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Authors

Liao, Irene T
Fulford, Avery H
Ostevik, Kate L
et al.

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Crossability and Genetic Characterization of a North American Representative of *Ipomoea grandifolia* (Convolvulaceae), a Member of *Ipomoea* Series *Batatas*

Irene T. Liao,[1,2,4] Avery H. Fulford,[1] Kate L. Ostevik,[1,3] Mark D. Rausher[1]

[1]Department of Biology, Duke University, Durham, North Carolina 27708, USA; irene.liao@alumni.duke.edu; avery.fulford@duke.edu; kate.ostevik@gmail.com; mrausher@duke.edu
[2]Department of Molecular, Cell, and Developmental Biology, University of California – Los Angeles, Los Angeles, California 90095, USA; ireneliao@ucla.edu
[3]Department of Evolution, Ecology, and Organismal Biology, University of California – Riverside, Riverside, California 92521, USA; kostevik@ucr.edu
[4]Author for correspondence (irene.liao@alumni.duke.edu)

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Abstract—Species in the genus *Ipomoea* are often difficult to identify due to their similar morphologies and their ability to hybridize with one another. An undescribed North American *Ipomoea* morphotype in *Ipomoea* series *Batatas*, referred here as *Ipomoea* Carolina morphotype, was found to be morphologically, genetically, and reproductively isolated from other locally co-occurring *Ipomoea* species. A previous phylogenetic analysis that included a broader sampling of species in *Ipomoea* series *Batatas* suggested that *Ipomoea* Carolina morphotype may be *Ipomoea grandifolia*, a species described as found only in South America. To evaluate these findings, we tested intrinsic cross-compatibility between *Ipomoea* Carolina morphotype and *I. grandifolia* as well as with three other co-localizing North American *Ipomoea* species: *Ipomoea cordatotriloba*, *Ipomoea lacunosa*, and *Ipomoea leucantha*. We also examined genetic differentiation using single nucleotide polymorphisms from leaf transcriptomes from multiple individuals of all five species and several outgroup species. We find no cross-incompatibility and little genetic differentiation between *Ipomoea* Carolina morphotype and *Ipomoea grandifolia*, suggesting that *Ipomoea* Carolina morphotype is a representative of *Ipomoea grandifolia*. This finding raises additional questions about the origins of *Ipomoea grandifolia* in North America and how its disjunct distribution could play a role in the divergence of *Ipomoea grandifolia* in the future.

Keywords—Genetic differentiation, reproductive isolation, species delimitation.

Ipomoea L. is a genus in the family Convolvulaceae composed of 650–900 species distributed worldwide, with species commonly known as "morning glories" or "bindweeds" (Eserman et al. 2020; Wood et al. 2020). Two species have agricultural importance – the sweetpotato *I. batatas* (L.) Lam. and the leafy vegetable *I. aquatica* Forsskål (Austin 2007) – and several species are popular horticultural plants, including *I. purpurea* (L.) Roth and *I. nil* (L.) Roth (Hoshino et al. 2016). Additionally, many species are noxious weeds (Webster and Nichols 2012; Alvarado-Serrano et al. 2019), often found in areas of human disturbance, including roadsides and fields, which affect overall agricultural yield (Takao et al. 2011).

Ipomoea species historically have had taxonomical and nomenclatural problems due to similarities in morphological characteristics (Austin and Huáman 1996; Muñoz-Rodríguez et al. 2019; Eserman et al. 2020; Wood et al. 2020). Widespread species have been frequently misidentified and often have had multiple synonyms or name changes, especially when species identification historically depended on comparisons with regional species rather than with species found more broadly (Muñoz-Rodríguez et al. 2019; Wood et al. 2020). Recent taxonomic efforts have combined phylogenetics, population genetics, and morphology as an approach to delimit species (Muñoz-Rodríguez et al. 2019; Wood et al. 2020). Because these efforts will potentially lead to broad nomenclatural changes, including a possible scientific renaming of the sweetpotato, a proposal has been made to change the type specimen of *Ipomoea* to prevent these nomenclatural changes (Eserman et al. 2020).

Of the clades within the tribe Ipomoeeae (Eserman et al. 2014), researchers have been particularly interested in the Batatas clade (also referred to as *Ipomoea* series *Batatas* (Choisy) D.F.Austin) because the clade includes the economically important sweetpotato (Austin 1978, 1988). Many studies have focused on understanding the origins of the sweetpotato (Austin 1978; Jarret et al. 1992; Yang et al. 2017; Muñoz-Rodríguez et al. 2018; Wu et al. 2018; Gao et al. 2020), and by association its wild relatives, in part to assist in sweetpotato breeding efforts and genetic conservation (Khoury et al. 2015; Guerrero-Zurita et al. 2020). Taxa comprising this clade were historically grouped together based on a few distinguishing morphological characters, including: sepal characteristics (equality of sepal length, presence/absence of margin hairs, and shape of the apex); corollas that have a campanulate or funnel-like shape, and white to pink in color; hirsuteness of ovary; and chartaceous capsule (Austin 1978; McDonald and Austin 1990; Wood et al. 2020). Molecular phylogenies from plastome and bait-capture sequencing have subsequently supported the hypothesis that these taxa form a monophyletic group (Eserman et al. 2014; Muñoz-Rodríguez et al. 2018). Based on the most recent *Ipomoea* monograph, the clade comprises the following taxa: *I. australis* (O'Donell) J.R.I.Wood & P.Muñoz, *I. batatas* (L.) Lam. *I. cordatotriloba* Dennst., *I. cynanchifolia* Meisn., *I. grandifolia* (Dammer) O'Donell, *I. lactifera* J.R.I.Wood & Scotland, *I. lacunosa* L., *I. leucantha* Jacq., *I. littoralis* Blume, *I. ramosissima* (Poir.) Choisy, *I. splendor-sylvae* House, *I. tabascana* McDonald & Austin, *I. tenuissima* Choisy, *I. tiliacea* (Willd.) Choisy, *I. trifida* (Kunth) G.Don., and *I. triloba* L.

Taxa in the *Ipomoea* series *Batatas* clade can be further broken into three to four subgroups based on a crossability study among 11 species (Diaz et al. 1996) and recent molecular phylogenies (Eserman et al. 2014; Muñoz-Rodríguez et al. 2018), with the following consensus groups: Group 1 comprises *I. batatas* and *I. trifida*; Group 2 includes *I. grandifolia*, *I. cynanchifolia*, *I. australis*, *I. tenuissima*, *I. triloba*, *I. lacunosa*, *I. leucantha*, *I. cordatotriloba*; Group 3 includes *I. tiliacea*, *I. ramosissima*, *I. littoralis*, and *I. lactifera*, and Group 4 consists of *I. splendor-sylvae*, the sister species to all other taxa. Although some taxa

within these groups often display morphologies that appear to be intermediate between two taxa, morphological similarities can also be found in taxa between different subgroups (e.g. *I. cynanchifolia* as an intermediate of *I. ramosissima* and *I. grandifolia*). Despite the difficulty in identifying these taxa morphologically, taxa can generally be correctly identified through genetic analysis and/or geographic location (Wood et al. 2020).

The evolutionary relationships among these taxa are complex and species delimitation difficult, particularly for taxa in Group 2. A population genetics analysis demonstrated that taxa from Group 2 are indistinguishable from one another (Muñoz-Rodríguez et al. 2018). Additionally, many taxa are cross-compatible with others in the group, but the strength of crossability ranges from 0.06 to 1.00 (Diaz et al. 1996). This variation in reproductive barrier strength indicates that hybridization may likely occur. For sister species *I. lacunosa* and *I. cordatotriloba*, genomic signatures revealed that *I. cordatotriloba* experienced introgression from *I. lacunosa* in the recent past (Rifkin et al. 2019). This asymmetric introgression has contributed to changes in the strength of reproductive isolation between the two species (Ostevik et al. 2021). While not supported by genetic evidence and extensive investigation into reproductive barriers, several taxa have morphological characters that are intermediate between two taxa and are proposed to be have hybrid origins: *I. grandifolia* is described as an intermediate of *I. australis* and *I. triloba* or even as a larger form of *I. triloba* (Austin 1978; Wood et al. 2020), while *I. leucantha* is proposed to be a stable hybrid of *I. lacunosa* and *I. cordatotriloba* (Austin 1978; Abel and Austin 1981). Given that these closely related taxa may be hybridizing, describing an unknown *Ipomoea* requires combining several lines of evidence that do not solely depend on morphological characteristics (De Queiroz 2007; Carstens et al. 2013).

An unknown morphotype belonging to *Ipomoea* series *Batatas* was identified in the southeastern parts of the United States by Duncan and Rausher (2013). The authors proposed it as a possible new species that they termed *I. "austinii."* However, this morphotype was not formally described, and because the name *Ipomoea austinii* Infante-Bet. describes a species from Colombia (Infante-Betancour 2014), to avoid confusion, this North American *Ipomoea* will be referred to as "*Ipomoea* Carolina morphotype."

Duncan and Rausher (2013) compared *Ipomoea* Carolina morphotype with three other *Ipomoea* species found in North and South Carolina: *I. lacunosa*, *I. cordatotriloba*, and *I. leucantha*. Through floral morphological characterization, genetic analysis using microsatellites, and an artificial crossing study to determine intrinsic reproductive barriers, they found that *Ipomoea* Carolina morphotype was distinct and possibly a new species. This study included many individuals per species but lacked comparisons with other *Ipomoea* species in *Ipomoea* series *Batatas*. A phylogenetic analysis investigating the evolutionary relationships of *Ipomoea* series *Batatas* included two samples of *Ipomoea* Carolina morphotype and other *Ipomoea* series *Batatas* species and revealed that *Ipomoea* Carolina morphotype was nested within a clade that included *I. grandifolia* (Eserman 2017). This result suggests that *I.* Carolina morphotype may not be a novel species, but is rather a North American representative of *I. grandifolia*, a species that has only been described from South America (Khoury et al. 2015; Wood et al. 2020).

Based on these considerations, we embarked on a study to determine whether *Ipomoea* Carolina morphotype is a novel species versus a representative of *I. grandifolia* by assessing species separation in terms of 1) whether there is intrinsic reproductive isolation between *I.* Carolina morphotype and *I. grandifolia* in the form of cross incompatibility; and 2) whether the two taxa are genetically differentiated. To quantify reproductive isolation, we performed crosses within and among five species in *Ipomoea* series *Batatas* subgroup 2, to determine whether *I.* Carolina morphotype and *I. grandifolia* are more cross-compatible with each other than with the other species. To determine genetic similarity, we generated genetic markers by sequencing transcriptomes from individuals of the same five taxa and several additional *Ipomoea* species in *Ipomoea* series *Batatas* as outgroups. Both approaches reveal that *Ipomoea* Carolina morphotype has no cross-incompatibility and little genetic divergence from *I. grandifolia*, suggesting that they represent the same species. We also provide additional support for the possibility of ongoing hybridization among taxa in *Ipomoea* series *Batatas* group 2 as evidenced by varying levels of reproductive isolation among the taxa, even though many are genetically distinguishable from one another.

Materials and Methods

Materials and Planting—We planted seeds of accessions from different populations representing all species used in this study: 10 *I. grandifolia* obtained from the International Potato Center (CIP), five *I.* Carolina morphotype, four *I. leucantha*, 10 *I. lacunosa*, and seven *I. cordatotriloba* from collections made by previous Rausher lab members and the USDA (Fig. 1). For a subset of these (all *I. grandifolia*, *I.* Carolina morphotype, and *I. leucantha*; two *I. cordatotriloba* and three *I. lacunosa*), two seeds from the same accession were planted and used for the crosses. For the remaining (seven *I. lacunosa*, five *I. cordatotriloba*), two seeds from different accessions from the same population were planted (Table S1, Liao et al. 2022). These seeds were planted in September 2018.

Additional samples (three purple-flowered *I. lacunosa*, one *I. cynanchifolia*, one *I. tenuissima*, one *I. splendor-sylvae*, one *I. ramosissima*, three *I. grandifolia*, one *I. trifida*) were planted in 2019 and 2020 for population genetic analyses. As taxa outside of Group 2, *I. splendor-sylvae*, *I. ramosissima*, and *I. trifida* were specifically chosen as outgroups for the analyses. See Appendices 1, 2, and 3, for voucher and sequence information.

Seeds were scarified with a razor blade, planted directly into soil (Farfard 4P) in 4-inch square pots, and were grown in a growth room at under a photoperiod of 12-hr light:12-hr dark at 21–23°C for one month to induce flowering before being transferred into the Duke Research Greenhouses.

Crossing Design and Statistical Analyses—Pairwise crosses were made between all possible combinations of species except *I. lacunosa* and *I. cordatotriloba* as there was a previous in-depth study into the reproductive barriers between these two species (Ostevik et al. 2021). For each pair of species, we performed approximately 100 reciprocal crosses, 50 in each direction. Within each pair of species, we assigned individuals from different populations in species 1 to be paired randomly with individuals from different populations in species 2 such that there were 10 sets of unique population pairs. At least five crosses made for each population pair in each direction (10 reciprocal crosses). We also performed crosses within species and within individuals for each species. Finally, we also tagged flowers that were left to be self-pollinated (no anther removal the day before) or applied self pollen on the day of flowering if there was anther-stigma separation as a control for seed set without any manual removal of anthers the day before flowering.

To perform crosses, we removed the anthers from the female parent on the afternoon before the flower opened using forceps, taking care to not damage the style. We tagged the emasculated flower with lab tape indicating the individual and the date of the cross. The following morning, we removed the entire flower for individuals representing the male parent and applied pollen directly on the stigma and noted the cross on the lab tape tag. These crosses were also noted on "crossing cards" and in a crossing log. Because some extraneous pollen may have inadvertently landed on the stigma, either selfed-pollen grains from anther removal (anthers can begin to dehisce the evening before the flower opens) or contaminating pollen from other individuals, we used a handheld 10 × magnifying lens to confirm the lack of pollen grains on the stigma before

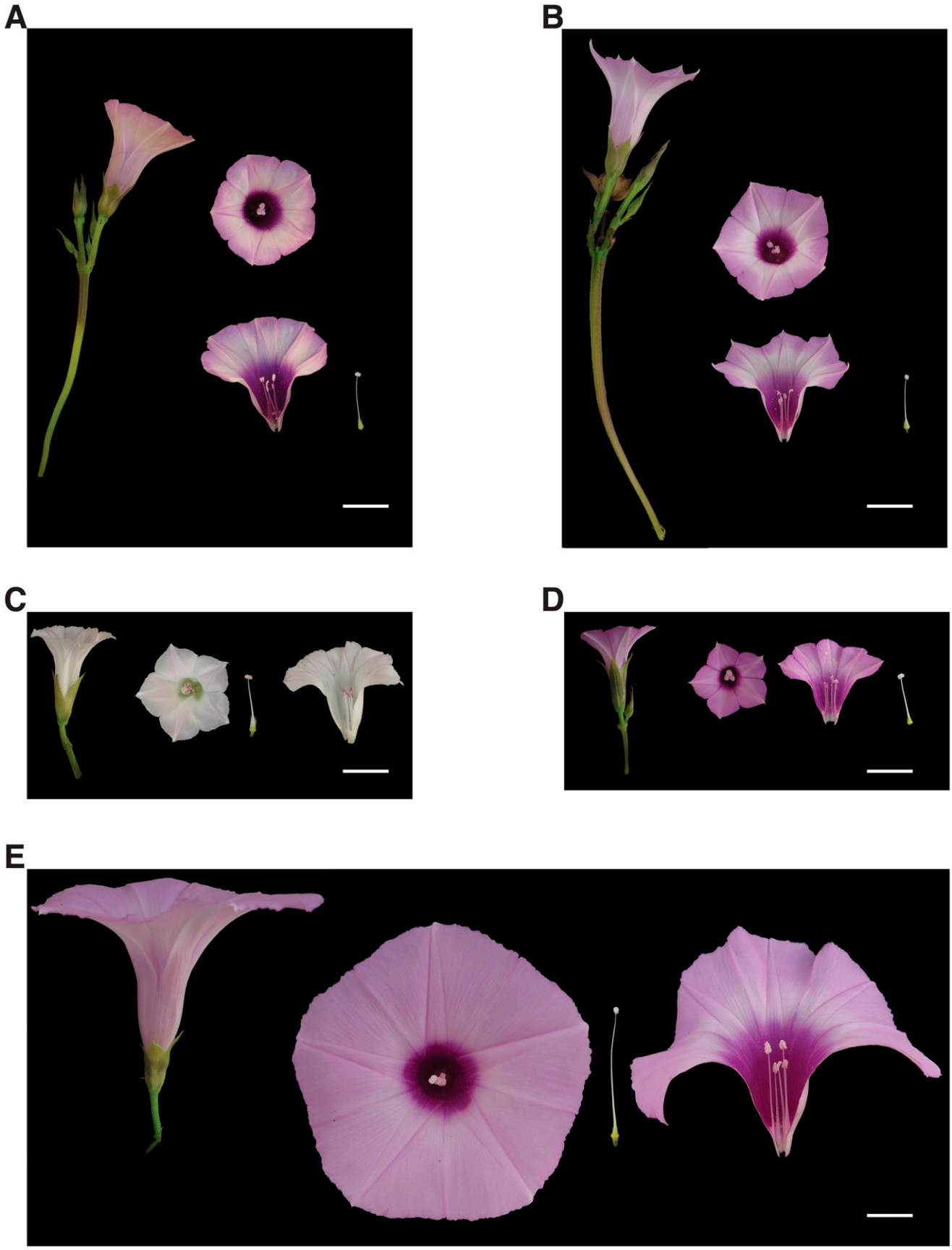

FIG. 1. Images of the five *Ipomoea* species used in the crossing study. Each set of images displays the species in three angles: on the side, top down, and with the flower slit open to see the corolla color banding pattern. A. *Ipomoea* Carolina morphotype. B. *Ipomoea grandifolia*. C. *Ipomoea lacunosa*. D. *Ipomoea leucantha*. E. *Ipomoea cordatotriloba* (from an allopatric population). Scale bar: 1 cm.

hand-pollination. We also left emasculated flowers unpollinated to examine whether there was any pollen contamination, as manifested by fruit or seed development. Of those emasculated, approximately 5% fruited or produced at least one seed (Table S2, Liao et al. 2022).

We collected as many buds and fruits representing unsuccessful fertilizations as possible (we missed a few because they fell off the plant or were lost within the plant) and cross-checked these with the notes on the crossing cards and log. For successful crosses, we counted the number of seeds per fruit (generally, the maximum is four seeds for these species, with some rare occurrences of 5–6 seeds per fruit). We considered crosses that produced at least one seed to have set fruit (1) and those with no seeds to have not set fruit (0). Because fruits could often be hidden and missed in the bushy plants, and unfertilized buds often fall off the plant and are lost, approximately 116 crosses (out of 1531; 7.6%) in the log without a bud or a fruit found were removed from the dataset prior to statistical analyses.

One way to test whether *I.* Carolina morphotype is the same species as *I. grandifolia* is to examine whether there are reproductive barriers that prevent successful seed set between the two species (Diaz et al. 1996; Tiffin et al. 2001; Briscoe Runquist et al. 2014; Melo et al. 2014; Ostevik et al. 2021). If *I.* Carolina morphotype and *I. grandifolia* are the same species, we would expect them to be cross compatible and that average fruit and seed set from crosses between the two taxa would be equal to that for intraspecific crosses. If *I.* Carolina morphotype and *I. grandifolia* are separate species, we would expect either that interspecific compatibility is less than intraspecific compatibility or that interspecific and intraspecific compatibility are equal. In the latter case, other intrinsic and extrinsic reproductive barriers would need to be examined to determine if these are two distinct species.

We examined two additional expectations if *I. grandifolia* and *I.* Carolina morphotype are the same species. First, we expect that *I.* Carolina morphotype × *I. grandifolia* would produce more seeds than both *I.* Carolina morphotype × species B and *I. grandifolia* × species B, where species B is one of the other three species (*I. lacunosa*, *I. leucantha*, *I. cordatotriloba*). We tested this expectation after fitting linear models and using appropriate contrast statements in a one-way analysis of variance (see below). Second, we expect that *I.* Carolina morphotype × species B would produce similar number of seeds as *I. grandifolia* × species B. In other words, we expect *I.* Carolina morphotype and *I. grandifolia* to be interchangeable and that there would be no difference whether *I.* Carolina morphotype or *I. grandifolia* was used as a parent in the cross. We tested the second expectation using a two-way ANOVA with *I.* Carolina morphotype or *I. grandifolia* as a parent as factor 1 and whether species B was used as a female or male parent as factor 2.

We performed statistical analyses using programs and packages in R version 4.0.2 (R Core Team 2020). We first calculated the average number of seeds per fruit for each population pair and used this value to calculate the average and the standard error number of seeds produced per fruit for each species pair. For the analyses for each set of comparisons, we first tested whether the fruit set was different among the comparisons. We then removed the crosses that produced no fruit and asked whether the mean number of seeds per fruit differed among crosses. Finally, to summarize the collective effect of the first two, we used the results of all the crosses (regardless if the cross produced at least 1 seed) and tested whether the mean number of seeds per fruit differed among the crosses.

To test for differences in fruit set, we performed a contingency test and corrected for multiple comparisons using a false discovery rate of 0.05 (Benjamini and Hochberg 1995). To test for differences in the mean number of seeds from crosses that produced a fruit, we fitted a linear model. For mean number of seeds from all crosses, because there were many crosses with zero seeds, much of the data was zero-inflated, so we tested models with and without a zero-inflated term. We used generalized linear mixed models with female parent as a random effect and the cross type (e.g. *I.* Carolina morphotype × *I.* Carolina morphotype; *I.* Carolina morphotype × *I. grandifolia*) as a fixed effect and mean seed number as the dependent variable. We used the glmmTMB function from glmmTMB v. 1.0.2.1 (Brooks et al. 2017) with a tweedie or gaussian distribution. For all models, we used DHARMa v. 0.3.3.0 (Hartig 2020) to assess the fit of the model before using emmeans v. 1.5.3 (Lenth 2020) to calculate the estimated marginal means and 95% confidence intervals and tested whether the differences in reproductive output in each type of comparison were significant.

RNA Extraction, Library Preparation, and Sequencing—To obtain genomic data for analyzing genetic differentiation between *I.* Carolina morphotype and *I. grandifolia* and among other species in *Ipomoea* series *Batatas*, RNA was extracted from young leaf tissue using TRI-Reagent as detailed in Rifkin et al. (2019). This was done for all *I. grandifolia* and *I.* Carolina morphotype accessions and two of the *I. leucantha* accessions used for the crosses. We also included three purple-flowered *I. lacunosa* from our own collections as well as additional accessions from the USDA (three additional *I. grandifolia*, one *I. cynanchifolia*, one *I. tenuissima*, one *I. splendor-sylvae*, one *I. ramosissima*, and one *I. trifida* accessions; Table S1). Total RNA was run on a 0.8% agarose gel to determine that there was no degradation and stored at -80°C before library construction. Libraries were made following the manufacturer's instructions using the KAPA Stranded mRNA-Seq Kit (KAPA Biosystems) with NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs) Barcodes (New England Biolabs). These were sequenced on one Illumina NovaSeq 6000 S2 150bp PE flow cell at the Duke Center for Genomic and Computational Biology Sequencing and Genomic Technologies Core. Raw sequence data are deposited in the NCBI Sequence Read Archive (SRA) [accession: PRJNA735523]. A summary of the sequencing output can be found in Table S3 (Liao et al. 2022).

Sequence Processing—In addition to analyzing genetic differentiation between *I.* Carolina morphotype and *I. grandifolia*, we also tested whether these two taxa are genetically distinct from the three other species in *Ipomoea* series *Batatas* found occurring with *I.* Carolina morphotype: *I. lacunosa*, *I. cordatotriloba,* and *I. leucantha*. We had previously sequenced 31 *I. lacunosa*, 30 *I. cordatotriloba*, and one *I.* Carolina morphotype samples for a different study (NCBI SRA accession: PRJNA769750; Rifkin et al. 2019) and included these sequences in this study. Although we did not have leaf transcriptomes for two *I. leucantha* individuals used in the crossing study (Le_GMT, Le_Good), we had whole genome sequences from another study (NCBI SRA accession PRJNA732922; Liao 2021) and included these sequences. In total, we aligned a total of 90 leaf transcriptomes to the *I. lacunosa* draft genome using STAR v. 2.7.5c (Dobin et al. 2013) and aligned the two *I. leucantha* genome sequences to the *I. lacunosa* draft genome using NextGenMap v0.5.5 (Sedlazeck et al. 2013). All aligned sequences, transcripts or whole genome, were then sorted and cleaned using Picard Tools v. 2.19.2–1-g0d1e881 (http://broadinstitute.github.io/picard/); sequences from multiple lanes were merged together using samtools v. 1.9; finally, merged and non-merged BAM files were validated with ValidateBAM before variant calling. We used mpileup in bcftools v. 1.10.2 to call variants, which were then filtered in the following expressions to extract biallelic SNPs: 'QUAL >= 20 & AVG(FMT/GQ)>10 & INFO/DP>=900 & INFO/DP<=2700 & TYPE="snp" & F_MISSING<0.1'. Using these criteria, a total of 438729 SNPs from 92 samples were called and used for downstream population genetic analyses.

Descriptive Population Genetic Analyses—To determine the genetic relationships among the species in this study, we took three approaches. First, we performed a principal components analysis (PCA) that also accounted for the linkage disequilibrium (LD) among the variants. Following Rifkin et al. (2019), we used a set of programs and functions in R. First, we used the snpgdsLDpruning function from SNPRelate v. 1.22.0 (Zheng et al. 2012) to prune the SNPs with an LD threshold of 0.3, which resulted in 14,113 SNPs. This set of variants was used to run the PCA using snpgdsPCA function as well as a cluster analyses of the identity-by-state pairwise distances. We also calculated the Euclidean distances using the full set of 438,729 SNPs with the bitwise.dist function from poppr v. 2.8.7 (Kamvar et al. 2015) and clustered them with the "average" method using hclust. Finally, we constructed a neighbor-joining tree with phangorn v. 2.5.5 (Schliep 2011) using *I. splendor-sylvae* as the rooted outgroup species and 1000 bootstraps.

We also inferred population structure and ancestry using fastSTRUCTURE (Raj et al. 2014) and ADMIXTURE v. 1.3.0 (Alexander and Lange 2011). First, we further filtered the VCF SNP dataset by removing loci with minor allele frequencies less than 0.05 and then converted the remaining 119,505 SNPs into the three input files (.bed .fam .bim) using PLINK 1.9 (Purcell and Chang 2022; Chang et al. 2015). For both fastSTRUCTURE and ADMIXTURE, we ran 10 runs with different starting seeds using the default settings for each number of genetic groups, K, from 2 to 10. For each of the 10 runs, we ran fastSTRUCTURE with the simple prior and determined the best K (referred to as model complexity) by running the chooseK.py script. Similarly, for each of the 10 runs, we ran ADMIXTURE with cross-validation to also determine the best K for the run. The runs for each K were merged and then visualized using pophelper v. 2.3.1 (Francis 2017). We chose the most common best K from 10 runs from fastSTRUCTURE and ADMIXTURE to represent the overall best K from each analysis.

Results

Data Availability—Scripts and datasets are openly available on GitHub at https://github.com/itliao/Grandifolia and are also available on Dryad (Liao et al. 2022).

No Cross-Incompatibility Between I. Carolina Morphotype and I. grandifolia—If *I.* Carolina morphotype and *I. grandifolia* are the same species, we would expect them to behave similarly in crosses. Specifically, we would expect to see three patterns in interspecific crosses: (1) Crosses between *I.* Carolina morphotype and *I. grandifolia* should be as successful as intraspecific crosses for these species; (2) Crosses between *I.* Carolina morphotype and *I. grandifolia* should be more successful than between either of these species and any of the other species used in this study (e.g. *I.* Carolina morphotype × *I. grandifolia* crosses should be more successful than *I.* Carolina morphotype × species B, where species B is *I. lacunosa*, *I. leucantha*, or *I. cordatotriloba*); and (3) Crosses between *I.* Carolina morphotype and species B and between *I. grandifolia* and species B should be equally successful (i.e. the two species should behave similarly in crosses with other species). In the following sections, we examine these expectations, using both fruit set and mean number of seeds produced per fruit as measures of cross success.

(1) Interspecific crosses between *I.* Carolina morphotype and *I. grandifolia* are significantly more successful than intraspecific crosses. For fruit set, there is no detectable difference between species for intraspecific crosses and no effect of reciprocal interspecific crosses (Table 1). Fruit set for the interspecific crosses (83%) is actually significantly higher than that for intraspecific crosses (64% and 58% for *I.* Carolina morphotype and *I. grandifolia*, respectively; Table 1; Fig. 2A). The same pattern is also reflected in the average seeds per fruit: for both the analyses with and without crosses with 0 seeds removed, there was no detectable difference between species or between reciprocal interspecific crosses (Table 1). The interspecific crosses set more seeds per fruit than either intraspecific cross (Fig. 2B, D; Tables 1, 2; Table S4, Liao et al. 2022). This result is explained mostly by lower fruit set in intraspecific crosses because there was no detectable difference in the mean number of seeds per fruit when the crosses that set zero seeds were removed (Fig. 2C; Table 1). It thus appears that there is no detectable cross-incompatibility between the two species.

(2) Crosses between *I.* Carolina morphotype and *I. grandifolia* have the highest success of any interspecific crosses involving these two species. For this comparison, we compared the pooled reciprocal crosses between *I.* Carolina morphotype and *I. grandifolia* with individual interspecific crosses and pooled reciprocal crosses for a given pair of species, depending on whether the reciprocal crosses were different for each cross. This pooling is justified for *I.* Carolina morphotype and *I. grandifolia* because as mentioned above, reciprocal crosses were not significantly different (Table 1). However, this symmetrical result does not apply for every interspecific cross. For instance, *I.* Carolina morphotype × *I. cordatotriloba* reciprocal crosses were significantly different for fruit set, but not for the mean number of seeds per fruit (Table 3A). On the other hand, *I.* Carolina morphotype × *I. lacunosa* reciprocal crosses were significantly different when considering mean number of seeds for crosses with at least one seed per fruit, but not so for fruit set and overall mean number of seeds for all crosses.

Fruit set was significantly higher for the *I.* Carolina morphotype × *I. grandifolia* crosses than for the other interspecific crosses involving these two species (Table 3B, C; Fig. S1, Table S5, Liao et al. 2022). This result holds regardless of whether or not we pooled the reciprocal crosses involving *I. cordatotriloba*.

With all crosses, including those that produced no seeds, mean seed set was significantly greater for the *I.* Carolina morphotype × *I. grandifolia* crosses than for all other crosses involving these species whether reciprocal crosses are pooled or not, with one exception: *I.* Carolina morphotype× *I. grandifolia vs. I. grandifolia* (female) × *I. lacunosa* (male) (Figs. 3A, B; Fig. S2, Liao et al. 2022; Table 3B, C). Because these patterns could be explained by reduced fruit set in the "other" crosses, we also compared seed production using only fruits that produced at least one seed (crosses with 0 seeds removed). In this case, the pattern was more complex. In no case was there less seed set for *I.* Carolina morphotype × *I. grandifolia* than for another cross. When reciprocal crosses are pooled, mean seed number for *I.* Carolina morphotype × *I. grandifolia* was significantly higher than for all the "other" crosses except *I.* Carolina morphotype × *I. lacunosa* (Fig. 3A; Table 3C). When reciprocal crosses are not pooled, at least one cross involving each of the "other" species has significantly lower mean seed number than *I.* Carolina morphotype × *I. grandifolia* (Fig. 3A; Table 3B). Together, these results indicate that both fruit set and seed number independent of fruit set are higher for *I.* Carolina morphotype × *I. grandifolia* than for crosses involving one of these species and one of the other species.

(3) Crosses between *I.* Carolina morphotype and species B and between *I. grandifolia* and species B are equally successful. Fruit set was generally not significantly differently when species B was used as a male or female parent in crosses with *I.* Carolina morphotype or *I. grandifolia*, except in crosses with *I. cordatotriloba* (Table 4). The mean number of seeds, whether 0 seeds were removed or not, is generally significantly different when comparing between crosses with species B as a female parent and crosses with species B as a male parent, regardless of whether *I.* Carolina morphotype or *I. grandifolia* was used in the cross (Fig. 4; Table 4). This pattern indicates that the direction of the cross is important for comparing

TABLE 1. Comparison of fruit set and mean number of seeds for *I.* Carolina morphotype and *I. grandifolia* intra- and interspecific crosses. Asterisks indicate significance at $P < 0.05$ after a FDR adjustment for multiple comparisons. M = *I.* Carolina morphotype; G = *I. grandifolia*. First row: comparison of species difference for intraspecific crosses. Second row: comparison of reciprocal interspecific reciprocal crosses. Third row: comparison of intraspecific vs. interspecific crosses. t.ratio is the test statistic used to calculate the *p* value. Corresponds to Fig. 2.

			Mean number of seeds per fruit					
	Fruit set		Crosses with 0 seeds removed			All crosses		
Cross	G	p.value	df	t.ratio	p.value	df	t.ratio	p.value
M×M vs. G×G	0.460	0.498	35	0	0.873	30	0.195	0.847
M×G vs. G×M	0.150	0.699	35	−0.891	0.379	30	−0.299	0.767
M×M + G×G vs. M×G + G×M	13.293	0.000266*	35	0.057	0.955	30	−3.273	0.003*

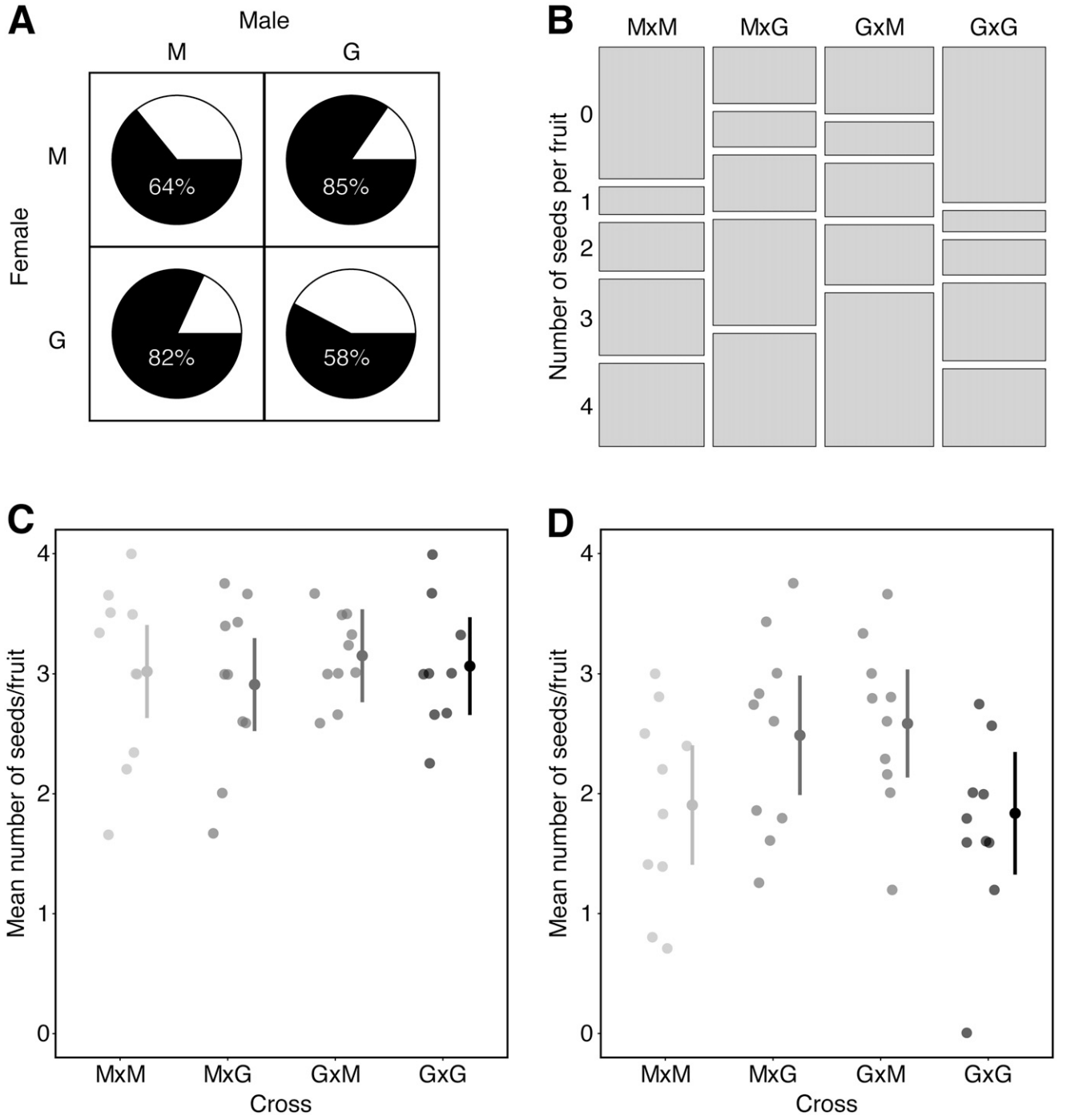

FIG. 2. Fruit set and seed set from *Ipomoea* Carolina morphotype and *I. grandifolia* intraspecific and interspecific crosses. A. Pie chart of the percent of fruit set (e.g. crosses that resulted in at least one seed per fruit). Black indicates successful fruit set and white represents failed fruit set. B. Matrix of the number of seeds per fruit produced from each type of cross. C–D. Plots of the mean number of seeds produced per fruit for each type of cross. C. Only crosses producing at least 1 seed. D. All crosses. Each point represents the mean number of seeds produced by approximately five crosses made between the same individuals, and the estimated marginal means and 95% confidence intervals from a linear mixed model are displayed. M = *I.* Carolina morphotype and G = *I. grandifolia.* Light gray represents M×M crosses, dark gray represents M×G and G×M interspecific crosses, and black represents G×G crosses. Corresponds to Table 1.

whether crosses with *I.* Carolina morphotype and *I. grandifolia* result in similar fruit and seed set. Fruit set was not significantly different when *I.* Carolina morphotype and *I. grandifolia* were crossed to the same third species in the same cross direction (Table 4). A similar pattern is shown by the mean number of seeds per fruit regardless of whether crosses with zero seeds were removed (Fig. 4A–C; Table 4) or not (Fig. 4D–F; Table 4). It thus seems that *I.* Carolina morphotype and *I. grandifolia* behave similarly in crosses with other species.

Lack of Genetic Differentiation of I. Carolina Morphotype and I. grandifolia—Analysis of our genetic data indicate that *Ipomoea* Carolina morphotype and *I. grandifolia* are not genetically distinct. Although the optimal number of groups (K)

TABLE 2. Weighted average seed set per fruit for crosses from unique population pairs. The average seed set per fruit for each unique population pair was first calculated before averaging across the pairs to determine the average seed set per fruit for each species cross. The number of unique population pairs and the standard error values are found within the parenthesis for each species pair. The species on the left represents the female parent, and the species on the top represents the male parent. No crosses were performed within and between *I. lacunosa* and *I. cordatotriloba* because these crosses were done in a previous study (notated with two asterisks, Ostevik et al. 2021). Results from all cross combinations between *I.* Carolina morphotype and *I. grandifolia* are shaded in gray. M = *I.* Carolina morphotype; C = *I. cordatotriloba*; G = *I. grandifolia*; La = *I. lacunosa*; Le = *I. leucantha*; S = within individual cross with anther removal (a control); X = within individual self (without anther removal, for most cases, just self-pollination without manipulation unless there was some distance between the anther and the stigma).

		Male						
		M	G	C	La	Le	S	X
Female	M	1.905 (10, 0.255)	2.487 (10, 0.261)	0.65 (11, 0.180)	1.88 (10, 0.265)	1.61 (10, 0.284)	1.89 (5, 0.5)	3.514 (5, 0.154)
	G	2.585 (10, 0.224)	1.712 (10, 0.241)	0.33 (10, 0.116)	1.88 (10, 0.339)	1.528 (10, 0.222)	2.297 (10, 0.232)	3.5 (10, 0.179)
	C	0.236 (11, 0.120)	0.217 (12, 0.183)	1.476 (80, 0.116)**	0.072 (79, 0.028)**	0.0833 (12, 0.083)	2.212 (5, 0.519)	2.403 (7, 0.365)
	La	1.267 (9, 0.334)	0.85 (10, 0.176)	0.065 (79, 0.016)**	1.913 (80, 0.092)**	2.302 (10, 0.291)	2.581 (10, 0.258)	3.082 (10, 0.185)
	Le	1.177 (10, 0.176)	0.718 (10, 0.148)	0.157 (10, 0.068)	2.095 (10, 0.348)	0.787 (10, 0.16)	0.875 (4, 0.217)	3.301 (4, 0.497)

from fastSTRUCTURE and ADMIXTURE differ (fastSTRUCTURE K = 6 or 7, both from 4/10 runs; ADMIXTURE K = 5 from 7/10 runs; see Table S7 (Liao et al. 2022) for the output inferring the optimal number of groups for both analyses), *I.* Carolina morphotype and *I. grandifolia* are placed in the same group for both analyses (Fig. 5A, primarily yellow, left side of the plots). The fastSTRUCTURE results suggest a low amount of admixture with *I. leucantha*, but the magnitude is the

TABLE 3. Comparison of fruit set and mean number of seeds for *I.* Carolina morphotype and *I. grandifolia* interspecific crosses with other species. Cross within parentheses (e.g. (M × G)) indicates data pooled across reciprocal crosses. Cross not within parentheses (e.g. M × C) indicates the first species was the female parent and the second species was the male parent. Asterisks indicate significant at overall $p < 0.05$ after a FDR adjustment for multiple comparisons. A. Comparison of reciprocal interspecific crosses. B. Comparison of *I.* Carolina morphotype × *I. grandifolia* (M × G) with interspecific crosses involving either *I.* Carolina morphotype or *I. grandifolia* and one of the other three species. C. Comparison of *I.* Carolina morphotype × *I. grandifolia* (M × G) with pooled interspecific crosses involving either *I.* Carolina morphotype or *I. grandifolia* and one of the other three species. M = *I.* Carolina morphotype; C = *I. cordatotriloba*; G = *I. grandifolia*; La = *I. lacunosa*; Le = *I. leucantha*. Corresponds to Fig. 3.

A.

	Fruit set		Mean number of seeds per fruit: Crosses with 0 seeds removed			All crosses		
Cross	G	p.value	df	t.ratio	p.value	df	t.ratio	p.value
M × La vs. La × M	1.5351	0.2154	103	2	0.016*	111	1.391	0.167
G × La vs. La × G	2.337	0.1263	103	3.913	0.0002*	111	4.250	<.0001*
M × Le vs. Le × M	0.0353	0.851	103	2.134	0.035	111	0.811	0.419
G × Le vs. Le × G	0.9462	0.3307	103	4.257	<.0001*	111	2.677	0.009*
M × C vs. C × M	8.483	0.003585*	103	−1.550	0.124	111	0.822	0.413
G × C vs. C × G	2.8869	0.0893	103	−1.988	0.049	111	−2.760	0.007*

B.

	Fruit set		Mean number of seeds per fruit: Crosses with 0 seeds removed			All crosses		
Cross	G	p.value	df	t.ratio	p.value	df	t.ratio	p.value
(M × G) vs. M × La	4.2106	0.04017*	103	−0.1	0.9214	111	2.167	0.032*
(M × G) vs. La × M	12.169	0.000486*	103	2.695	0.0082*	111	3.402	0.001*
(M × G) vs. G × La	8.9741	0.002738*	103	−0.17	0.8646	111	1.420	0.159
(M × G) vs. La × G	23.437	1.29E-06*	103	4.465	<.0001*	111	5.848	<.0001*
(M × G) vs. M × Le	14.499	0.0001402*	103	0.544	0.5874	111	3.070	0.003*
(M × G) vs. Le × M	13.24	0.0002741*	103	2.968	0.0037*	111	3.492	0.001*
(M × G) vs. G × Le	15.985	6.38E-05*	103	0.238	0.8123	111	3.219	0.002*
(M × G) vs. Le × G	25.975	3.46E-07*	103	5.103	<.0001*	111	5.308	<.0001*
(M × G) vs. M × C	36.908	1.24E-09*	103	4.077	0.0001*	111	5.430	<.0001*
(M × G) vs. C × M	80.401	2.20E-16*	103	1.381	0.1702	111	4.249	<.0001*
(M × G) vs. G × C	54.539	1.52E-13*	103	5.991	<.0001*	111	6.060	<.0001*
(M × G) vs. C × G	82.024	2.20E-16*	103	1.26	0.2105	111	2.136	0.035*

C.

	Fruit set		Mean number of seeds per fruit: Crosses with 0 seeds removed			All crosses		
Cross	G	p.value	df	t.ratio	p.value	df	t.ratio	p.value
(M × G) vs. (M × La)	11.49	0.0006999*	103	1.670	0.10	111	3.811	2.00E-04*
(M × G) vs. (G × La)	22.919	1.69E-06*	103	2.590	0.011*	111	5.068	<.0001*
(M × G) vs. (M × Le)	20.475	6.04E-06*	103	2.187	0.031*	111	4.449	<.0001*
(M × G) vs. (G × Le)	30.093	4.12E-08*	103	3.338	0.001*	111	5.781	<.0001*
(M × G) vs. (M × C)	82.496	2.20E-16*	103	3.247	0.002*	111	6.333	<.0001*
(M × G) vs. (G × C)	100.96	2.20E-16*	103	3.788	3.00E-04*	111	5.640	<.0001*

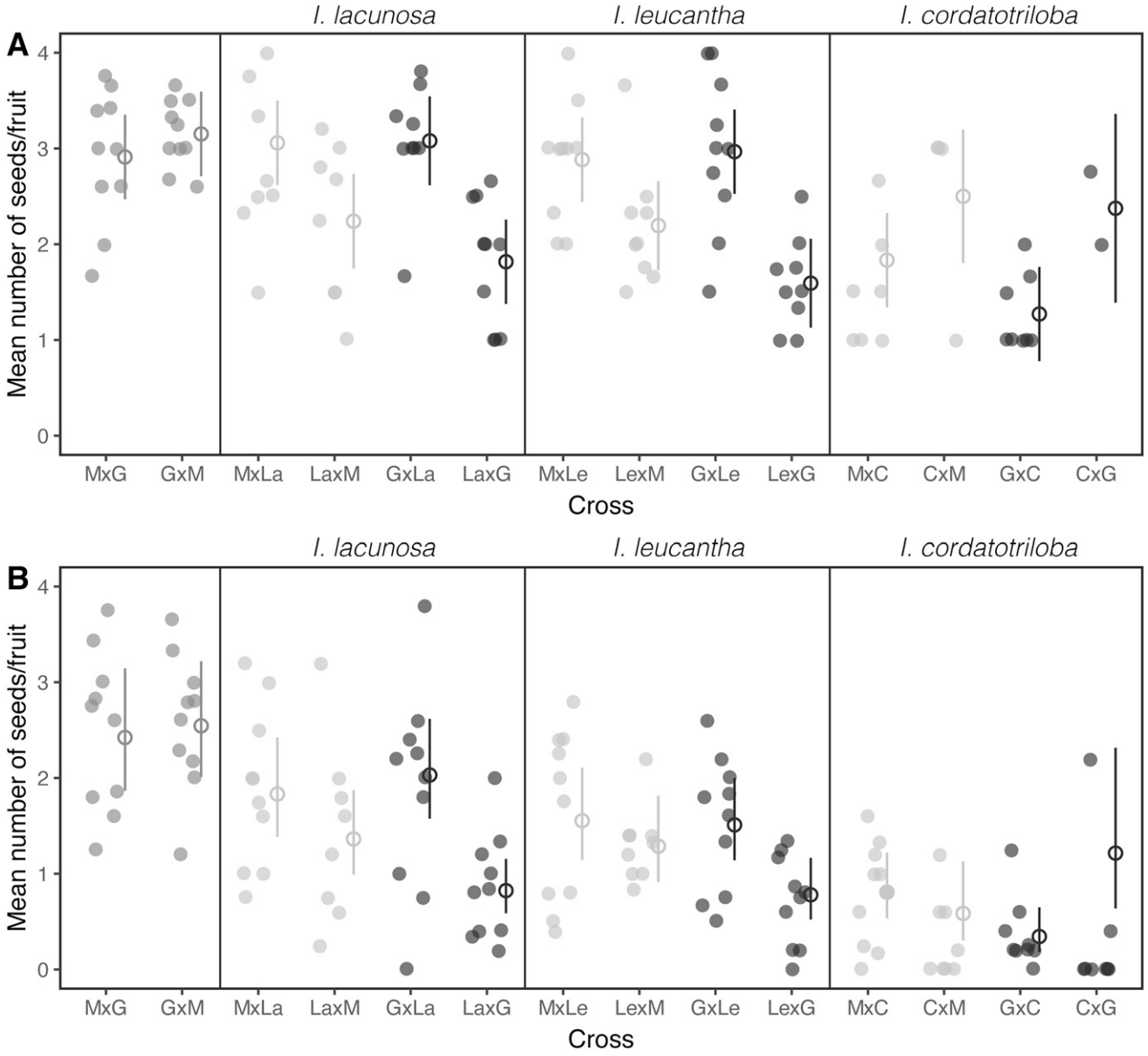

FIG. 3. *Ipomoea* Carolina morphotype and *I. grandifolia* interspecific crosses generally produce more seeds per fruit than many of the interspecific crosses made with either *I.* Carolina morphotype or *I. grandifolia* as one of the parents and the indicated species as the other parent. Each point represented the mean number of seeds produced by approximately five crosses made between the same individuals, and the estimated marginal means (open circles) and 95% confidence intervals from a linear mixed model are displayed. A. Crosses with zero seeds were removed before analysis. B. All crosses were included in the analysis. Crosses between *Ipomoea* Carolina morphotype and *I. grandifolia* are in dark gray (on the left). Interspecific crosses including *I.* Carolina morphotype are in light gray. Interspecific crosses including *I. grandifolia* are in black. M = *I.* Carolina morphotype; G = *I. grandifolia*; C = *I. cordatotriloba*; La = *I. lacunosa*; Le = *I. leucantha*. Corresponds to Table 3.

same for the two taxa. We have chosen to display the fastSTRUCTURE results from K = 7 (Fig. 5A), but results from fastSTRUCTURE show that *Ipomoea* Carolina morphotype and *I. grandifolia* are consistently in the same group regardless of K groups (Fig. S3A, Liao et al. 2022). However, ADMIXTURE results suggest that *I.* Carolina morphotype and *I. grandifolia* could potentially be different when examining K > 7 (Fig. S3B). This difference could have arisen from classifying the lone *I. cynanchifolia* as a distinct group (right of *I. grandifolia* group), which results in an interpretation that *I. grandifolia* is genetically a mixture of *I.* Carolina morphotype and *I. cynanchifolia*. However, in cases of K < 7 in ADMIXTURE, and for all the fastSTRUCTURE results, *I. cynanchifolia* does not appear to be genetically distinct from *I. grandifolia* and *I.* Carolina morphotype. This analysis also shows that *I.* Carolina morphotype and *I. grandifolia* are genetically different from all the other *Ipomoea* species considered in this study, including *I. lacunosa*, *I. cordatotriloba*, and *I. leucantha*, three species that *I.* Carolina morphotype co-occurs with in the Carolinas (Fig. 5A, middle to right side).

Principal components analysis (PCA), cluster analysis of the identity-by-state pairwise distances and Euclidean distances, and the neighbor-joining tree all yield similar results. In the PCA, *I.* Carolina morphotype clusters tightly with most *I. grandifolia* rather than having a separate cluster from any of the species examined (Fig. 5B). Because the first two eigenvalues each only explain about 5% of the overall variation, we

TABLE 4. Comparison of fruit set and mean number of seeds for crosses with either *I.* Carolina morphotype or *I. grandifolia* as a parent with another species. "M vs. G" is factor 1, which tests whether fruit or seed set from crosses with *I.* Carolina morphotype or *I. grandifolia* as a parent with another species is significantly different. "Female vs. Male" is factor 2, which tests whether fruit or seed set from using the other species (species A) as a female or male parent is significantly different. Asterisks indicate significant at overall $p < 0.05$ after a FDR adjustment for multiple comparisons. M = *I.* Carolina morphotype; G = *I. grandifolia*. Corresponds to Fig. 4.

			Mean number of seeds per fruit			
	Fruit set		Crosses with 0 seeds removed		All crosses	
Crosses Compared	G	p.value	F	p.value	χ^2	p.value
I. lacunosa						
M vs. G	1.7353	0.1877	0.621	0.436	1.907	0.167
Female vs. Male	3.9509	0.04685	18.174	1.51E-04*	9.346	0.002*
I. leucantha						
M vs. G	0.73921	0.3899	1.218	0.277	2.192	0.139
Female vs. Male	0.32022	0.5715	22.205	3.82E-05*	5.183	0.023
I. cordatotriloba						
M vs. G	1.1764	0.2781	1.691	0.209	2.319	0.128
Female vs. Male	10.907	9.58E-04*	4.547	0.046	1.211	0.271

examined the next two eigenvalues. These graphs also show that the clustering between *I.* Carolina morphotype and *I. grandifolia* remains consistent, even with the low percentage of variation (Fig. S4, Liao et al. 2022). Because the top four eigenvalues explain less than 20% of the genetic variation among all the species with the remaining 80% unexplained, we calculated the Euclidean distances between the species, which revealed that there are fewer differences between *I.* Carolina morphotype and *I. grandifolia* than with other *Ipomoea* species (Fig. S5, Liao et al. 2022). Finally, the neighbor-joining tree (Fig. 6), and to a large extent, the dendrogram of the identity-by-state pairwise distances (Fig. S6, Liao et al. 2022), also demonstrate similar clustering, where *I.* Carolina morphotype is found to be within the *I. grandifolia* cluster rather than a separate, monophyletic group. This *I.* Carolina morphotype and *I. grandifolia* monophyletic cluster has a 100% bootstrap support (Fig. 6).

DISCUSSION

Ipomoea species in *Ipomoea* series *Batatas* have similar leaf and floral morphologies that make them difficult to distinguish from these characteristics alone (Austin 1978; Jarret et al. 1992). This similarity has led to misidentification in both historical and contemporary herbarium and germplasm records. Genetic and genomic resources have assisted in species identification and in disentangling evolutionary relationships, but these recent phylogenetic studies have not been entirely conclusive for the taxa represented in this clade, yielding conflicting results and interpretations (Muñoz-Rodríguez et al. 2018; Wu et al. 2018). As revealed by these studies, members of this group have complex histories with multiple cases of introgression, hybridization, and polyploidization (Yang et al. 2017; Wu et al. 2018; Rifkin et al. 2019; Gao et al. 2020). Attempting to describe a new species in this group thus requires careful considerations based on several lines of evidence (De Queiroz 2007; Carstens et al. 2013). Here, instead of formally describing *I.* Carolina morphotype as a new species, we report that these individuals are in fact *I. grandifolia*, a species heretofore only described from across South America. Below, we evaluate the results, present scenarios for the dispersal and distribution of *I. grandifolia* in North America, and discuss more broadly the taxonomic complications that remain to be resolved in *Ipomoea* series *Batatas*.

The study proposing *I.* Carolina morphotype as a new species presented morphological, genetic, and reproductive evidence, but it only compared *I.* Carolina morphotype with other co-occurring *Ipomoea* clade Batatas (Duncan and Rausher 2013). A phylogenetic study of *Ipomoea* series *Batatas* hinted that *I.* Carolina morphotype may in fact be *I. grandifolia*, but lacked morphological and reproductive analyses (Eserman 2017). While we have not presented morphological evidence, our personal observations indicate that *I.* Carolina morphotype and *I. grandifolia* are remarkably similar in floral shape and size, corolla color patterning, and inflorescence structure (Fig. 1A, B; images found on Dryad, Liao et al. 2022). Our crossing study also reveals that there is no reproductive barrier in the form of cross incompatibility between these two taxa. First, *I.* Carolina morphotype × *I. grandifolia* interspecific crosses are significantly more successful than the intraspecific crosses. Second, interspecific crosses with the other three *Ipomoea* species (*I. lacunosa*, *I. leucantha*, *I. cordatotriloba*) also reveal that overall seed set was higher for *I.* Carolina morphotype × *I. grandifolia* crosses than any interspecific cross that included either of these species. Third, when crossed with other species, *I.* Carolina morphotype and *I. grandifolia* yield similar results, suggesting that these two taxa are interchangeable. Finally, our population genetic analyses also reveal that there has been little genetic differentiation between the two, consistent with the phylogenetic findings (Eserman 2017). These species are also genetically different from the other species included in our study. While we did not examine other reproductive barriers that may separate these species, based on these criteria, we propose that *I.* Carolina morphotype should be considered to be a North American representative of *I. grandifolia*.

Ipomoea grandifolia is a species that has a complicated taxonomic history. It was originally identified as a taxon in the genus *Jacquemontia* until it was placed in *Ipomoea* by O'Donell (O'Donell 1952; Wood et al. 2015, 2020). Morphologically, it is described as having a larger flower than *I. triloba* and also having shorter corollas than *I. australis* (Wood et al. 2015, 2020). Because the floral morphology appears to be in between that of *I. triloba* and *I. australis*, *I. grandifolia* has been hypothesized to be a hybrid, with *I. australis* as one of the parents and possibly *I. triloba* as the other (Austin 1978). Whether *I. grandifolia* is a hybrid of these two species still requires corroboration (Wood et al. 2020). Chloroplast phylogenies indicate that specimens identified as *I. grandifolia* are polyphyletic, often found sister to specimens identified as *I. australis* and *I. cynanchifolia*, although the nuclear phylogeny suggests that most specimens identified as *I. grandifolia* form a monophyletic group (Muñoz-Rodríguez et al. 2018). Our study included three of the same *I. grandifolia* accessions from CIP (CIP_460316, CIP_460332, CIP_460453) that were found in the Muñoz-Rodríguez study.

Recognizing *I.* Carolina morphotype as *I. grandifolia* raises some questions regarding its disjunct distribution. Until now, *I. grandifolia* has only been reported from northern Argentina, southern Brazil, and parts of Bolivia, Paraguay, and Uruguay (Khoury et al. 2015; Wood et al. 2020). Given the high degree of genetic similarity between individuals from North America and South America, it is likely that *I. grandifolia* was

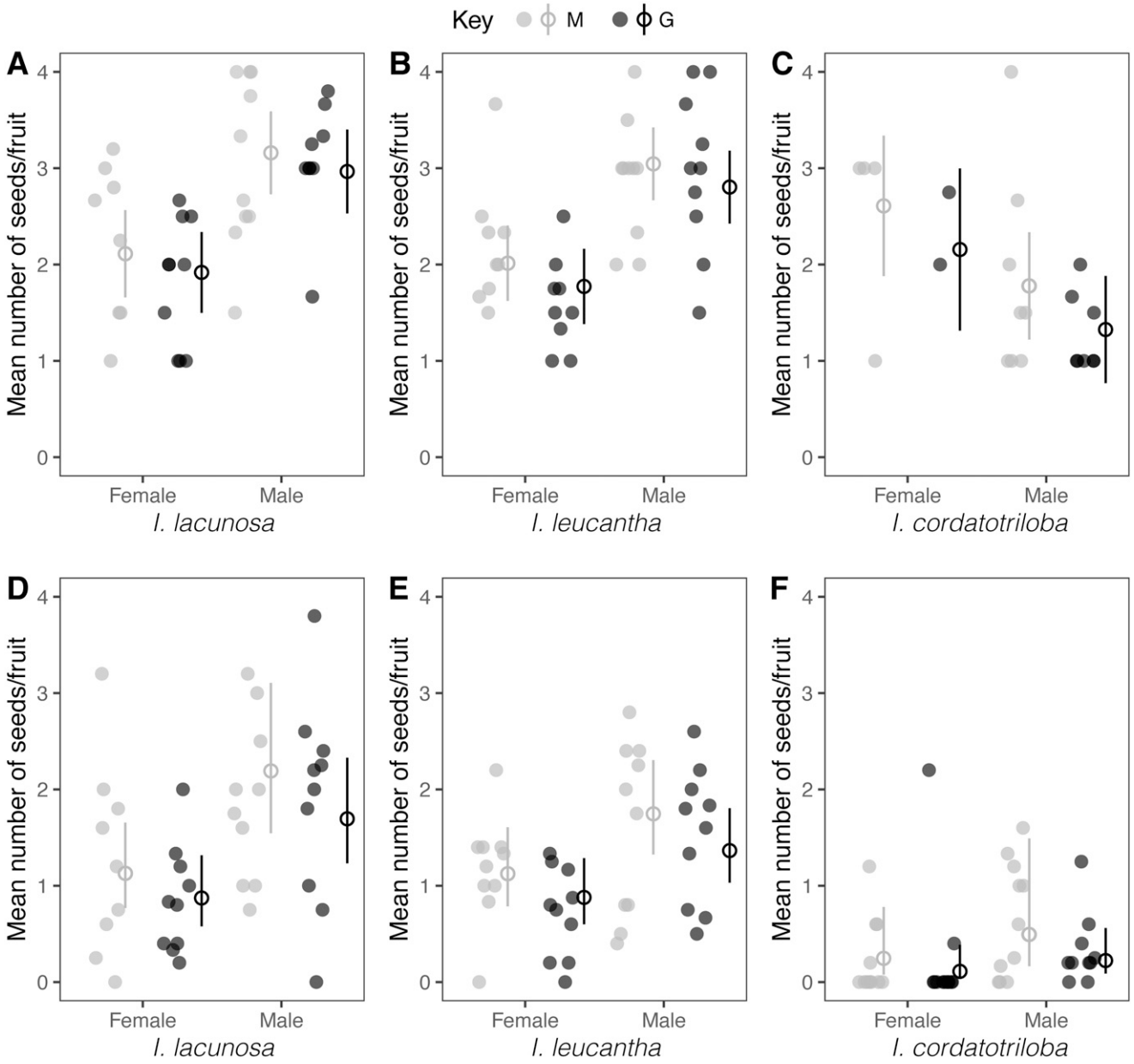

FIG. 4. Mean seed set in crosses made with either *I.* Carolina morphotype or *I. grandifolia* as one parent and the indicated species as the other parent. Each point represents the mean number of seeds produced by approximately five crosses made between the same individuals, and the estimated marginal means (open circles) and 95% confidence intervals from a linear mixed model are displayed. A–C. Crosses with zero seeds were removed before analysis. D–F. All crosses were included in the analysis. A, D. Crosses made with *I. lacunosa* as the female or male parent. B, E. Crosses made with *I. leucantha* as the female or male parent. C, F. Crosses made with *I. cordatotriloba* as the female or male parent. M = *I.* Carolina morphotype (light gray). G = *I. grandifolia* (black). Corresponds to Table 4.

recently introduced to North America, but timing of this introduction will require demographic modeling and more population sampling across both regions (Martin et al. 2019). Although the process by which this species was introduced is unclear, we hypothesize that *I. grandifolia* may have been inadvertently introduced through some form of agricultural trade given that many plant introductions have been made in this manner (Austin 1978; Mühlenbach 1979). We believe that this scenario is possible because of increased movement of agricultural goods in modern times and that *I. grandifolia* is a common weed that grows with crop species (e.g. soybean and sugarcane) in Brazil (Takao et al. 2011; Pereira et al. 2015; Pagnoncelli et al. 2017; Barroso et al. 2019).

Although currently there is little detectable genetic variation that distinguishes North American and South American *I. grandifolia*, these two groups may become more divergent in the future. The geographic isolation of these populations may further facilitate the divergence of these two groups. If there is little reintroduction of South American *I. grandifolia* into North America, over time both groups of *I. grandifolia* may diverge by adapting to local environments. Gene flow from other local co-occurring *Ipomoea* series *Batatas* species (e.g. *I. lacunosa*, *I. cordatotriloba*, *I. leucantha* in North America; *I. cynanchifolia* and *I. australis* in South America) may also contribute to increased differentiation between the two groups. One of our genetic analyses suggests that such genetic

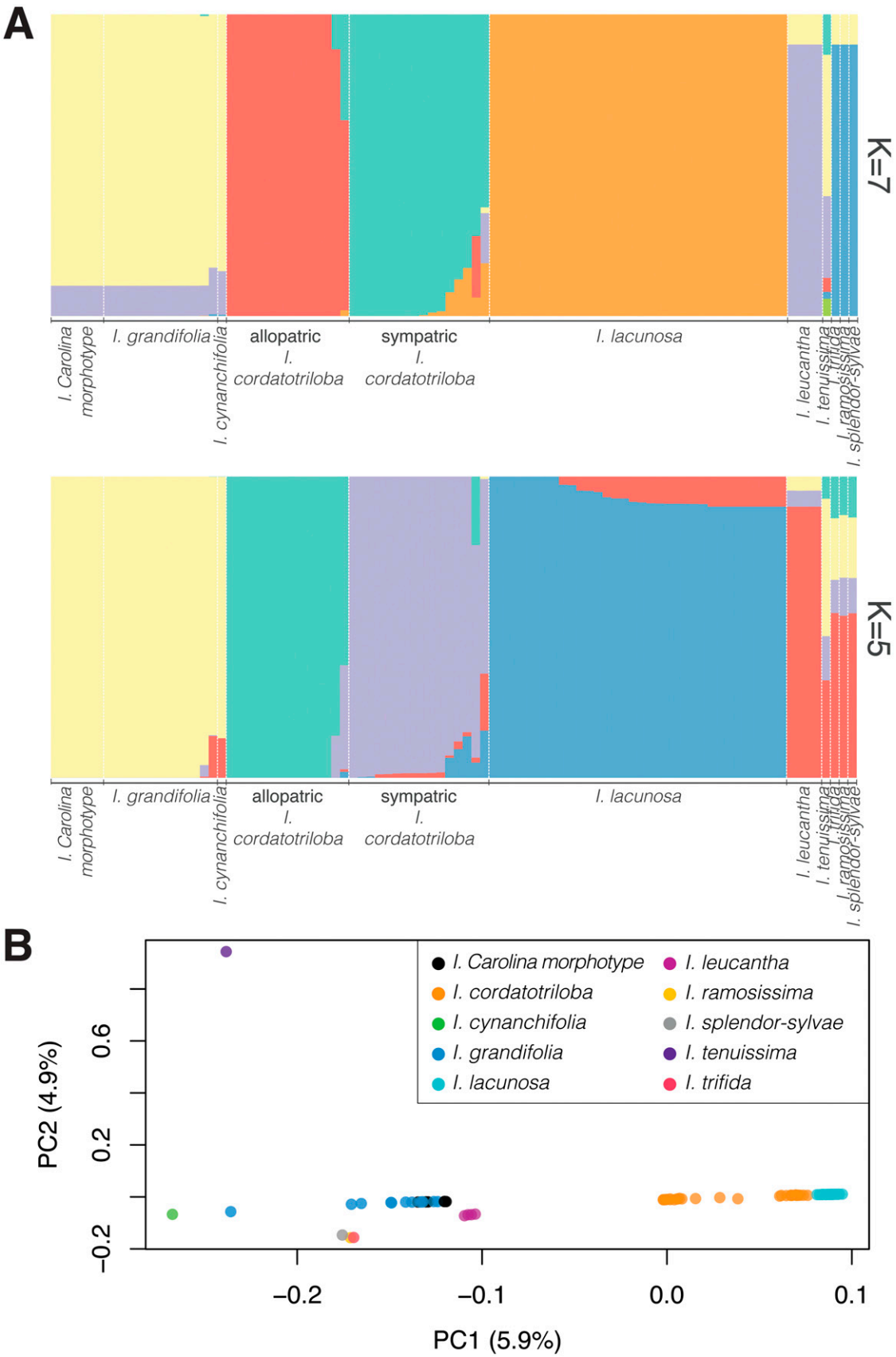

FIG. 5. *Ipomoea* Carolina morphotype and *I. grandifolia* are not genetically differentiated. A. Plots from the most common number of K groups from fastSTRUCTURE (top; K = 7 for 4/10 runs) and ADMIXTURE (bottom; K = 5 for 7/10 runs). *Ipomoea* Carolina morphotype and *I. grandifolia* are found on the left, represented by the yellow-colored group for both analyses. The outgroup species, *I. ramosissima*, *I. trifida*, and *I. splendor-sylvae*, are found on the right side. The *I. cordatotriloba* and *I. lacunosa* samples are the same as that from Rifkin et al. (2019), includes three additional purple *I. lacunosa*, and recapitulates the INSTRUCT and STRUCTURE plots from Rifkin et al. (2019) (see Figs. 2, S2). B. PCA supports the fastSTRUCTURE and ADMIXTURE results with *I.* Carolina morphotype (black dots) clustering in the middle bottom with *I. grandifolia* (dark blue dots).

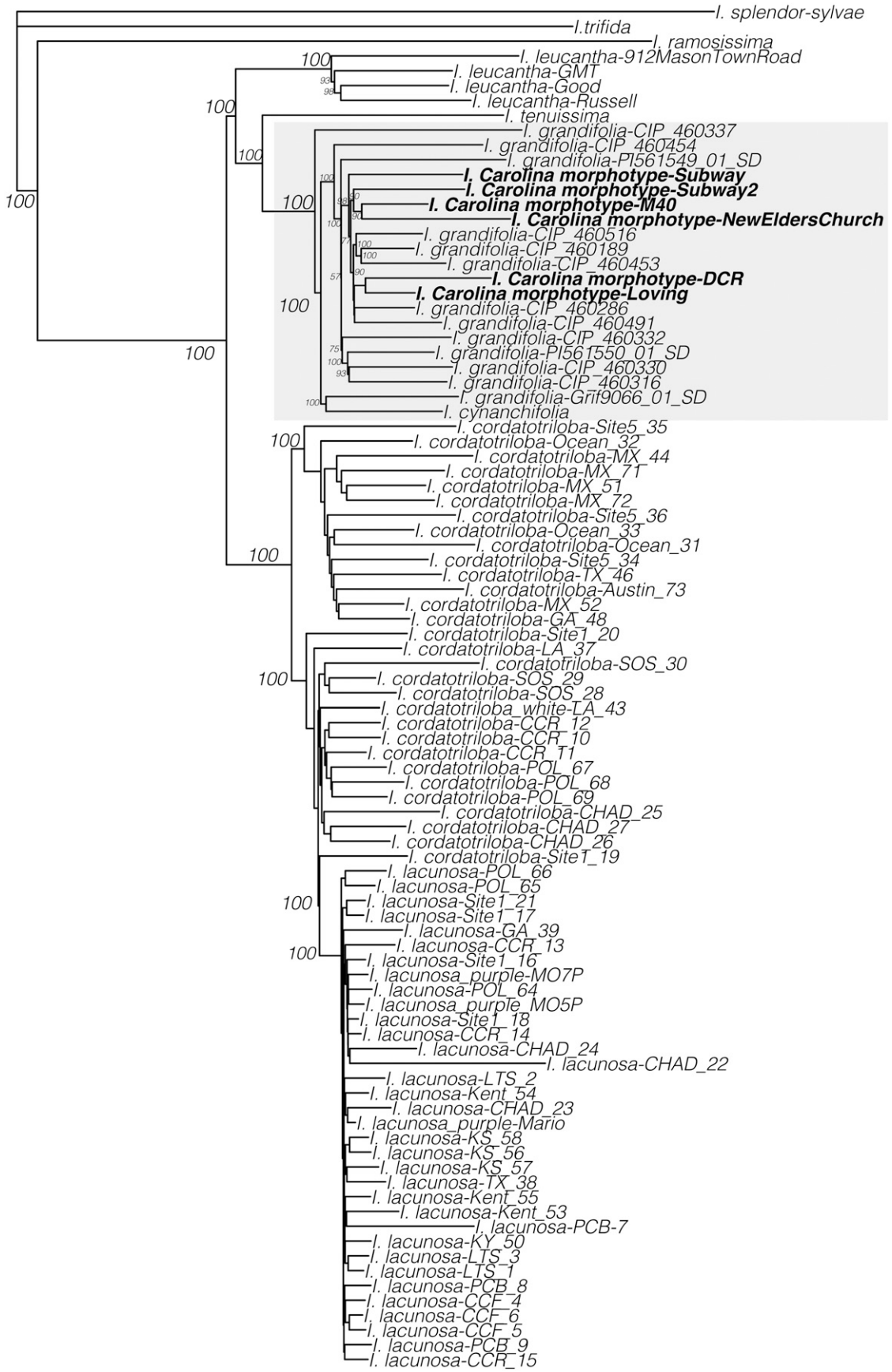

FIG. 6. A neighbor-joining tree also indicates that *I.* Carolina morphotype (bolded) clusters with most *I. grandifolia* samples with 100% bootstrap support after 1000 bootstraps (cluster highlighted with light gray background).

divergence could already be occurring. While we only included one *I. cynanchifolia* individual in our study as a sister species to *I. grandifolia*, the ADMIXTURE analysis suggests that ancestry from *I. cynanchifolia* may contribute to genetic differences between North and South American *I. grandifolia* (Fig. S3B; ADMIXTURE clusters K7–9). Alternatively, it could be one or a few accessions of *I. grandifolia* was misidentified (PI561550_01_SD, Grif9066_01_SD; see clustering for Figs. 6, S5, S6). A broader and more extensive sampling is needed to examine these hypotheses and their possible evolutionary trajectories.

While complications often arise in taxonomic and evolutionary studies due to species misidentifications in germplasm, herbarium specimens, and personal collections (Goodwin et al. 2015), phylogenetic analyses, population sampling, quantitative morphological descriptions, and global comparisons have begun to clarify many species identities, including those of *Ipomoea* species. Genetic analyses have led to several updates in species nomenclature in *Ipomoea* series *Batatas*. For instance *I. cordatotriloba* had been described to have a disjunct distribution with North and South American populations (Khoury et al. 2015), but a phylogenetic analysis showed that these two populations are genetically distinct, with the North American *I. cordatotriloba* more closely related to *I. lacunosa* and the South American *I. cordatotriloba* more closely related to *I. grandifolia* and *I. cynanchifolia* (Muñoz-Rodríguez et al. 2018). This realization led to changing the name of South American *I. cordatotriloba* var. *australis* to its own species name of *I. australis* (Wood et al. 2020). *Ipomoea leucantha* has been proposed to be a hybrid with two different origins. The first hypothesis suggests that *I. leucantha* may be a hybrid between *I. cordatotriloba* and *I. lacunosa* (Austin 1978; Abel and Austin 1981); a second, more recent hypothesis suggests *I. leucantha* may share ancestry with *I. australis* and *I. triloba* (Muñoz-Rodríguez et al. 2018). Because we did not include the same *I. leucantha* samples from the Muñoz-Rodríguez study or the other two species, *I. australis* and *I. triloba*, we are unable to verify the second hypothesis. We are also unable to verify that the *I. leucantha* used in our study and the Muñoz-Rodríguez study are the same species. In any case, the *I. leucantha* used in our study indicate that *I. leucantha* is genetically distinct from all the species included in this study, including *I. cordatotriloba* and *I. lacunosa*, consistent with the findings from Duncan and Rausher (2013). There also appears to be no evidence of admixture from *I. lacunosa* or *I. cordatotriloba* into *I. leucantha*, although there may be a breakdown in reproductive barrier to seed set between *I. lacunosa* and *I. leucantha* (Fig. S7, Table S6, Liao et al. 2022) (Diaz et al. 1996). Further studies are needed to tease apart the genetic ancestry of samples identified as *I. leucantha* and whether gene flow is occurring between *I. lacunosa* and North American *I. leucantha*.

Although molecular analyses of taxa in *Ipomoea* series *Batatas* may differ somewhat in the relationships they reveal, the overall patterns from the studies are remarkably similar. Muñoz-Rodríguez and colleagues (2018) also performed population genetic analysis on many individuals of taxa in *Ipomoea* series *Batatas* with STRUCTURE and found that individuals in Group 2 (*I. grandifolia*, *I. cynanchifolia*, *I. australis*, *I. tenuissima*, *I. triloba*, *I. lacunosa*, *I. leucantha*, *I. cordatotriloba*) are genetically indistinguishable, counter to the STRUCTURE-like findings in our analysis. Differences in our results could be attributed to the number of variable molecular sites used in the analyses and the number of K optimal groups examined; we used ~119,500 variable sites from aligning leaf transcriptomes to *I. lacunosa* draft genome and ran K 2–10 optimal groups while the Muñoz-Rodríguez study used 3000 randomly selected variable sites from 605 putative single copy nuclear genes and ran K 1–5 optimal groups (Muñoz-Rodríguez et al. 2018). However, for both our studies, we were unable to molecularly distinguish the taxa outside of those in Group 2 (e.g. *I. ramosissima*, *I. trifida*, and *I. splendor-sylvae*). While the lower number of variable sites could be the cause in the Muñoz- Rodríguez study, for our analysis, we had further filtered the SNP dataset by removing loci with minor allele frequencies less than 0.05. Given that we only used one individual to represent *I. ramosissima*, *I. trifida*, and *I. splendor-sylvae*, variation that may have distinguished these three species were not included in the fastSTRUCTURE and ADMIXTURE analyses (Fig. 5A; Fig. S3). For both studies, other analyses (e.g. molecular phylogenies for Muñoz- Rodríguez et al. 2018; PCA, neighbor-joining tree; Figs. 5B, 6; Figs. S4–S6) reveal molecular differentiation among taxa within and outside of Group 2. Overall, the predicted evolutionary relationships among taxa in *Ipomoea* series *Batatas* from both studies are consistent.

Patterns of crossability and reproductive isolation within *Ipomoea* series *Batatas* are more difficult to assess given that we only focused on four of the eight taxa in Group 2 and only examined one form of reproductive isolation. While we cannot directly compare this study with Diaz et al. (1996) due to few overlapping taxa used between the two studies, we can make direct comparisons with the study by Duncan and Rausher (2013). Duncan and Rausher included the same four taxa used in this study (except for that of *I. grandifolia*) and revealed that all interspecific crosses yielded lower average seed set than intraspecific crosses. Additionally, their study suggests that the direction of the interspecific cross could be important to consider as reciprocal crosses did not necessarily yield similar average seed sets. Our study, which includes more accessions and more crosses made between taxa, suggests that the reproductive barrier to seed set among these taxa is more complicated than the Duncan and Rausher study revealed. While most intraspecific crosses have a greater average seed set than interspecific crosses, this pattern was not found for crosses between *I. lacunosa* and *I. leucantha* (Fig. S7; Table 2). Additionally, crosses between *I. grandifolia*/*I.* Carolina morphotype with *I. lacunosa* as the male parent often yielded similar average seed set as that of the intraspecific crosses for *I. grandifolia*/*I.* Carolina morphotype (Tables 2, 3). These results suggest that there may be little reproductive isolation in the form of reduced seed set among these three taxa such that hybridization can occur among these species. However, because we did not examine other forms of reproductive barriers, such as hybrid inviability (Coughlan et al. 2020), additional study is warranted to examine the strength of the reproductive barriers among taxa in Group 2 of *Ipomoea* series *Batatas*, the extent of hybridization among the taxa, and whether these taxa maintain the potential to hybridize with one another.

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Author Contributions

ITL conceived of the project, performed artificial crosses, extracted RNA, analyzed all data, and wrote the manuscript. AHF performed artificial crosses and collected data on the resulting crosses. KLO made leaf transcriptome libraries and advised in data analyses. MDR conceived of and advised on the project, data analyses, and writing the manuscript. All authors read, edited, and approved the final manuscript.

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APPENDIX 1. Vouchers and SRA accessions in which the leaf transcriptome sequences were obtained from the exact voucher individual: taxon, collection locality, voucher collector and number (herbarium acronym), seed accession, SRA accession, NCBI project accession.

Ingroup: *Ipomoea grandifolia*, Argentina, *Liao 19-04* (DUKE), CIP_460189, SRR14748296, PRJNA735523. *Ipomoea grandifolia*, Paraguay, *Liao 19-01* (DUKE), CIP_460316, SRR14748317, PRJNA735523 *Ipomoea grandifolia*, Paraguay, *Liao 19-17* (DUKE), CIP_460330, SRR14748316, PRJNA735523. *Ipomoea grandifolia*, Argentina, *Liao 19-02* (DUKE), CIP_460453, SRR14748313, PRJNA735523. *Ipomoea grandifolia*, Argentina, *Liao 19-21* (DUKE), CIP_460454, SRR14748312, PRJNA735523. *Ipomoea grandifolia*, Paraguay, *Liao 19-03* (DUKE), CIP_460516, SRR14748310, PRJNA735523. *Ipomoea grandifolia* (Carolina morphotype), USA-Carolinas, *Liao 19-08* (DUKE), Rausher Lab - Subway, SRR14748320, PRJNA735523. *Ipomoea grandifolia* (Carolina morphotype), USA-Carolinas, *Liao 19-06* (DUKE), Rausher Lab - Loving, SRR14748319, PRJNA735523. *Ipomoea grandifolia* (Carolina morphotype), USA-Carolinas, *Liao 19-16* (DUKE), Rausher Lab - New Elder's Church, SRR14748308, PRJNA735523. *Ipomoea grandifolia* (Carolina morphotype), USA-Carolinas, *Liao 19-20* (DUKE), Rausher Lab – DCR (IL21), SRR14748299, PRJNA735523. *Ipomoea grandifolia* (Carolina morphotype), USA-Carolinas, *Liao 19-09* (DUKE), Rausher Lab - M40, SRR14748298, PRJNA735523. *Ipomoea leucantha*, USA-Carolinas, *Liao 19-05* (DUKE), Rausher Lab - 912MasonTownRoad, SRR14748304, PRJNA735523. *Ipomoea leucantha*, USA-Carolinas, *Liao 19-18* (DUKE), Rausher Lab - Russell, SRR14748304, PRJNA735523.

APPENDIX 2. Vouchers and SRA accessions in which the leaf transcriptome sequences were obtained from the parent or sibling of the voucher individual: taxon, collection locality, voucher collector and number (herbarium acronym), seed accession, SRA accession, NCBI project accession.

Ingroup: *Ipomoea cordatotriloba*, USA-Carolinas, *Liao 19-07* (DUKE), Rausher Lab - PC_CHAD_1, SRR15373711, PRJNA769750. *Ipomoea cordatotriloba*, USA-Carolinas, *Liao 19-14* (DUKE), Rausher Lab - PC_SOS_2, SRR15373707, PRJNA769750. *Ipomoea cordatotriloba*, USA-Carolinas, *Liao 19-23* (DUKE), Rausher Lab - PC_SOS_3, SRR15373706, PRJNA769750. *Ipomoea cordatotriloba*, USA-Carolinas, *Liao 19-25* (DUKE), Rausher Lab - PC_Ocean_2, SRR15373704, PRJNA769750. *Ipomoea cordatotriloba*, USA - Georgia (approx), *Liao 19-26* (DUKE), PI_645627_02_SD, SRR15373673, PRJNA769750. *Ipomoea grandifolia*, Paraguay, *Liao 19-22* (DUKE), CIP_460337, SRR14748314, PRJNA735523. *Ipomoea lacunosa*, USA-Carolinas, *Liao 19-12* (DUKE), Rausher Lab - L_CCR_1, SRR15373721, PRJNA769750. *Ipomoea lacunosa*, USA-Carolinas, *Liao 19-10* (DUKE), Rausher Lab - L_CHAD_3, SRR15373690, PRJNA769750. *Ipomoea lacunosa*, USA-Carolinas, *Liao 19-28* (DUKE), Rausher Lab - L_Site1_1, SRR15373718, PRJNA769750. *Ipomoea lacunosa*, USA-Carolinas, *Liao 19-15* (DUKE), Rausher Lab - L_CCF_1, SRR15373708, PRJNA769750. *Ipomoea lacunosa*, USA-Kentucky, *Liao 19-31* (DUKE), Rausher Lab - L_KY, SRR15373672, PRJNA769750. *Ipomoea lacunosa*, USA-Kansas, *Liao 19-29* (DUKE), Rausher Lab - L_Kent_7, SRR15373667, PRJNA769750. *Ipomoea lacunosa*, USA-Kansas, *Liao 19-11* (DUKE), Rausher Lab - L_KS_5, SRR15373686, PRJNA769750.

APPENDIX 3. Samples in which vouchers were not made, but the seeds were obtained from a germplasm (CIP or USDA): taxon, collection locality, germplasm, seed accession, SRA accession, NCBI project accession.

Ingroup: *Ipomoea cordatotriloba*, USA-Texas, USDA, Grif_15931_01_SD, SRR15373670, PRJNA769750. *Ipomoea cordatotriloba*, USA-Texas, USDA, GRIF_6183_01_SD, SRR15373693, PRJNA769750. *Ipomoea cordatotriloba*, Mexico, USDA, PI_518495_02_SD, SRR15373676, PRJNA769750. *Ipomoea cordatotriloba*, USA-Louisiana (approx), USDA, PI_645624_01_SD, SRR15373698, PRJNA769750. *Ipomoea cordatotriloba*, USA-Louisiana (approx), USDA, PI_645625_01_SD, SRR15373695, PRJNA769750. *Ipomoea cordatotriloba*, USA-Texas, USDA, PI_675061, SRR15373675, PRJNA769750. *Ipomoea cynanchifolia*, Brazil (CIP_460149), USDA, PI549093_01_SD, SRR14748297, PRJNA735523. *Ipomoea grandifolia*, Argentina, CIP, CIP_460286, SRR14748318, PRJNA735523. *Ipomoea grandifolia*, Paraguay, CIP, CIP_460332, SRR14748315, PRJNA735523. *Ipomoea grandifolia*, Argentina, CIP, CIP_460491, SRR14748311, PRJNA735523. *Ipomoea grandifolia*, Brazil (CIP_460106), USDA, Grif9066_01_SD, SRR14748294, PRJNA735523. *Ipomoea grandifolia*, Argentina (CIP_460189), USDA, PI561549_01_SD, SRR14748296, PRJNA735523. *Ipomoea grandifolia*, Paraguay (CIP_460190), USDA, PI561550_01_SD, SRR14748295, PRJNA735523. *Ipomoea lacunosa*, USA-Georgia (approx), USDA, PI_645621_02_SD, SRR15373696, PRJNA769750. *Ipomoea lacunosa*, USA-Texas, USDA, PI_645623_01_SD, SRR15373697, PRJNA769750.

Outgroup: *Ipomoea ramosissima*, Bolivia (CIP_460036), USDA, PI552786_01_SD, SRR14748303, PRJNA735523. *Ipomoea splendor-sylvae*, Mexico, USDA, PI561557_01_SD, SRR14748302, PRJNA735523. *Ipomoea tenuissima*, USA-Florida (approx), USDA, PI553012_01_SD, SRR14748301, PRJNA735523. *Ipomoea trifida*, Venuzuela (CIP_460007), USDA, PI561543_01_SD, SRR14748300, PRJNA735523.