the E. Desmond Lee and Family Endowment, the Missouri Botanical Garden, and the American Society of Plant Taxonomists through the Shirley and Alan Graham Research Grant.

Author Contributions

SFS conducted field work, provided the genetic data, conducted phylogenetic analyses, and was the primary author for all parts of the manuscript. EK assisted in project design, provided the lab facilities, greatly supported many aspects of the research, and reviewed and edited the manuscript.

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Appendix 1. Taxa investigated in the study, with associated voucher information and GenBank accession information. Herbarium acronyms follow Thiers (2020). Voucher information is listed as follows: scientific name, origin or source of sample, primary collector(s) and collection number in italics, herbarium in parentheses, and GenBank accession numbers as follows: *ndhF*, ITS: clone name, accession number, *LD:* clone name, accession number. Underscore symbols indicate that the corresponding gene region was not sampled.

Ingroup: *Dimorphocarpa wislizeni* (Engelm.) Rollins, USA, New Mexico, *Fuentes-Soriano et al. 267* (MO), JQ323046, ITS: Dwis_1_A_a, MT175753, Dwis_1_A_b, MT175754, *LD*: Dwis_1_D_a, MT306018, Dwis_1_D_b, MT306019; New Mexico, *Fuentes-Soriano et al. 304* (MO),_, ITS: Dwis_2_F_a, MT175755, Dwis_2_F_b, MT175756, *LD*: Dwis_2_G_a, MT306020, Dwis_2_G_b, MT306021. *Dimorphocarpa membranacea* (Payson) Rollins, Mexico, Tamaulipas, Mexico, *Palmer 87* (MO), JQ323047, ITS: Dmem_2_I_a, MT175751, Dmem_2_I_b, MT175752, *LD*: Dmem_2_H_a, MT306016, Dmem_2_H_b, MT306017; Tamaulipas, Mexico, *Palmer 87* (US),_, ITS: Dmem_1_H_a, MT175749, Dmem_1_H_b, MT175750, *LD*: Dmem_1_H_a, MT306014, Dmem_1_H_b, MT306015. *Dithyrea californica* Harv., USA, California, *Rollins & Rollins 7836* (GH),_, ITS: Dcal_1_F_a, MT175741, Dcal_1_F_b, MT175742, *LD*: Dcal_2_B_a, MT306022, Dcal_2_B_b, MT306023; Mexico, Baja California, *Shreve 6963* (MO),_, ITS: Dcal_2_A_a, MT175743, Dcal_2_A_b, MT175744,_. *Dithyrea maritima* (Davidson) Davidson, USA, California, *Raven & Thompson 20706* (MO), JQ323048, ITS: Dmar_1_E_a, MT175745, Dmar_1_E_b, MT175746, *LD*: Dmar_1_B_a, MT306024, Dmar_1_B_b, MT306025; California, *Thorne & Tilforth 52437* (MO),_, ITS: Dmar_2_A_c, MT175747, Dmar_2_A_d, MT175748,_. *Lyrocarpa coulteri* Hook. & Harv., Mexico, Baja California, *Fuentes-Soriano et al. 117* (MO), JQ323049, ITS: Lcoul_1_A_a, MT175757, Lcoul_1_A_b, MT175758, *LD*: Lcou_1_D_a, MT306026, Lcou_1_D_b, MT306027; Baja California, *Fuentes-Soriano et al. 148* (MO),_, ITS: Lcoul_2_F_a, MT175759, Lcoul_2_I_a, MT175760, *LD*: Lcou_2_D_a, MT306028, Lcou_2_D_b, MT306029. *Lyrocarpa linearifolia* Rollins, Mexico, Baja California, *Wiggins 17235* (MO), JQ323050, ITS: Llin_1_C_a, MT175761, Llin_1_E_a, MT175762, Llin_1_E_b, MT175763, Llin_1_F_a, MT175764, *LD*: Llin_1_D_a, MT306030, Llin_1_D_b, MT306031; Baja California, *Moran 10387* (NY),_,_, *LD*: Llin_2_G_a, MT306032, Llin_2_G_b, MT306033. *Lyrocarpa xantii* Brandegee, Mexico, Baja California, *Fuentes-Soriano et al. 131* (MO), JQ323051, ITS: Lxan_1_B_a, MT175765, Lxan_1_B_b, MT175766,_; Baja California, *Fuentes-Soriano et al. 132* (MO),_, ITS: Lxan_2_B_a, MT175767, Lxan_2_B_b, MT175768,_. *Nerisyrenia gypsophila* J.D.Bacon, Mexico, Coahuila, *Fuentes-Soriano et al. 171a* (MO), JQ323055, ITS: Ngyp_1_I_a, MT175769, Ngyp_1_I_b, MT175770, *LD*: Ngyp_1_H_a, MT306034, Ngyp_1_H_b, MT306035; Coahuila, *Fuentes-Soriano et al.* 171b (MO),_, ITS: Ngyp_2_I_a, MT175771, Ngyp_2_I_b, MT175772, *LD*: Ngyp_2_H_c, MT306036, Ngyp_2_H_d, MT306037. *Nerisyrenia incana* Rollins, Mexico, Coahuila, *Fuentes-Soriano et al. 99* (MO), JQ323052, ITS: Ninc_1_E_a, MT175773, Ninc_1_E_b, MT175774, *LD*: Ninc_1_D_a, MT306038, Ninc_1_D_b, MT306039; Coahuila, *Fuentes-Soriano et al. 101* (MO),_, ITS: Ninc_2_G_a, MT175775, Ninc_2_G_b, MT175776, *LD*: Ninc_2_H_a, MT306040, Ninc_2_H_b, MT306041.

Nerisyrenia johnstonii J.D.Bacon, Mexico, Coahuila, *Fuentes-Soriano et al. 98a* (MO), JQ323053, ITS: Njoh_1_B_a, MT175777, Njoh_1_B_b, MT175778, *LD*: Njoh_1_B_a, MT306042, Njoh_1_B_b, MT306043; Coahuila, *Fuentes-Soriano et al. 98b* (MO),_, ITS: Njoh_2_G_a, MT175779, Njoh_2_G_b, MT175780, *LD*: Njoh_2_B_c, MT306044, Njoh_2_B_d, MT306045. *Nerisyrenia mexicana* (J.D.Bacon) B.L.Turner, Mexico, Coahuila, *Fuentes-Soriano et al. 96a* (MO), JQ323054, ITS: Nmex_1_B_a, MT175781, Nmex_1_B_b, MT175782, *LD*: Nmex_1_B_b, MT306046; Coahuila, *Fuentes-Soriano et al. 96b* (MO),_, ITS: Nmex_2_G_a, MT175783, Nmex_2_G_b, MT175784, *LD*: Nmex_1_B_c, MT306047, Nmex_1_B_d, MT306048. *Paysonia auriculata* (Engelm. & A.Gray) O'Kane & Al-Shehbaz, USA, Arizona, *Fuentes-Soriano 300b* (MO),_, ITS: Pyaur_1_B_a, MT175785, Pyaur_1_B_b, MT175786,_; Oklahoma, *Fuentes-Soriano 300a* (MO), JQ323056, ITS: Pyaur_2_G_a, MT175787, Pyaur_2_G_b, MT175788, *LD*: Pyaur_2_H_a, MT306049, Pyaur_2_H_b, MT306050. *Paysonia densipila* (Rollins) O'Kane & Al-Shehbaz, USA, Tennessee, *Fuentes-Soriano et al. 271* (MO), JQ323057, ITS: Pyden_1_E_a, MT175789, Pyden_1_E_b, MT175790, *LD*: Pyden_1_D_a, MT306051, Pyden_1_D_b, MT306052; Tennessee, *Rollins & Rollins 9008* (MO),_, ITS: Pyden_2_G_a, MT175791, Pyden_2_G_b, MT175792, Pyden_2_G_c, MT175793, *LD*: Pyden_2_E_a, MT306053, Pyden_2_E_b, MT306054. *Paysonia grandiflora* (Hook.) O'Kane & Al-Shehbaz, USA, Texas, *Nesom & Nesom 6858* (ENCB),_, ITS: Pygra_1_B_a, MT175794, Pygra_1_B_b, MT175795, *LD*: Pygra_1_B_a, MT306055, Pygra_1_B_b MT306056; Texas, *Rollins 5556* (MO), JQ323058, ITS: Pygra_2_C_a, MT175796, Pygra_2_C_b, MT175797, *LD*: Pygra_2_G_b, MT306057, Pygra_2_H_a, MT306058. *Paysonia lasiocarpa* (Hook. ex A.Gray) O'Kane & Al-Shehbaz, Mexico, Coahuila, *Higgins 2712* (ENCB), JQ323059, ITS: Pylas_1_G_a, MT175798, *LD*: Pylas_1_F_a, MT306059, Pylas_1_F_b, MT306060; Tamaulipas, *Gonzalez-Romo 339* (ENCB),_, ITS: Pylas_2_G_a, MT175799, Pylas_2_G_b, MT175800, *LD*: Pylas_2_F_a, MT306061, Pylas_2_F_b, MT306062. *Paysonia lescurii* (A.Gray) O'Kane & Al-Shehbaz, USA, Tennessee, *Fuentes-Soriano & Rogers 308* (MO), JQ323060, ITS: Pyles_1_I_a, MT175801, Pyles_1_I_b, MT175802, *LD*: Pyles_1_G_a, MT306063, Pyles_1_G_b, MT306064; Tennessee, *Rollins & Rollins 55117* (MO),_, ITS: Pyles_2_I_a, MT175803, Pyles_2_I_b, MT175804, *LD*: Pyles_2_G_c, MT306065, Pyles_2_G_d, MT306066. *Paysonia lyrata* (Rollins) O'Kane & Al-Shehbaz, USA, Alabama, *Fuentes-Soriano 301a* (MO) JQ323061, ITS: Pylyr_1_F_a, MT175805, Pylyr_1_F_b, MT175806, *LD*: Pylyr_1_E_a, MT306067, Pylyr_1_E_b, MT306068; Alabama, *Fuentes-Soriano 301b* (MO),_, ITS: Pylyr_2_I_a, MT175807, Pylyr_2_I_b, MT175808, *LD*: Pylyr_2_H_a, MT306069, Pylyr_2_H_b, MT306070. *Paysonia perforata* (Rollins) O'Kane & Al-Shehbaz, USA, Tennessee, *Fuentes-Soriano et al. 242* (MO),_, ITS: Pyper_1_E_a, MT175809, Pyper_1_E_b, MT175810, *LD*: Pyper_1_B_a, MT306071, Pyper_1_B_b, MT306072, Pyper_1_C_a, MT306073, Pyper_1_C_b, MT306074; Tennessee, *Fuentes-Soriano 272a* (MO),_, ITS: Pyper_2_E_a, MT175811, Pyper_2_E_b, MT175812,_; Tennessee, *Rollins and Rollins 9006* (GH),_,_, *LD*: Pyper_2_H_a, MT306075, Pyper_2_H_b, MT306076. *Paysonia stonensis* (Rollins) O'Kane & Al-Shehbaz, USA, Missouri, *Fuentes-Soriano et al. 243* (MO),_, ITS: Pysto_2_I_a, MT175817, Pysto_2_I_b, MT175818, *LD*: Pysto_1_D_a, MT306077, Pysto_1_D_b, MT306078; Missouri, *Fuentes-Soriano et al. 273* (MO), JQ323062, ITS: Pysto_1_C_a, MT175813, Pysto_1_C_b, MT175814, Pysto_1_F_a, MT175815, Pysto_1_F_b, MT175816, *LD*: Pysto_2_D_a, MT306079, Pysto_2_D_b, MT306080. *Physaria acutifolia* Rydb., USA, Arizona, *Fuentes-Soriano 274* (MO), JQ323063, ITS: Pacu_1_G_a, MT175819, Pacu_1_G_b, MT175820, *LD*: Pacu_1_D_a, MT306081, Pacu_1_D_b, MT306082; New Mexico, *Fuentes-Soriano 275* (MO),_, ITS: Pacu_2_G_a, MT175821, Pacu_2_G_b, MT175822, *LD*: Pacu_2_D_c, MT306083, Pacu_2_D_d, MT306084. *Physaria alpina* Rollins, USA, Colorado, *O'Kane 3736* (ISTC), JQ323064, ITS: Palp_1_G_a, MT175823, Palp_1_G_b, MT175824, *LD*: Palp_2_F_a, MT306087, Palp_2_F_b, MT306088; Colorado, *O'Kane 3982* (ISTC),_, ITS: Palp_2_G_a, MT175825, Palp_2_G_b, MT175826, *LD*: Palp_1_D_a, MT306085, Palp_1_D_b, MT306086. *Physaria angustifolia* (Nutt. ex Torr. & A.Gray) O'Kane & Al-Shehbaz, USA, Oklahoma, *Fuentes-Soriano 299* (MO), JQ323065, ITS: Pang_1_F_a, MT175827, Pang_1_F_b, MT175828, *LD*: Pang_1_C_a, MT306089, Pang_1_C_b, MT306090; Oklahoma, *Fuentes-Soriano 303* (MO),_, ITS: Pang_2_G_a, MT175829, Pang_2_G_b, MT175830, *LD*: Pang_2_E_a, MT306091, Pang_2_E_b, MT306092. *Physaria argentea* (S.Schauer) O'Kane & Al-Shehbaz, Mexico, Hidalgo, *Fuentes-Soriano et al. 238a* (MO), JQ323066, ITS: Parg_1_B_a, MT175831, Parg_1_B_b, MT175832, *LD*: Pargn_1_E_a, MT306093, Pargn_1_E_b, MT306094; Hidalgo, *Fuentes-Soriano et al. 238b* (MO),_, ITS: Parg_2_F_a, MT175833, Parg_2_F_b, MT175834, *LD*: Pargn_2_B_a, MT306095, Pargn_2_B_b, MT306096. *Physaria argyraea* (A.Gray) O'Kane & Al-Shehbaz, Mexico, San Luis Potosi, *Fuentes-Soriano et al. 42* (MO), JQ323068, ITS: Pargy_1_G_a, MT175835, Pargy_1_G_b, MT175836, Pargy_2_G_a, MT175837, Pargy_2_G_b, MT175838, *LD*: Pargy_1_D_a, MT306097, Pargy_1_D_b, MT306098, Pargy_1_D_c, MT306099, Pargy_1_D_d, MT306100. *Physaria arizonica* (S.Watson) O'Kane & Al-Shehbaz, USA, Arizona, *Fuentes-Soriano et al. 260* (MO), JQ323067, ITS: Pari_1_E_a, MT175839, Pari_1_E_b, MT175840, *LD*: Pariz_1_D_a, MT306101, Pariz_1_D_b, MT306102; Arizona, *Fuentes-Soriano 277* (MO),_, ITS: Pari_2_E_a, MT175841, Pari_2_E_b, MT175842, *LD*: Pariz_2_E_a, MT306103, Pariz_2_E_b, MT306104. *Physaria bellii* G.A.Mulligan, USA, B & T world seeds, *JC-1* (MO),_, ITS: Pbell_1_D_a, MT175843, Pbell_1_D_b, MT175844, *LD*: Pbell_1_D_a, MT306105, Pbell_1_D_b, MT306106; B & T world seeds, *JC-2* (MO),_, ITS: Pbell_2_F_a, MT175845, Pbell_2_F_b, MT175846, *LD*: Pbell_2_E_a, MT306107, Pbell_2_E_b, MT306108. *Physaria eburniflora* Rollins, USA, B & T world seeds, *969-1JC* (MO), JQ323069, ITS: Pebu_1_D_a, MT175847, Pebu_1_D_b, MT175848, *LD*: Pebu_1_E_a, MT306109, Pebu_1_E_b, MT306110; B & T world seeds, *969-2JC* (MO),_, ITS: Pebu_2_F_a, MT175849, Pebu_2_F_b, MT175850, *LD*: Pebu_2_G_a, MT306111, Pebu_2_G_b, MT306112. *Physaria fendleri* (A.Gray) O'Kane & Al-Shehbaz, Mexico, Coahuila, *Fuentes-Soriano et al. 190f* (MO),_, ITS: Pfen_1_B_a, MT175851, Pfen_1_B_b, MT175852, Pfen_2_E_a, MT175853, *LD*: Pfen_2_E_a, MT306113, Pfen_2_E_b, MT306114. *Physaria filiformis* (Rollins) O'Kane & Al-Shehbaz, USA, Missouri, *Fuentes-Soriano et al. 240a* (MO), JQ323070, ITS: Pfil_1_D_a, MT175855, Pfil_1_D_b, MT175856, *LD*: Pfil_1_E_a, MT306115, Pfil_1_E_b, MT306116; Missouri, *Fuentes-Soriano et al. 240b* (MO),_, ITS: Pfil_2_G_a, MT175857, Pfil_2_G_b, MT175858, *LD*: Pfil_2_B_a, MT306117, Pfil_2_B_b, MT306118. *Physaria floribunda* Rydb., USA, New Mexico, *Beilstein 01-17* (MO), DQ288813, ITS: Pflo_1_G_a, MT175859, Pflo_1_G_b, MT175860, *LD*: Pflo_1_H_a, MT306119; New Mexico, *Rollins et al. 8339*, (MO),_, ITS: Pflo_2_G_a, MT175861, Pflo_2_G_b, MT175862, LD: Pflo_2_G_a, MT306120, Pflo_2_G_b, MT306121, Pflo_2_G_c, MT306122, Pflo_2_G_d, MT306123. *Physaria globosa* (Desv.) O'Kane & Al-Shehbaz, USA, Missouri, *Fuentes-Soriano 241a* (MO), JQ323071, ITS: Pglo_1_D_a, MT175863, Pglo_1_D_b, MT175864, *LD*: Pglo_1_E_a, MT306124, Pglo_1_E_b, MT306125; Missouri, *Fuentes-Soriano 241b* (MO),_, ITS: Pglo_2_F_a, MT175865, Pglo_2_F_b, MT175866, *LD*: Pglo_2_H_a, MT306126, Pglo_2_H_b, MT306127. *Physaria gordonii* (A.Gray) O'Kane & Al-Shehbaz, U. S. A, New Mexico, *Fuentes-Soriano et al. 268* (MO), JQ323072, ITS: Pgor_1_E_a, MT175867 Pgor_1_E_b, MT175868, *LD*: Pgor_1_G_a, MT306128 Pgor_1_G_b, MT306129; Arizona, *Fuentes-Soriano 302* (MO),_, ITS: Pgor_2_G_a, MT175869 Pgor_2_G_b, MT175870, *LD*: Pgor_2_H_a, MT306130 Pgor_2_H_b, MT306131. *Physaria gracilis* (Hook.) O'Kane & Al-Shehbaz, USA, Oklahoma, *Fuentes-Soriano 296* (MO),_, ITS: Pgra_1_F_a, MT175871 Pgra_1_F_b, MT175872, *LD*: Pgra_1_E_a, MT306132 Pgra_1_E_b, MT306133; Oklahoma, *Fuentes-Soriano 298* (MO),_, ITS: Pgra_2_G_a, MT175873 Pgra_2_G_b, MT175874,_. *Physaria intermedia* (S.Watson) O'Kane & Al-Shehbaz, USA, Arizona, *Fuentes-Soriano et al. 265* (MO), JQ323073, ITS: Pint_1_E_a, MT175875, Pint_1_E_b, MT175876, *LD*: Pint_1_D_a, MT306136, Pint_1_D_b, MT306137; Arizona, *Fuentes-Soriano 280* (MO),_, ITS: Pint_2_E_a, MT175877, Pint_2_E_b, MT175878, Pint_2_D_a, *LD*: MT306138, Pint_2_D_b, MT306139. *Physaria johnstonii* (Rollins) O'Kane & Al-Shehbaz, Mexico, Coahuila, *Fuentes-Soriano et al. 194a* (MO),_, ITS: Pjoh_1_F_a, MT175879 Pjoh_1_F_b, MT175880, *LD*: Pjohn_1_D_a, MT306140 Pjohn_1_D_b, MT306141; Coahuila, Mexico, *Fuentes-Soriano et al. 194b* (MO),_, ITS: Pjoh_2_F_a, MT175881 Pjoh_2_F_b, MT175882, *LD*: Pjohn_2_D_e, MT306142 Pjohn_2_D_f, MT306143. *Physaria kingii* (S.Watson) O'Kane & Al-Shehbaz, USA, Arizona, *Fuentes-Soriano et al. 264a* (MO), JQ323074, ITS: Pkin_1_F_a, MT175883, Pkin_1_F_b, MT175884, *LD*: Pkin_1_E_a, MT306144 Pkin_1_E_b, MT306145; Arizona, *Fuentes-Soriano et al. 264b* (MO),_, ITS: Pkin_2_G_a, MT175885, Pkin_2_G_b, MT175886, *LD*: Pkin_2_E_a, MT306146, Pkin_2_E_b, MT306147. *Physaria mexicana* (Rollins) O'Kane & Al-Shehbaz, Mexico, Coahuila, *Fuentes-Soriano et al. 294a* (MO), JQ323075, ITS: Pmex_1_E_a, MT175887, Pmex_1_E_b, MT175888, *LD*: Pmex_3_D_a, MT306152; Coahuila, *Fuentes-Soriano et al. 294b* (MO),_, ITS: Pmex_2_E_a, MT175889, Pmex_2_E_b, MT175890,_; Coahuila, *Purpus 1148* (MO),_,_, *LD*: Pmex_1_D_b, MT306148, Pmex_1_D_c, MT306149; San Luis Potosi, Torres-Colín 15811 (MO),_,_, *LD*: Pmex_2_D_d, MT306150, Pmex_2_D_e, MT306151. *Physaria mirandiana* (Rollins) O'Kane & Al-Shehbaz, Mexico, Nuevo León, *Steward 8630* (MEXU), JQ323076, ITS: Pmir_1_G_a, MT175891, Pmir_1_G_b, MT175892, *LD*: Pmir_1_E_a, MT306153, Pmir_1_E_b, MT306154; Nuevo León, *Villareal & Betancourt 8630* (MEXU),_, ITS: Pmir_2_I_a, MT175893, Pmir_2_I_b, MT175894, *LD*: Pmir_2_E_a, MT306155,

Pmir_2_E_b, MT306156. *Physaria navajoensis* (O'Kane) O'Kane & Al-Shehbaz, USA, New Mexico, *O'Kane & Heil 3850a* (ISTC), JQ323077, ITS: Pnav_1_E_a, MT175895, Pnav_1_E_b, MT175896, *LD*: Pnav_1_D_a, MT306157, Pnav_1_D_b, MT306158; New Mexico, *O'Kane & Heil 3850b* (ISTC),_, ITS: Pnav_2_F_a, MT175897, Pnav_2_F_b, MT175898, *LD*: Pnav_2_H_a, MT306159, Pnav_2_H_b, MT306160. *Physaria ovalifolia* (Rydb.) O'Kane & Al-Shehbaz, USA, *Texas*, *Waller 1305* (ENCB), JQ323078, ITS: Pova_1_E_a, MT175899, Pova_1_E_b, MT175900, *LD*: Pova_1_E_a, MT306161, Pova_2_E_a, MT30616; Oklahoma, *Fuentes-Soriano 291* (MO),_, ITS: Pova_2_F_a, MT175901, Pova_2_F_b, MT175902, *LD*: Pova_2_D_a, MT306162, Pova_2_D_b, MT306163. *Physaria pallida* (Torr. & A.Gray) O'Kane & Al-Shehbaz, USA, Texas, *Fuentes-Soriano 297a* (MO),_, ITS: Ppal_1_B_a, MT175903, Ppal_1_B_b, MT175904, *LD*: Ppal_1_E_a, MT306165; Texas, *Fuentes-Soriano 297b* (MO),_, ITS: Ppal_2_I_a, MT175905, Ppal_2_I_b, MT175906,_. *Physaria pueblensis* (Payson) Rollins, Mexico, Puebla, *Fuentes-Soriano et al. 109a* (MO),_, ITS: Ppue_1_F_a, MT175907, Ppue_1_F_b, MT175908, *LD*: Ppue_1_E_a, MT306166, Ppue_1_E_b, MT306167; Puebla, *Fuentes-Soriano et al. 109b* (MO),_, ITS: Ppue_2_F_a, MT175909, Ppue_2_F_b, MT175910, *LD*: Ppue_2_E_c, MT306168, Ppue_2_E_d, MT306169. *Physaria purpurea* (A.Gray) O'Kane & Al-Shehbaz, Mexico, Coahuila, *Fuentes-Soriano et al. 167a* (MO), JQ323079, ITS: Ppur_1_B_a, MT175911, Ppur_1_B_b, MT175912, *LD*: Ppur_2_D_a, MT306172, Ppur_2_D_b, MT306173; Coahuila, Mexico, *Fuentes-Soriano et al. 167b* (MO),_, ITS: Ppur_2_F_a, MT175913, Ppur_2_F_b, MT175914, *LD*: Ppur_1_D_c, MT306170, Ppur_1_D_d, MT306171. *Physaria rectipes* (Wooton & Standl.) O'Kane & Al-Shehbaz, USA, Arizona, *Fuentes-Soriano 292a* (MO),_, ITS: Prec_1_E_a, MT175915, Prec_1_E_b, MT175916, *LD*: Prec_1_D_a, MT306174, Prec_1_E_b, MT306175; Arizona, *Fuentes-Soriano 292b* (MO),_, ITS: Prec_2_F_a, MT175917, Prec_2_F_b, MT175918, *LD*: Prec_2_E_c, MT306176, Prec_2_E_d, MT306177. *Physaria rosei* (Rollins) O'Kane & Al-Shehbaz, Mexico, Puebla, *Fuentes-Soriano et al. 222a* (MO), JQ323080, ITS: Pros_1_E_a, MT175919, Pros_1_E_b, MT175920, *LD*: Pros_1_E_a, MT306178, Pros_1_E_b, MT306179; Puebla, *Fuentes-Soriano et al. 222d* (MO),_, ITS: Pros_2_F_a, MT175921, Pros_2_F_b, MT175922, *LD*: Pros_2_D_a, MT306180, Pros_2_D_b, MT306181. *Physaria schaffneri* (S.Watson) O'Kane & Al-Shehbaz, Mexico, Guanajuato, *Ventura & Lopez 8201* (MEXU), JQ323081, ITS: Psch_1_G_a, MT175923, Psch_1_G_b, MT175924, *LD*: Pscha_1_H_a, MT306182, Pscha_1_H_b, MT306183; Guanajuato, *Ventura & Lopez 8297* (MEXU),_, ITS: Psch_2_G_a, MT175925, Psch_2_G_b, MT175926, *LD*: Pscha_2_H_c, MT306184, Pscha_2_H_d, MT306185. *Physaria tenella* (A.Nelson) O'Kane & Al-Shehbaz, USA, Arizona, *Rolllins & Rollins 7814* (ENCB), JQ323082, ITS: Pten_3_F_a, MT175927, Pten_3_G_a, MT175928, Pten_3_G_b, MT175929,_; New Mexico, *Fuentes-Soriano et al. 266* (MO),_, ITS: Pten_2_F_a, MT175854, *LD*: Pten_1_D_a, MT306186, Pten_1_D_b, MT306187. *Physaria wyndii* (Rollins & E.A.Shaw) O'Kane & Al-Shehbaz, Mexico, Coahuila, *Fuentes-Soriano et al. 193a* (MO),_, ITS: Pwyn_1_G_a, MT175930, Pwyn_1_G_b, MT175931, *LD*: Pwyn_1_D_a, MT306188, Pwyn_1_D_b, MT306189; Coahuila, *Fuentes-Soriano et al. 193b* (MO),_, ITS: Pwyn_2_G_a, MT175932, Pwyn_2_G_b, MT175933, *LD*: Pwyn_2_D_c, MT306190, Pwyn_2_D_d, MT306191. *Synthlipsis greggii* A.Gray, Mexico, Coahuila, *Fuentes-Soriano et al. 105* (MO),_, ITS: Sgre_1_B_a, MT175934, Sgre_1_B_b, MT175935, *LD*: Sgre_1_D_a, MT306192, Sgre_1_D_b, MT306193; Coahuila, *Fuentes-Soriano et al. 162* (MO), JQ323083, ITS: Sgre_2_I_a, MT175936, Sgre_2_I_b, MT175937, *LD*: Sgre_2_G_a, MT306194, Sgre_2_G_b, MT306195.

Outgroup: *Alyssum alyssoides* (L.) L., USA, New Mexico, *Fuentes-Soriano et al. 269* (MO), JQ323084, ITS: Alys_1_E_a, MT175938, Alys_1_E_b, MT175939, Alys_2_I_a, MT175940, Alys_2_I_b, MT175941, *LD*: Alys_1_D_a, MT306196, Alys_1_D_b, MT306197, Alys_1_H_a, MT306198, Alys_1_H_b, MT306199. *Arabidopsis thaliana* (L.) Heynh., USA, Missouri, *Fuentes-Soriano et al. s.n.* (MO), JQ323085, ITS: Atha_1_A_a, MT175942, Atha_1_A_b, MT175943, Atha_1_B_a, MT175944, Atha_1_B_b, MT175945, *LD*: Atha_1_A_a, MT306200. *Capsella bursa-pastoris* (L.) Medik, Sweden, Harnosand, *Holm 14* (OSUB),_,_, DQ343328. *Boechera laevigata* (Muhl. ex. Willd.) Al-Shehbaz, USA, Missouri, *Beilstein 01-06* (MO), DQ288739, ITS: Blae_1_B_a, MT175946, Blae_1_B_b, MT175947, *LD*: Blae_1_D_a, MT306201, Blae_1_D_b, MT306202, Blae_1_D_c, MT306203, Blae_1_D_d, MT306204. *Descurainia sophia* (L.) Webb. ex Prantl, USA, New Mexico, *Beilstein 01-19* (MO), DQ288759, ITS: Desop_1_H_a, MT175948, Desop_1_H_b, MT175949,_. *Exhalimolobos palmeri* (Hemsl.) Al-Shehbaz and C.D.Bailey, Mexico, Puebla, *Fuentes-Soriano et al. 224* (MO), JQ323086,_,_; Puebla, *Fuentes-Soriano et al. 31* (MO),_, ITS: Eber_1_F_a, MT175950, Eber_1_F_b, MT175951; Puebla, *Fuentes-Soriano et al. 219* (MO),_, ITS: Eber_2_E_a, MT175952, Eber_2_E_b, MT175953, *LD*: Eber_1_A_a, MT306205. *Lepidium draba* L., USA, Arizona, *Fuentes-Soriano et al. 249* (MO), JQ323087, ITS: Ldra_1_E_a, MT175954, Ldra_1_E_b, MT175955, *LD*: Ldra_1_D_a, MT306206, Ldra_1_D_b, MT306207, Ldra_1_D_c, MT306208, Ldra_1_D_d, MT306209. *Neslia paniculata* (L.) Desv., Unknown (B),_,_, DQ343348. *Pennellia lasiocalycina* (O.E.Schulz) Rollins, Mexico, Chihuahua, *Fuentes-Soriano et al. 56* (MO), JQ323088, ITS: Pela_1_A_a, MT175956, Pela_1_B_a, MT175957, LD: Pelas_1_D_a, MT306210, Pelas_1_D_b, MT306211. *Sphaerocardamum nesliiforme* S.Schauer, Hidalgo, Mexico, *Fuentes-Soriano et al. 231a* (MO), JQ323090, ITS: Snes_1_F_a, MT175958, Snes_1_F_b, MT175959, *LD*: Spha_1_H_a, MT306212, Spha_1_H_b, MT306213. *Transberingia bursifolia* (DC.) Al-Shehbaz & O'Kane, unknown; *Price 1385* (GA),_,_, DQ343347.