A Phylogeny of the Genus *Amaranthus* (Amaranthaceae) Based on Several Low-Copy Nuclear Loci and Chloroplast Regions

Katherine E. Waselkov,[1,4] Alexis S. Boleda,[2] and Kenneth M. Olsen[3]

[1]Department of Biology, Mail Stop SB 73, 2555 East San Ramon Avenue, California State University, Fresno, California 93740, USA; kwaselkov@csufresno.edu

[2]Department of Mathematics, 4176 Campus Drive, University of Maryland, College Park, Maryland 20742, USA; aboleda@math.umd.edu

[3]Department of Biology, Campus Box 1137, One Brookings Drive, Washington University, Saint Louis, Missouri 63130, USA; kolsen@wustl.edu

[4]Author for correspondence

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Abstract—The genus *Amaranthus* (pigweeds) is a group of ~74 monoecious or dioecious annual species native to every continent but Antarctica, frequently associated with natural and human disturbance, with several economically important domesticated and weedy species. We set out to reconstruct the phylogeny of *Amaranthus,* with broad geographic sampling, in order to answer questions about biogeographic relationships in the genus and the monophyly of the subgenera. Fifty-eight species were included in maximum parsimony and Bayesian phylogenetic analyses based on ITS and three low-copy nuclear genes (*A36*, *G3PDH*, and *Waxy*), as well as two chloroplast regions (*trnL5′-trnL3′* and *matK/trnK*). Topology tests were also employed to test taxonomic hypotheses about incongruence between trees and the monophyly of clades containing Galápagos species. Our analyses support the origin of the genus in the Americas, with a single long-distance dispersal event to the Old World, and both nuclear and chloroplast trees recover three to four major clades, roughly corresponding to three subgenera recognized based on morphology. However, there are species in all of these clades that were not predicted based on morphology, and we discover previously unsuspected relationships between Galápagos species and species from the North American Southwest, which comprise small monophyletic groups outside of the three recognized subgenera. Additionally, an important herbicide resistant weed species (*A. palmeri*) and its sister species are placed into different large clades based on nuclear or chloroplast data, suggesting a chloroplast capture event. These results will provide a basis for further exploration of the evolution of weedy ecological strategies in the group.

Keywords—*Amaranthus palmeri*, chloroplast capture, dioecy, pigweeds, topology testing.

The plant genus *Amaranthus* L. (Amaranthaceae) includes ~74 species, with the bulk of the species (~55) native to the Americas, and the remaining 19 native to Eurasia, South Africa, and Australia/Oceania (Sauer 1955; Bayón 2015). The greatest species diversity occurs in warm temperate, subtropical, and tropical regions of the world (Mosyakin and Robertson 2003). The English colloquial name for plants in this genus is "pigweed," and many species in the group are human commensals (Sauer 1957). The entire genus is both edible and nutritious, and a number of *Amaranthus* species have been important in the diet of Old and New World human cultures for millennia (Kent 1991; Gremillion 2004; Jin et al. 2014). Several species are currently economically important: three species (*A. caudatus* L., *A. cruentus* L., and *A. hypochondriacus* L.) are domesticated pseudocereals popular in South America and South Asia, two species (*A. blitum* L. and *A. tricolor* L.) are grown as vegetable crops in Asia, and around eight species are problematic agricultural weeds worldwide (Mosyakin and Robertson 2003). At the other end of the ecological spectrum are several island endemics (*A. minimus* Standl. in Cuba, *A. brownii* Christoph. & Caum in Hawaiʻi, and *A. anderssonii* J.T. Howell, *A. furcatus* J.T.Howell, and *A. sclerantoides* (Andersson) Andersson in the Galápagos archipelago; Bayón 2015) and the federally threatened beach species *A. pumilus* Raf. from the Atlantic coast of the USA (Weakly et al. 1996; Nolan et al. 2010). There is at present no well-supported phylogeny of the genus that includes broad geographic and taxonomic sampling, despite its worldwide distribution and close association with human activities. Understanding the evolutionary relationships between the species of *Amaranthus* has important implications for studies of the origins and improvement of the domesticated species, the evolution of weedy traits in the agricultural pest species, and clarification of current taxonomy and recognition of as-yet undescribed species.

Amaranthus is characterized by the following traits: an annual or (rarely) short-lived perennial life history; alternate leaves; inflorescences terminal and/or axillary; imperfect flowers (plants monoecious or dioecious) in compound dichasia subtended by bracts and packed into inflorescences; flowers usually with three to five membranaceous tepals; fruit a utricle or pyxidium; and a base chromosome number of 16 or 17 (Mosyakin and Robertson 2003). Although the whole genus is hypothesized to be paleoallotetraploid (Greizerstein and Poggio 1992; Clouse et al. 2016), *Amaranthus dubius* Mart. ex Thell. is the only known polyploid species (allotetraploid, $2n = 64$) with respect to the base chromosome number $n = 16$, based on numerous chromosome counts from a variety of *Amaranthus* species (e.g. Murray 1940; Grant 1959a, c; Song et al. 2002). In addition, every examined species in the genus has C_4 photosynthesis, unlike the closest extant related genera (Sage et al. 2007). The group has garnered interest in the past mainly for its domesticated species and its agricultural weed species. *Amaranthus caudatus* (domesticated in the Andes), *A. cruentus* (domesticated in southern Mexico or Guatemala), and *A. hypochondriacus* (domesticated in central Mexico; Sauer 1950, 1967) have been the subject of many studies aiming to resolve the question of their phylogenetic origin, as well as many studies interested in crop potential and improvement (e.g. Mallory et al. 2008; Kietlinski et al. 2014; Clouse et al. 2016; Stetter et al. 2017). These grain (or more correctly, pseudograin) amaranths were important in the Aztec and Incan empires, but were suppressed by the Spanish during colonial times (Sauer 1950). Their nutritional and agricultural properties were not rediscovered until the 1970s: researchers found an almost complete complement of amino acids in the grains, as well as relatively high disease resistance and some drought resistance (Grubben and van Sloten 1981). Two *Amaranthus* species have also been semi-domesticated as vegetable crops in

Europe, Asia, and Africa: *A. tricolor* (Asian origin) and *A. blitum* (Eurasian origin) (Sauer 1967), with several other species harvested in the wild or cultivated for vegetable use (including *A. dubius*, *A. cruentus*, *A. thunbergii* Moq., and *A. hypochondriacus*) (Brenner et al. 2000).

Nine *Amaranthus* species are listed as "introduced, invasive, and noxious plants" in the USDA Plants Database (Southern Weed Science Society 1998), and an additional 21 species are listed as "agricultural weeds" in the Global Compendium of Weeds (Randall 2007). The genus is the focus of more USA weed science research than any other plant genus (Tranel and Trucco 2009). The *Amaranthus* species of greatest concern to agriculture are infamous for their ability to rapidly evolve herbicide resistance: as of June 2017, 11 species in the genus were resistant to at least one herbicide chemical mode of action, and three species (*A. hybridus* L., *A. palmeri* S.Watson, and *A. tuberculatus* (Moq.) J.D.Sauer) had evolved resistance to four or more modes, including glyphosate (RoundUp®) (Heap 2017). Herbicide-resistant waterhemp (*A. tuberculatus*) and Palmer amaranth (*A. palmeri*) are among the most economically damaging weeds of soybeans, corn, and cotton in the midwestern and southern USA (Beckie 2011; Ward et al. 2013; Chatham et al. 2015). The success of these two species as agricultural weeds is all the more interesting considering their dioecious mating system, which makes selfing impossible, in defiance of Baker's Law concerning colonization probability (Baker 1955, 1974; but see Trucco et al. 2007 for evidence of agamospermy in *A. palmeri*). From the perspective of weed evolution, a well-sampled *Amaranthus* phylogeny would be very useful for studying morphological, physiological, and genomic changes associated with invasion of agricultural ecosystems. It is currently unknown how many times weeds evolved independently within the group.

Müller and Borsch (2005) and Sage et al. (2007) placed the genus in the Amaranthaceae sensu lato (including the Chenopodiaceae) and Amaranthaceae sensu stricto, subfamily Amaranthoideae, tribe Amarantheae, subtribe Amaranthinae, closely related to the genera *Pleuropterantha* Franch. and *Chamissoa* Kunth. The genus *Amaranthus* was first established by Linnaeus in 1753. Various parts of the genus were at one time recognized as separate genera, particularly the dioecious species and the monoecious species with dehiscent or indehiscent fruits (Linnaeus 1753; Kunth 1838). These genera were later placed within *Amaranthus* by Grenier and Godron (1855), Sauer (1955), and Robertson (1981), and are presently recognized as subgenera in the group by most authorities: *Amaranthus* includes *Amaranthus* subgenus *Amaranthus*, *Amaranthus* subgenus *Acnida* (L.) Aellen ex K.R.Robertson, and *Amaranthus* subgenus *Albersia* (Kunth) Gren. & Godr. (Mosyakin and Robertson 1996; Costea et al. 2001a). *Amaranthus* subgenus *Acnida* includes all of the dioecious species of genus *Amaranthus*, and the monoecious species are divided into *Amaranthus* subgenus *Amaranthus* and *Amaranthus* subgenus *Albersia* using a combination of morphological characters, including inflorescence position, number of tepals, and fruit dehiscence (Mosyakin and Robertson 1996; Bayón 2015). Several experts have suggested that this infrageneric taxonomy may not correspond well to evolutionary history (Eliasson 1988; Mosyakin and Robertson 2003). The most recent taxonomic revision of all nine of the dioecious species in *Amaranthus* was completed in the 1950s and updated in subsequent years by the same author (Sauer 1955, 1957, 1972; see Pratt and Clark 2001 and Costea and Tardif 2003 for

demotion of *A. rudis* J.D.Sauer to varietal status within *A. tuberculatus* (Moq.) J.D.Sauer), and a fully illustrated taxonomic revision of all 65 of the described monoecious species in the genus (based solely on morphology) was recently completed (Bayón 2015).

Most previous phylogenetic work in the genus *Amaranthus* has either involved a restricted sample of species, or produced low-resolution results. The exception is the recent genotyping-by-sequencing (GBS)-based phylogeny by Stetter and Schmid (2017), which sampled 35 species of *Amaranthus* available from germplasm banks. The resulting phylogenies were neighbor-joining distance trees and networks, and a species tree based on the multispecies coalescent, using a variable number of single nucleotide polymorphisms (SNPs) depending on calling method (de novo or reference-based). The authors relied on existing taxonomic designations from germplasm banks, without performing independent morphological verifications of species identity. Due to some errors in species identification, the interpretation of the authors' results concerning relationships in the genus as a whole is somewhat difficult (see Discussion). The main objective of that study was to infer the domestication history of the grain amaranth species (which were heavily represented among the accessions included) (Stetter and Schmid 2017). An earlier study examined 30 species available from the USDA using restriction-site analysis of three PCR-amplified loci (one nuclear and two chloroplast loci); these markers yielded very low-resolution results due to low levels of polymorphism (Lanoue et al. 1996). Both of these previous studies showed several conserved groups in all phylogenetic trees, with species of *Amaranthus* subgenus *Acnida* in two separate clades. Therefore, the best-sampled phylogenetic studies in the genus to date do not support the monophyly of the taxonomic subgenera. Experiments in hybridization between weedy *Amaranthus* species have determined that *A. palmeri* and *A. tuberculatus* (both dioecious species) yield practically no fertile hybrid offspring when crossed, whereas *A. hybridus* (a monoecious species) and *A. tuberculatus* produce up to 3% F_1 individuals with seed output comparable to parents in backcrosses (Murray 1940; Trucco et al. 2005, 2007). These results suggest that the dioecious weed species may not be each other's closest relatives, and that deciphering the phylogenetic relationships between *Amaranthus* species could generate new hypotheses about the potential for gene flow between agricultural weed species.

Many other studies have involved limited reconstruction of relationships between species in *Amaranthus* subgenus *Amaranthus* (especially the *A. hybridus* species complex) using a variety of molecular markers, including RAPDs and isozymes (Chan and Sun 1997); low-C_OT DNA sequences (Sun et al. 1999); ITS DNA sequences, AFLPs, and ISSRs (Xu and Sun 2001); SNPs (Maughan et al. 2011); and microsatellites (Mallory et al. 2008; Kietlinski et al. 2014). These studies supported the origin of the domesticated grain amaranths (*A. hypochondriacus*, *A. cruentus*, and *A. caudatus*) from *A. hybridus*, although some reported evidence for lesser contributions from other species (e.g. Xu and Sun 2001). Another study used AFLPs to explore relationships among eight agricultural weeds (Wassom and Tranel 2005), and yet another surveyed 15 domesticated and weedy species found in China using ITS data (Song et al. 2000). A number of species in the genus have never been analyzed genetically or phylogenetically, including all of the species from Australia and South Africa.

The unusual pan-global distribution of *Amaranthus* raises interesting biogeographical questions about the history of diversification in the genus. The geographical region of origin of the genus and the relationships of the Old World species to the New World species are both unknown. Long-distance dispersal between continents is almost definitely involved in the radiation of the genus, as it is estimated to be less than 65 million years old (Kadereit et al. 2003). The ~46 monoecious species native to the Americas have been placed into two distinct subgenera (*Amaranthus* subgenera *Albersia* and *Amaranthus*), which have overlapping geographic and ecological distributions, but the relationship of these putative clades to each other and to the dioecious species (all New World) is unclear. Furthermore, there are four *Amaranthus* species native or endemic to the Galápagos Islands. Morphological similarities between these species and various other species in the genus have been noted by previous authors, but it is currently unknown whether the genus radiated in the islands after a single colonization event, or arrived in the Galápagos multiple times (Eliasson 1985, 1987).

We set out to reconstruct the phylogeny of *Amaranthus*, with broad geographic sampling, in order to answer questions about biogeographic relationships in the genus and the monophyly of the subgenera, and provide a basis for further exploration of the evolution of various ecological strategies in the group.

Materials and Methods

Taxon Sampling—The genus *Amaranthus* contains 74 species, according to the taxonomic authorities Sauer (1955), Pratt and Clark (2001), and Bayón (2015). Fifty-eight species were sampled here, 57 of which are recognized by Bayón (we treat *A. quitensis* Kunth. as a separate species from *A. hybridus* L., following Sauer 1967, Kietlinski et al. 2014, and Iamonico 2015, but unlike Costea et al. 2001a and Bayón 2015), as well as subspecies of *A. blitum* L., *A. graecizans* L., and *A. powellii* S. Watson recognized by Costea et al. (2001a, b, 2003). Multiple accessions of each species were sampled when material was available and good sequence data could be obtained, for a maximum of 100 specimens of *Amaranthus* included in our phylogenetic reconstructions. This sampling represents each subgenus and section of *Amaranthus* defined by Mosyakin and Robertson (1996), and also represents each broad geographical region in which the genus occurs (see Table 1). In addition, two outgroup species from closely-related genera were included: two specimens of *Chamissoa altissima* (Jacq.) Kunth, a Neotropical clambering shrub, and one specimen of *Pleuropterantha revoilii* Franch., a North African shrub. These C3 genera are the closest relatives of *Amaranthus* based on Sage et al.'s (2007) *matK/trnK*-based phylogeny of Amaranthaceae, with *Pleuropterantha* being the sister taxon to *Amaranthus* and *Chamissoa* being the sister taxon to *Pleuropterantha + Amaranthus*.

Species and subspecies included in the phylogenetic reconstruction are listed in Table 1, along with their taxonomic authorities (hereafter omitted in the text). The voucher information for each specimen is provided in Appendix 1; many specimens were obtained from the USDA Germplasm Resources Information Network (GRIN) database (http://www.ars-grin.gov), which has an extensive collection of wild and cultivated *Amaranthus* species. Seeds from GRIN were grown in the Washington University Plant Growth Facility until flowering, for tissue collection and confirmation of species identity. When herbarium material or field-collected material was used, the collector, collection number, and herbarium (and herbarium accession number when available) are listed instead of a PI number as voucher information. Also listed in Table 1 is the classification of each species in two recent taxonomic treatments of the genus, the *Flora of North America* treatment (Mosyakin and Robertson 1996, 2003) and the complete treatment of the monoecious species (Bayón 2015). The geographical provenances of specimens are given in a separate column, along with the abbreviation ID used for each specimen in the phylogenetic trees (i.e. "*A. blitoides* IA" from Iowa, USA). Finally, the native geographic range (with references) of each species is listed by continent and by area within continent.

DNA Extraction and Sequencing—DNA was extracted from each sample with Qiagen DNeasy Plant Mini Kits (Qiagen Inc., Valencia, California), except for the herbarium specimens. These were ground with liquid nitrogen, and then processed using a modification of Doyle and Doyle's (1990) CTAB plant extraction protocol. The modification was as follows: after the chloroform extraction step, reagents and columns from the Invitrogen PureLink PCR Purification Kit (Thermo Fisher Scientific Inc., Waltham, Massachusetts) were used to clean the DNA. Five volumes of Invitrogen binding buffer were added to the aqueous phase and mixed. Then this mixture was loaded onto the columns provided in the kit, and the columns were washed and eluted with Invitrogen wash buffer and then elution buffer. This procedure produced higher-quality, cleaner *Amaranthus* DNA from well-preserved herbarium material than did the Qiagen DNeasy Plant Mini Kit.

Four nuclear genes (partial or complete) and two chloroplast regions were amplified and sequenced for each specimen. The nuclear genes were *A36* (a predicted DEAD-box ATP-dependent RNA helicase), *G3PDH* (glyceraldehyde 3-phosphate dehydrogenase), ITS (internal transcribed spacers 1 and 2 and the intervening 5.8S ribosomal gene), and *Waxy* (granule-bound starch synthase I). The chloroplast regions were *matK/trnK* (the maturase K gene and surrounding *trnK* intron) and *trnL5'-trnL3'* (part of the *trnL* intron). Primers and their published sources are listed in Table S1. The *G3PDH* primers were redesigned after amplification with primers from Strand et al. (1997) to amplify one specific gene copy of the two *G3PDH* copies detected by cloning. Internal primers were designed for *A36* and *G3PDH* and were used only if the DNA quality was too poor to obtain a high-quality sequence read from the external primers alone. In contrast, the internal primers designed for *matK/trnK* and *Waxy* were used for all specimens, due to the length of the amplified region.

The PCR was performed on ABI GeneAmp 9700 thermocyclers (Applied Biosystems, Carlsbad, California), in 25 μL reactions containing: 1X GoTaq Flexi Buffer (Promega, Madison, Wisconsin), 2 mM MgCl₂, 0.2 mM each dNTPs, 0.8 μM each forward and reverse primers, 0.125 μL GoTaq, 7.125 μL nanowater, 6.25 μL betaine, and from 2–4 μL genomic DNA. Amplification conditions were: 94°C for 5 min, then 35 cycles of 94°C (30 s) denaturation, 50°C (30 s) annealing, 68°C (2 min) extension, and 72°C (7 min) final extension. The PCR cleanup was performed with Invitrogen PureLink PCR Purification Kits, according to the manufacturer's instructions but starting with 20–25 μL PCR products. Direct sequencing was performed in 12 μL reactions containing: 0.625 × sequencing buffer, 0.27 μM primer, 1.0 μL PCR product, 1.0 μL BigDye v. 3.0 terminator (Applied Biosystems), and 6.9 μL ddH₂O. Sequencing reaction conditions were: 96°C for 1 min, then 50 cycles of 96°C (10 s) denaturation, 50°C (5 s) annealing, and 60°C (4 min) extension. Sequences were cleaned with Sephadex columns (GE Healthcare, Piscataway, New Jersey) and sequenced on the ABI Prism 3130x Genetic Analyzer (Applied Biosystems). Cloning was performed for the dioecious species (which are obligately outcrossing and therefore highly heterozygous, unlike the monoecious species, which are highly selfing [Murray 1940]) and for *A. dubius*, the lone polyploid species. Sequencing of cloned PCR products was similar to direct sequencing, except with the intermediate steps of transformation of ligated PCR products into Z competent *E. coli* cells (Zymo Research Co., Irvine, California), followed by plating and colony PCR. We obtained at least eight clones per species to distinguish and phase the two alleles for heterozygotes, and to eliminate SNPs and haplotypes resulting from PCR recombination or other replication error during cloning.

All sequences were combined into contigs using Sequencher 5.2 DNA sequence analysis software (Gene Codes Co., Ann Arbor, Michigan) and ambiguous base calls were edited by visual inspection of chromatograms. If after several sequencing attempts, the quality of a particular base call was still ambiguous, this site was coded as missing data ("N"). Alignments were constructed automatically using the Muscle function in Sequencher and then proofread by eye and edited if necessary. Insertion and deletion variation in the sequence matrices was coded with SeqState 1.4.1 (Müller 2005), using the simple indel coding (SIC) and modified complex indel coding (MCIC) methods of Simmons and Ochoterena (2000) and Simmons et al. (2007). Mononucleotide repeats longer than 8 bp were identified at this stage and omitted in subsequent analyses, and ambiguously aligned gaps were treated as missing data.

Phylogenetic Analyses—Phylogenetic trees were constructed using single nuclear genes and also using the concatenated nuclear gene dataset and the concatenated chloroplast region dataset. For individual nuclear gene analyses, multiple alleles (if present) were included for the dioecious species and *A. dubius*. For concatenation of nuclear genes, multiple alleles for a single gene and single specimen were combined into a consensus sequence using IUPAC ambiguity codes for heterozygous sites. Because of

TABLE 1. Species and specimens sampled for the molecular phylogeny. The taxon name includes subspecies/variety (if applicable) and authority. "Subgenus Mosyakin and Robertson" lists the subgenus assignment according to Mosyakin and Robertson (1996); a question mark after the classification means that the authors did not specifically mention the species in their article, and we used their morphological criteria to place it into a subgenus and section. "Subgenus Bayón" lists the subgenus the species falls into according to the revision of the monoecious species by Bayón (2015). The geographical provenance of the specimen(s), given as country: state/province (if known), is followed by the abbreviation ID used in the phylogenetic trees. Geographical origin is the native range of the species; * = the original range is debated in the literature. Sources of geographical origin data: (1) Mosyakin and Robertson (2003); (2) Hunziker (1965); (3) Thellung (1914); (4) Costea and Tardif (2003); (5) Eliasson (1987); (6) Costea et al. (2001b); (7) Hunziker (1951); (8) Sauer (1967); (9) Palmer (2009); (10) Pedersen (1994); (11) Costea et al. (2003); (12) Bayón (2015); (13) Sauer (1957); (14) Costea et al. (2001a); (15) Brenan (1981); (16) Sohmer (1977); (17) Townsend (1993).

Taxon name	Subgenus Mosyakin and Robertson	Subgenus Bayón	Geographical provenance of specimens: Country: State/Province (ID in phylogeny)	Geographical origin of species: Continent(s): Area(s) within continent(s)
Amaranthus acanthochiton J.D.Sauer	*Acnida* sect. *Acanthochiton*	N/A	USA: Texas	North America: Southwestern U.S., northern Mexico (1)
Amaranthus acutilobus Uline & W.L.Bray	*Albersia* sect. *Pentamorion*	*Albersia*	Germany	North America: Southern Mexico (2)(3)
Amaranthus albus L.	*Albersia* sect. *Pyxidium*	*Albersia*	Canada: Saskatchewan (CA); South Africa (SA)	North America: Western and central U.S. and Canada (1)(4)
Amaranthus anderssonii J.T.Howell	*Albersia* sect. *Pentamorion*?	*Albersia*	Ecuador: Galápagos Islands	South America: Galápagos Islands (5)
Amaranthus arenicola I.M.Johnson	*Acnida* sect. *Saueranthus*	N/A	USA: Kansas	North America: U.S. Great Plains (1)
Amaranthus australis (A.Gray) J.D.Sauer	*Acnida* sect. *Acnida*	N/A	USA: Florida (FL1); USA: Florida (FL2)	Americas: Southeastern U.S., eastern Mexico, West Indies, northern South America (1)
Amaranthus blitoides S.Watson	*Albersia* sect. *Pyxidium*	*Albersia*	USA: Iowa (IA); USA: New Mexico (NM)	North America: Western and central U.S. and Canada (1)(4)
Amaranthus blitum L. subsp. *blitum*	*Albersia* sect. *Blitopsis*	*Albersia*	Switzerland	Eurasia* (1)(3)(6)
Amaranthus blitum L. subsp. *emarginatus* (Moq. ex Uline & W.L.Bray) Carretero, Muñoz Garm. & Pedrol	*Albersia* sect. *Blitopsis* (as *A. emarginatum*)	*Albersia* (as subsp. *polygonoides*)	USA: Florida (FL)	Eurasia* (1)(3)(6)
Amaranthus blitum L. subsp. *emarginatus* var. *pseudogracilis* (Thell.) Costea	N/A	*Albersia* (as subsp. *polygonoides* var. *pseudogracilis*)	USA: North Carolina (NC)	Eurasia* (1)(3)(6)
Amaranthus blitum L. subsp. *oleraceus* (L.) Costea	N/A	*Albersia*	Bangladesh	Eurasia* (1)(3)(6)
Amaranthus californicus (Moq.) S.Watson	*Albersia* sect. *Pyxidium*?	*Albersia*	USA: California	North America: Western U.S. and Canada (1)
Amaranthus cannabinus (L.) J.D.Sauer	*Acnida* sect. *Acnida*	N/A	USA: Virginia	North America: U.S. Atlantic Coast (1)
Amaranthus cardenasianus Hunz.	*Albersia* sect. *Pyxidium*?	*Amaranthus*	Bolivia	South America: Argentina, Bolivia (7)
Amaranthus caudatus L.	*Amaranthus* sect. *Amaranthus*	*Amaranthus*	Argentina (ARG); India (IND)	South America: Andean highlands (8)
Amaranthus centralis J.Palmer & Mowatt	*Albersia* sect. *Pentamorion*?	*Albersia*	Australia: Northern Territory	Australia: Central and northwestern regions (9)
Amaranthus clementii Domin	*Albersia* sect. *Pentamorion*	*Albersia*	Australia: Western Australia (AU1); Australia: Western Australia (AU2)	Australia: Western Australia (9)
Amaranthus cochleitepalus Domin	*Albersia* sect. *Pentamorion*?	*Albersia*	Australia: Northern Territory	Australia: Western and northern regions and Queensland (9)
Amaranthus crassipes Schltdl.	*Albersia* sect. *Pentamorion*	*Albersia*	Ecuador: Galápagos Islands (EC); USA: Texas (TX1); USA: Texas (TX2)	Americas: Southwestern U.S. and northern Mexico, Gulf of Mexico and surrounding coastal areas, to northern South America (1)
Amaranthus crispus (Lesp. & Thévenau) A.Braun ex J.M.Coult. & S.Watson	*Albersia* sect. *Pentamorion*	*Albersia*	Hungary	South America: Argentina (1)(10)
Amaranthus cruentus L.	*Amaranthus* sect. *Amaranthus*	*Amaranthus*	India (IND); Mexico (MX)	North and Central America: Southern Mexico and Guatemala (8)
Amaranthus cuspidifolius Domin	*Albersia* sect. *Pentamorion*?	*Albersia*	Australia: Western Australia (AU1); Australia: Western Australia (AU2)	Australia: Central and western regions (9)
Amaranthus deflexus L.	*Albersia* sect. *Blitopsis*	*Albersia*	Argentina (ARG1); Argentina: Buenos Aires (ARG2); Portugal (PT)	South America: pampas of Argentina (1)(10)

(*Continued*)

Table 1. (Continued).

Taxon name	Subgenus Mosyakin and Robertson	Subgenus Bayón	Geographical provenance of specimens: Country: State/Province (ID in phylogeny)	Geographical origin of species: Continent(s): Area(s) within continent(s)
Amaranthus dubius Mart. ex Thell.	*Amaranthus* sect. *Dubia*	*Amaranthus*	Cuba (CU); Venezuela (VZ)	Central and South America: West Indies and northern South America (1)
Amaranthus fimbriatus (Torr.) Benth. ex S.Watson	*Albersia* sect. *Pyxidium*?	*Amaranthus*	USA: Arizona (AZ1); USA: Arizona (AZ2)	North America: Southwestern U.S., northern Mexico (1)
Amaranthus floridanus (S.Watson) J.D.Sauer	*Acnida* sect. *Acnida*	N/A	USA: Florida	North America: Florida (1)
Amaranthus graecizans L. subsp. *aschersonianus* (Thell.) Costea, Brenner, & Tardif	*Albersia* sect. *Pyxidium*	*Albersia*	India	Eurasia: Mediterranean, South Asia, North Africa* (1)(6)(11)
Amaranthus graecizans L. subsp. *silvestris* (Villiers) Brenan	N/A	*Albersia*	Ecuador (EC); Portugal (PT)	Eurasia: Mediterranean, South Asia, North Africa* (1)(6)(11)
Amaranthus graecizans L. subsp. *thellungianus* (Nevski) Gusev	N/A	*Albersia*	Mauritania	Eurasia: Mediterranean, South Asia, North Africa* (1)(6)(11)
Amaranthus greggii S.Watson	*Acnida* sect. *Saueranthus*	N/A	USA: Louisiana (LA); USA: Texas (TX)	North America: Coastal Louisiana, Texas, Mexico (1)
Amaranthus hybridus L.	*Amaranthus* sect. *Amaranthus*	*Amaranthus*	Guatemala (GT); USA: Missouri (MO1); USA: Missouri (MO2)	Americas: eastern North America, Mexico, Central America, and northern South America (1)(8)
Amaranthus hypochondriacus L.	*Amaranthus* sect. *Amaranthus*	*Amaranthus*	India (IND); Mexico (MX)	North America: Western and central Mexico (8)
Amaranthus induratus C.A.Gardner ex J.Palmer & Mowatt	*Albersia* sect. *Pentamorion*?	*Albersia*	Australia: Western Australia	Australia: Northern and western regions (9)
Amaranthus interruptus R.Br.	*Albersia* sect. *Pentamorion*	*Albersia*	Australia: Northern Territory	Australia: Northern, northwestern, and central regions (9)
Amaranthus kloosianus Hunz.	*Albersia* sect. *Pentamorion*	*Albersia*	Argentina: Tucumán	South America: Argentina (7)
Amaranthus looseri Suess.	*Albersia* sect. *Pentamorion*?	*Albersia*	Chile	South America: Chile (2)
Amaranthus macrocarpus Benth. var. *macrocarpus*	*Albersia* sect. *Pentamorion*	*Albersia*	Australia	Australia: Eastern regions (9)
Amaranthus mitchellii Benth.	*Albersia* sect. *Pentamorion*	*Albersia*	Australia: Western Australia	Australia: Central and western regions (9)
Amaranthus muricatus (Moq.) Hieronymus	*Albersia* sect. *Pentamorion*	*Albersia*	Argentina: Buenos Aires (ARG1); Argentina: Salta (ARG2)	South America: Argentina, Bolivia, Paraguay, Uruguay (10)(12)
Amaranthus palmeri S.Watson	*Acnida* sect. *Saueranthus*	N/A	Mexico (MX); USA: Arizona (AZ1); USA: Arizona (AZ2)	North America: Southwestern U.S. and northern Mexico (13)
Amaranthus persimilis Hunz.	*Albersia* sect. *Pentamorion*	*Albersia*	Argentina	South America: Argentina (7)
Amaranthus polygonoides L.	*Albersia* sect. *Pentamorion*?	*Albersia*	USA: Texas	Americas: Southeastern U.S., Mexico and Central America, West Indies, northern South America (1)
Amaranthus powellii S.Watson subsp. *bouchonii* (Thell.) Costea & Carretero	*Amaranthus* sect. *Amaranthus* (as *A. bouchonii*)	N/A	Germany	North America and South America: Western Cordilleran region* (1)(8)(14)
Amaranthus powellii S.Watson subsp. *powellii*	*Amaranthus* sect. *Amaranthus*	*Amaranthus*	USA: Washington	North America and South America: Western Cordilleran region* (1)(8)(14)
Amaranthus praetermissus Brenan	*Albersia* sect. *Pyxidium*?	*Albersia*	South Africa	Africa: Angola, Botswana, Namibia, South Africa, Zimbabwe (15)
Amaranthus pumilus Raf.	*Albersia* sect. *Pentamorion*?	*Albersia*	USA: New Jersey (NJ); USA: North Carolina (NC); USA: South Carolina (SC)	North America: U.S. Atlantic Coast (1)
Amaranthus quitensis Kunth	*Amaranthus* sect. *Amaranthus*	*Amaranthus* (as subspecies of *A. hybridus*)	Argentina: Jujuy (ARG); Ecuador (EC)	South America: Andean highlands (8)
Amaranthus retroflexus L.	*Amaranthus* sect. *Amaranthus*	*Amaranthus*	US: Iowa	North America: Central and eastern North America (1)(8)
Amaranthus rhombeus R.Br.	*Albersia* sect. *Pyxidium*?	*Albersia*	Australia: Queensland (AU1); Australia: Northern Territory (AU2)	Australia: Coast of Northern Territory and Queensland (9)
Amaranthus scariosus Benth.	*Albersia* sect. *Pyxidium*?	*Amaranthus*	Nicaragua	North and Central America: Southern Mexico and Central America (8)
Amaranthus sclerantoides (Andersson) Andersson	*Albersia* sect. *Pyxidium*?	*Albersia*	Ecuador: Galápagos Islands	South America: Galápagos Islands (5)

(Continued)

Table 1.   (Continued).

Taxon name	Subgenus Mosyakin and Robertson	Subgenus Bayón	Geographical provenance of specimens: Country: State/Province (ID in phylogeny)	Geographical origin of species: Continent(s): Area(s) within continent(s)
Amaranthus spinosus L.	*Amaranthus* sect. *Centrusa*	*Amaranthus*	USA: Missouri (MO); USA: North Carolina (NC)	Americas: Neotropics (1)(8)
Amaranthus squamulatus (Andersson) B.L.Rob.	*Albersia* sect. *Pentamorion*?	*Albersia*	Ecuador: Galápagos Islands (EC1); Ecuador: Galápagos Islands (EC2)	South America: Galápagos Islands and coastal Ecuador (5)
Amaranthus standleyanus Parodi ex Covas	*Albersia* sect. *Pentamorion*	*Albersia*	Argentina: Tucumán (ARG1); Argentina (ARG2)	South America: Argentina, Paraguay (10)(12)
Amaranthus tamaulipensis Henrickson	*Albersia* sect. *Pyxidium*?	*Albersia*	Cuba	North America: Texas, northern Mexico (1)
Amaranthus thunbergii Moq.	*Albersia* sect. *Pyxidium*	*Albersia*	Namibia (NA1); Namibia (NA2)	Africa: Southern Africa and Eastern Africa (3)(15)
Amaranthus tricolor L.	*Albersia* sect. *Pyxidium*	*Albersia*	India: Madhya Pradesh (IND1); India: Tamil Nadu (IND2)	Eurasia: Tropical Asia (1)(3)
Amaranthus tuberculatus (Moq.) J.D.Sauer	*Acnida* sect. *Acnida*	N/A	USA: Illinois (IL); USA: Kansas (KS)	North America: Midwestern U.S., Ontario (1)
Amaranthus undulatus R.Br.	*Albersia* sect. *Pentamorion*	*Albersia*	Australia: Western Australia (AU1); Australia: Western Australia (AU2)	Australia: Northern and northwestern regions and Queensland (9)
Amaranthus urceolatus Benth.	*Albersia* sect. *Pentamorion*?	*Albersia*	Peru	South America: Peru, Ecuador (5)
Amaranthus viridis L.	*Albersia* sect. *Blitopsis*	*Albersia*	Brazil (BR); Indonesia: Java (INA)	South America: Tropical regions* (1)(3)(6)(10)
Amaranthus vulgatissimus Speg.	*Albersia* sect. *Pentamorion*	*Albersia*	Argentina: Salta (ARG1); Argentina: Tucumán (ARG2)	South America: Argentina (3)(10)
Amaranthus watsonii Standley	*Acnida* sect. *Saueranthus*	N/A	Mexico: Sonora	North America: Southwestern U.S. and northwestern Mexico (1)
Amaranthus wrightii S.Watson	*Amaranthus* sect. *Amaranthus*?	*Amaranthus*	USA: Texas (TX1); USA: Texas (TX2)	North America: Southwestern U.S. (1)
Chamissoa altissima (Jacq.) Kunth	N/A	N/A	Bolivia: Santa Cruz (BO); Mexico: Chiapas (MX)	Americas: Mexico to Argentina (16)
Pleuropterantha revoilii Franch.	N/A	N/A	Somalia	Africa: Ethiopia, Somalia (17)

the low phylogenetic informativeness of individual chloroplast genes and the complete linkage across the nonrecombining chloroplast genome, *trnL5′-trnL3′* and *matK/trnK* were always analyzed as a concatenated unit, after testing for topological conflict between individual chloroplast gene trees. The chloroplast and nuclear datasets support different phylogenetic positions for many species in the genus (see topology tests in Results); therefore, an analysis of all genes concatenated together was deemed inappropriate. Two methods were used to reconstruct trees: maximum parsimony (MP) and Bayesian inference (BI).

Maximum Parsimony Analyses—PAUP* v. 4.0b10 (Swofford 2002) was used to perform heuristic parsimony searches for each dataset. The parsimony search parameters were set to a tree bisection-reconnection branch swapping algorithm, with the starting tree obtained by stepwise addition and a simple addition sequence, and the maximum number of trees saved set to 20,000. The trees were rooted using specified outgroup sequences (*Chamissoa* and/or *Pleuropterantha*, depending on the sequences available for each dataset). Bootstrapping was performed in PAUP* using a full heuristic search with 1000 pseudoreplicates and 1000 maxtrees per pseudoreplicate, due to the extreme duration of heuristic searches with an unrestricted maximum number of trees. Decay indices (Bremer support) were calculated using TreeRot v. 3 (Sorenson and Franzosa 2007) from the PAUP* strict consensus tree for the concatenated chloroplast and concatenated nuclear datasets. TreeRot calculates the length of the shortest tree constrained to exclude each strict consensus node in turn, generating decay indices for each node. Trees were visualized using FigTree v. 1.4.2 (Rambaut 2012).

Bayesian Inference Analyses—We used the program MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway platform (Miller et al. 2010) with the "datablock" setting in order to use simple indel gap coding (which requires mixed data types). To choose the model of molecular evolution that best fit each data matrix, the program jModelTest v. 2.1.6 (Darriba et al. 2012) was run for each individual gene dataset and the model with the highest AIC (Akaike information criterion) value was selected. When MrBayes was inadequately parameterized to specify a model chosen by jModelTest, the most similar simpler model available was used (Table S2). For the concatenated datasets, a partitioned analysis was run (with a molecular model specified for each gene in the

dataset, and with parameters unlinked and rates set as variable among partitions to allow for independent evolution). A single outgroup sequence was specified for each dataset (from the accession *Chamissoa altissima* MX, except for the *G3PDH* dataset, for which *Pleuropterantha revoilii* was used). Each analysis consisted of two independent runs of four chains each (three heated, one cold), with 2,000,000 generations to start (first 25% of values discarded as burn-in), sampling the Markov chain every 1000 generations. All other MrBayes parameters were left set to the defaults. The program Tracer v. 1.6 (Rambaut et al. 2013) was used to assess convergence of model parameters. To determine whether the tree topology was affected by inclusion of *Amaranthus dubius*, a known allotetraploid, Bayesian analyses for each dataset were run with and without accessions of this species.

Topology Testing—We used Templeton's (1983) nonparametric test implemented in PAUP* to test taxonomic hypotheses about incomplete lineage sorting and incongruence between trees, and to test the monophyly of the Galápagos Island species and their closest relatives. The latter hypothesis was tested because of an intriguing pattern observed for the nuclear gene *G3PDH*, which supports all Galápagos species and their probable sister or progenitor species as belonging to the same small subgeneric clade in *Amaranthus*. Heuristic searches were conducted in the same way as above, except that topological constraints were applied to force certain groups to be monophyletic. The shortest constrained tree was compared to the shortest unconstrained tree using Templeton's test (the Wilcoxon signed-rank test). The following taxonomic hypotheses were tested. For each nuclear gene: alleles from the same dioecious accession form a monophyletic group; all sequences from dioecious species form a monophyletic group; or *Amaranthus dubius* sequences form a monophyletic group. For the concatenated nuclear dataset: *A. palmeri, A. watsonii,* and *A. pumilus* form a monophyletic group; or the former three species form a monophyletic group with *A. acanthochiton, A. arenicola, A. floridanus, A. greggii,* and *A. tuberculatus*. For the concatenated chloroplast dataset: *A. palmeri, A. watsonii,* and *A. spinosus* form a monophyletic group; or the former three species form a monophyletic group with the remainder of the Hybridus clade. For the concatenated nuclear, concatenated chloroplast, *A36,* and *Waxy* datasets: the Galápagos species and their close relatives (see below) form a monophyletic group. For *Waxy* and *G3PDH* datasets: all

dioecious species except *A. palmeri* and *A. watsonii* form a monophyletic group with *A. pumilus*.

Results

DNA Sequencing—In total, 650 new DNA sequences were generated for this study (635 *Amaranthus* sequences, 15 for the outgroups) and deposited in GenBank (accession numbers in Appendix 1). For *A36*, an aligned matrix of 762 bp was produced for 112 sequences, with 0.05% missing data. For *G3PDH*, an aligned matrix of 892 bp was produced for 112 sequences, with 0.67% missing data. Despite several attempts, neither accession of *Chamissoa altissima* could be sequenced for *G3PDH*, and a 110-bp section of *G3PDH* in *Pleuropterantha revoilii* was unalignable to *Amaranthus* and was coded as missing data. In addition, only a small fragment of one accession of *Amaranthus blitoides* (*A. blitoides* IA) could be sequenced for *G3PDH* and therefore the whole sequence was omitted (coded as missing data in the concatenated nuclear dataset). For ITS, an aligned matrix of 722 bp was produced for 107 sequences, with 0.10% missing data. For *Waxy*, an aligned matrix of 1350 bp was produced for 113 sequences, with 1.42% missing data. Despite several attempts, one accession of *C. altissima* (*C. altissima* BO) and one *Amaranthus* accession (*A. clementii* AU1) could not be sequenced for *Waxy* and were omitted from the dataset (coded as missing data in the concatenated nuclear dataset). For the other accession of *C. altissima* (*C. altissima* MX), only the last 420 bp were alignable, and the remainder of the sequence was coded as missing data. Additionally, a 140-bp section at the beginning of the sequence of *A. centralis* and a 330-bp intronic section of *P. revoilii* were both unalignable and were coded as missing data as well. Several smaller portions of the same intronic section of *Waxy* (< 100 bp) could not be sequenced for *A. centralis, A. cochleiptepalus, A. rhombeus* AU1, or *A. urceolatus*, and were coded as missing data. For the concatenated nuclear gene dataset, the aligned matrix was 3726 bp, and 103 sequences were included (2.14% missing data).

For the chloroplast regions, an aligned matrix of 668 bp was produced for *trnL5′-trnL3′* for 103 sequences, with 0.02% missing data. For *matK/trnK*, an aligned matrix of 2509 bp was produced for 103 sequences, with 0.43% missing data. For one accession of *C. altissima* (*C. altissima* BO) and for *A. urceolatus*, a 500-bp section in the *matK* region of *matK/trnK* could not be amplified successfully with the internal primers, and was coded as missing data. For the concatenated chloroplast dataset, the aligned matrix was 3177 bp, and 103 sequences were included (0.35% missing data).

For all subsequent phylogenetic analyses, mononucleotide repeats longer than 8 bp were omitted from the analysis for all datasets. These occurred a single time each in *G3PDH* (340–363 bp) and *trnL5′-trnL3′* (124–139 bp). Insertion and deletion variation was included in all subsequent analyses with simple indel coding (SIC). Modified complex indel coding was also used for each dataset for parsimony analysis; however, the topology of the resulting most parsimonious trees was identical to trees resulting from SIC datasets (K. Waselkov unpubl. data). Therefore, only the results from SIC datasets are reported here. Only unambiguously aligned indel variation was included in all analyses (with ambiguous gaps, found almost entirely relative to the outgroups, treated as missing data). Sequence alignments in FASTA format (including SIC and MrBayes command blocks) were submitted to the Dryad Digital Repository (Waselkov et al. 2018).

Phylogenetic Trees—Maximum Parsimony—The PAUP* analysis of the concatenated nuclear dataset was restricted to 20,000 most-parsimonious trees of 1881 steps. The consistency index (CI) of each tree was 0.736 (CI excluding autapomorphies = 0.647), and the retention index (RI) was 0.923. The strict consensus tree is shown in Fig. S1A, with bootstrap values from 1000 pseudoreplicates and Bremer support values. The PAUP* analysis of the concatenated chloroplast dataset was restricted to 20,000 most-parsimonious trees of 604 steps, with the CI = 0.859 (0.795 excluding autapomorphies), and the RI = 0.928. The strict consensus tree is shown in Fig. S1B. Maximum parsimony analyses were also conducted for each individual nuclear gene dataset, and bootstrap values for these analyses are mapped onto the Bayesian 50% majority-rule consensus trees (see below). Strict consensus tree files in NEXUS format from PAUP* analyses and TreeRot (Bremer support) analyses, as well as bootstrap support values (in the form of PAUP* log files) for all datasets, were submitted to the Dryad Digital Repository (Waselkov et al. 2018).

Bayesian Inference—Molecular models chosen for each species, based on highest AIC values in jModelTest, are listed in Table S2. For models that could not be implemented in MrBayes (which includes all four-by-four parameter models, such as TIM and TVM), the closest approximation was implemented (shown in Table S2). In all Bayesian analyses, simulations run for 2 million generations resulted in convergence of all parameters (verified with Tracer), with the exception of the *A36* dataset. For this single nuclear gene dataset, the Bayesian analysis was run for 20,000,000 generations. For all datasets, the topology of the Bayesian 50% majority-rule consensus tree is very similar to the strict consensus maximum parsimony tree, with slightly higher posterior probability values than parsimony bootstrap values for some clades (where > 85% is considered a high bootstrap value [70–84% is considered moderate support; Hillis and Bull 1993] and 0.98–1.0 is considered a high posterior probability value [0.90–0.97 is considered moderate support]). Bayesian 50% majority-rule consensus trees for the concatenated nuclear and concatenated chloroplast datasets are shown in Figs. 1A and 1B, and the equivalent trees for each individual nuclear gene dataset are shown in Supplementary Figs. S2A–D. Tree files in NEXUS format MrBayes analyses, as well as all Supplementary Tables and Figure images, were submitted to the Dryad Digital Repository (Waselkov et al. 2018).

Bayesian trees based on single nuclear genes show patterns suggesting incomplete lineage sorting (ILS) for alleles of the dioecious species and the allotetraploid *Amaranthus dubius*; alleles from the same accession are more closely related to alleles of another accession or species, rather than being monophyletic (Fig. S2A–D). These relationships differ between genes (as expected for ILS), and are consistent between molecular models of evolution (compare Figs. S2B and S2D to Waselkov 2013, Figs. 1.14b and 1.16b). Also, when *Amaranthus dubius* is excluded from each dataset, the Bayesian tree topologies are almost entirely identical (although posterior probabilities change slightly; K. Waselkov unpubl. data). The only exception is in the *Waxy* dataset tree, where one allele of *A. watsonii* switches from an unresolved position to join a clade consisting of *A. spinosus* and one *A. palmeri* MX allele with 0.92 posterior probability.
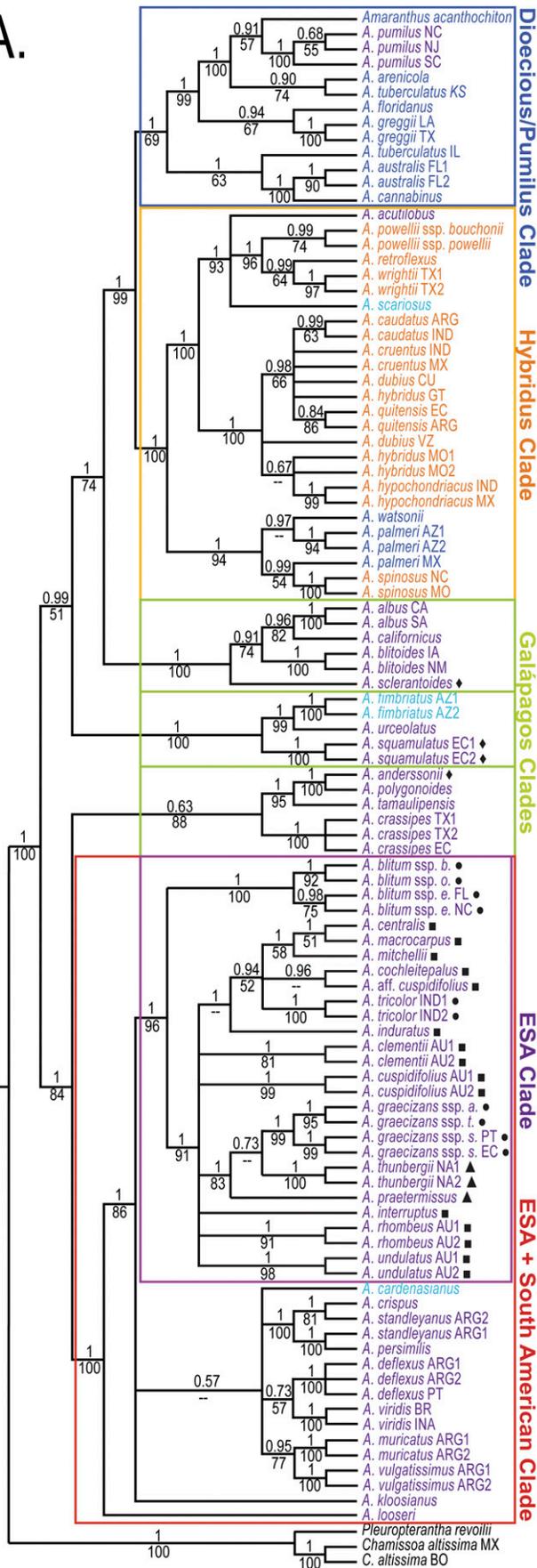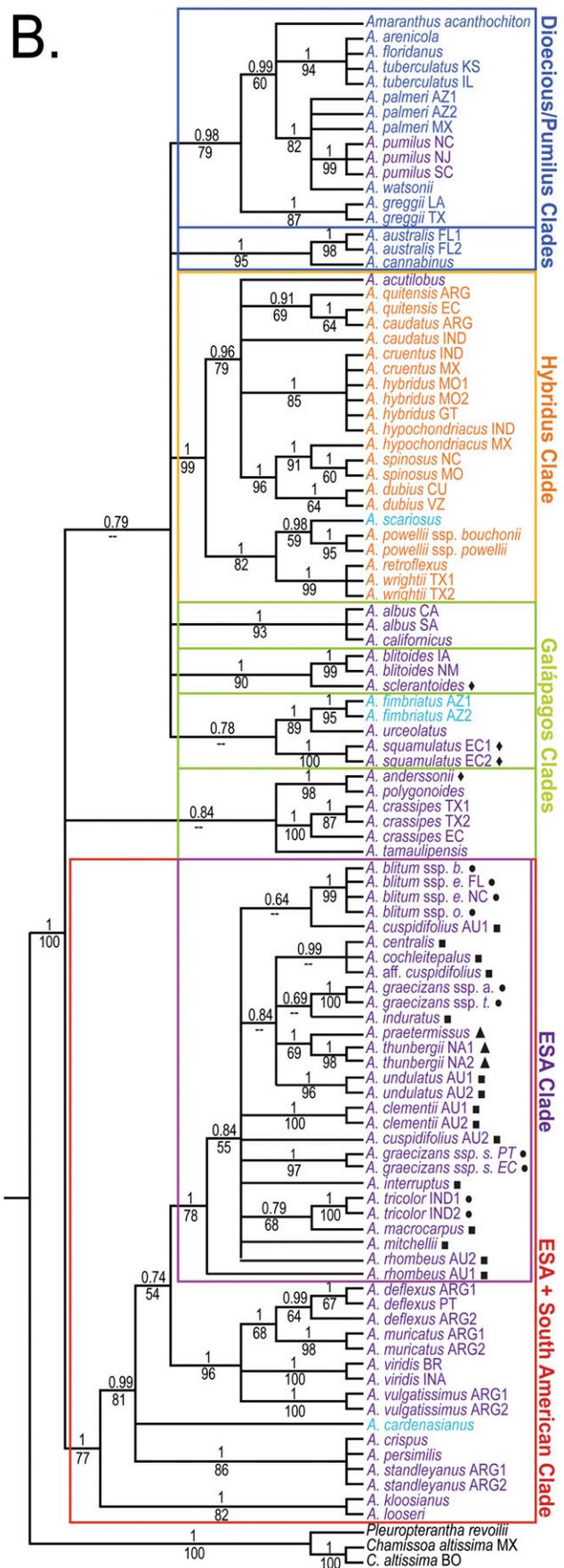
Fɪɢ. 1A, B.

Major Clades—The genus *Amaranthus* is supported as monophyletic, with *C. altissima* and *P. revoilii* supported as forming a clade when multiple outgroup sequences are included in the analysis (Bayesian posterior probability [PP]: 1.0 and parsimony bootstrap support [BS]: 100% in all unconstrained trees) (Figs. 1A, B). Additionally, all outgroup sequences are on long branches relative to the branch lengths within *Amaranthus* according to Bayesian analysis (see figures 1.1 and 1.2 in Waselkov 2013). There was no topological incongruence between trees reconstructed from *trnL5′-trnL3′* and *matK/trnK*; therefore, only the results of the concatenated chloroplast dataset are reported below. Within *Amaranthus*, several major clades are identifiable as supported (or at least not contradicted) by all genes. These are described below.

Eurasian/South African/Australian (ESA) + South American Clade—The Eurasian/South African/Australian (ESA) Clade contains all of the Old World species in *Amaranthus*. It is supported by the concatenated nuclear and chloroplast datasets (nuclear PP: 1.0/BS: 96%; chloroplast PP: 1.0/BS: 78%), and by the single nuclear genes *Waxy* (PP: 1.0/ BS: 95%), *A36* (PP: 1.0/BS: –), and *G3PDH* (PP: 0.99/BS: 66%). There are few well-supported relationships within this group. Species with multiple accessions are recovered as monophyletic (or unresolved) in all trees. Within the ESA clade, the two South African species are recovered as closely related to each other, but the concatenated datasets support different relationships between them: in the chloroplast tree, they are sister species (PP: 1.0/BS: 69%), while in the nuclear tree, they are in a clade with *A. graecizans*, a Eurasian species that occurs in North Africa (PP: 1.0/BS: 83%).

The remaining European and Australian species are largely unresolved: it appears that a specimen identified as having a morphological affinity to *A. cuspidifolius* (*A.* aff. *cuspidifolius*, Bates 50387) may be *A. cochleitepalus* or an unrecognized closely-related species, supported by the concatenated nuclear dataset (PP: 0.96/ BS: –), and the concatenated chloroplast dataset (PP: 0.99/BS: –). This chloroplast clade also places *A. centralis* with *A. cochleitepalus*, whereas the nuclear tree places *A. macrocarpus, A. mitchelli,* and *A. centralis* in a highly supported clade (PP: 1.0/BS: 58%).

The ESA clade is subtended by a grade of 11 South American *Amaranthus* species. The inclusion of these species in a larger clade with the ESA clade is supported by the concatenated nuclear (PP: 1.0/BS: 100%) and chloroplast (PP: 1.0/BS: 77%) datasets, and by the single nuclear genes *G3PDH* (PP: 1.0/BS: 99%) and *A36* (PP: 0.96/BS: –), and weakly by *Waxy* (PP: 0.76/ BS: 67%) (Figs. 1A, B, and S2A, B, D). The chloroplast dataset strongly supports *Amaranthus kloosianus* and *A. looseri* as sister taxa (PP: 1.0/BS: 82%) and together as the sister group to the remainder of the ESA + South American clade (PP: 0.99/BS: 81%), but in the nuclear tree, *A. looseri* is the sister taxon to the remainder of the clade [PP: 1.0/BS: 86%], and *A. kloosianus* has an unresolved position in the grade.

Among the remaining South American species, three species, *A. crispus, A. persimilis,* and *A. standleyanus*, form a monophyletic group (PP: 1.0 in the nuclear tree and the chloroplast tree). The relationships among *A. deflexus, A. muricatus, A. viridis,* and *A. vulgatissimus* are puzzling: various pairs of the species are highly supported as sister taxa to each other by different genes. The inclusion of all four species in a monophyletic group is supported strongly by the chloroplast dataset (PP: 1.0/BS: 86%), but not by any nuclear genes.

Hybridus Clade—The monophyletic group here called the Hybridus Clade consists of *A. hybridus* and its domesticated and wild or weedy relatives from the Americas, as well as two lesser-known Neotropical species, *A. acutilobus* and *A. scariosus*. It loosely corresponds to Mosyakin and Robertson's (1996) *Amaranthus* subgenus *Amaranthus*. It is supported by the concatenated nuclear (PP: 1.0/BS: 100%) and chloroplast (PP: 1.0/BS: 99%) datasets, and by three single nuclear genes: *A36* (PP: 0.99/ BS: –), *G3PDH* (PP: 1.0/BS: 99%), and *Waxy* (PP: 1.0/ BS: 90%) (Figs. 1A, B, and S2A, B, D). Relationships within this clade are also poorly resolved. Disagreement between the nuclear genes and the chloroplast regions complicates the placement of several species associated with this group, including *A. dubius, A. palmeri,* and *A. watsonii*; in fact, the latter two species are not included in this clade according to the chloroplast tree. These three species are discussed in more detail below.

All species of *Amaranthus* with published chromosome counts are $2n = 32$ or 34 ($n = 16$ or 17), except *Amaranthus dubius*, which is $2n = 64$ (Grant 1959c). *Amaranthus dubius* is a known allotetraploid that originated through hybridization between two species in this clade (Sauer 1967). This hybrid origin is reflected in the disagreement between the concatenated chloroplast and nuclear datasets in the placement of the species: *A. dubius* is strongly supported as the sister species to *A. spinosus* in the chloroplast tree (PP: 1.0/BS: 96%), while in the nuclear tree, the two *A. dubius* accessions appear in a clade with *A. hybridus, A. quitensis,* and the three domesticated pseudograin species (PP: 1.0/BS: 100%). *G3PDH, A36,* and *Waxy* were cloned for *A. dubius*, and one allele of each accession is supported as belonging to this same "core" *A. hybridus* group (with the inclusion of other species for *A36* and *G3PDH*), while the other is placed with *A. spinosus*, for each gene (Figs. S2A, B, D).

Another major disagreement between the chloroplast and nuclear trees is unexpected: *A. palmeri* and *A. watsonii* (considered probable sister species based on morphology) appear in a clade with *A. spinosus* alone with strong support in the nuclear tree (PP: 1.0/BS: 94%) and are a part of the larger Hybridus clade (PP: 1.0/BS: 100%). The inclusion of these two species in the Hybridus clade is also supported by three single nuclear genes (even with incomplete lineage sorting for alleles of these species [Figs. S2A, B, D]). The chloroplast dataset, in contrast, places *A. palmeri* and *A. watsonii* in a clade with *A. pumilus* (PP: 1.0/BS: 82%) and with

Fig. 1A, B. Bayesian (> 50%) majority rule consensus trees for *Amaranthus,* based on: A) four concatenated, partitioned nuclear genes; and B) two concatenated, partitioned chloroplast regions; both trees are rooted with two outgroups (*Chamissoa altissima* and *Pleuropterantha revoilii*). Subspecific taxa in *A. blitum* and *A. graecizans* are abbreviated to only the first letter; refer to Table 1 for full subspecific names, as well as the key to the geographical abbreviations in taxon names. Numbers above the branches are posterior probability values; numbers below the branches are bootstrap values from the maximum parsimony analysis (see Figs. S1A–B). Colors of taxon names indicate taxonomic subgenus: purple = subgenus *Albersia*; orange = subgenus *Amaranthus*; dark blue = subgenus *Acnida*; light blue = different subgenera depending on the taxonomic authority (*Albersia* according to Mosyakin and Robertson (1996) vs. *Amaranthus* according to Bayón (2015)). Colored boxes indicate clades discussed in this paper: purple = Eurasian/South African/Australian (ESA) clade; red = ESA + South American clade; orange = Hybridus clade; dark blue = Dioecious/Pumilus clade(s); light green = Galápagos clade(s). No symbol after the taxon name indicates a species native to the Americas; a square symbol indicates a species native to Australia; a triangle symbol indicates species native to Africa; a circle symbol indicates a species native to Eurasia; and a diamond symbol indicates a species native to the Galápagos Islands.

several of the other species in the Dioecious/Pumilus clade (*A. acanthochiton, A. tuberculatus, A. floridanus, A. arenicola*, PP: 0.99/BS: 60%) (see below).

In other relationships among species of the Hybridus clade, the nuclear tree supports *A. spinosus, A. palmeri*, and *A. watsonii* as the sister group to the remainder of the clade, but the chloroplast tree instead places a clade consisting of *A. retroflexus, A. wrightii, A. powellii*, and *A. scariosus* sister to the remaining Hybridus clade species. A clade of these same four species also occurs in the nuclear tree, except that it also includes *A. acutilobus*. Within this clade, *A. retroflexus* and *A. wrightii* are supported as sister taxa by the chloroplast dataset (PP: 1/BS: 99%), and the nuclear dataset (PP: 0.99/BS: 64%). Multiple accessions were sampled for *A. spinosus, A. powellii*, and *A. wrightii*, and each of these species is monophyletic or unresolved in every tree.

The core Hybridus group consists of *A. hybridus, A. hypochondriacus, A. caudatus, A. cruentus*, and *A. quitensis*. This group, with the inclusion of *A. dubius*, is well-resolved in the concatenated nuclear tree (PP: 1.0/BS: 100%), driven by ITS and *Waxy*, and unresolved in the *A36* and *G3PDH* trees. In the chloroplast tree, the core Hybridus clade is supported only with the inclusion of *A. dubius, A. spinosus*, and *A. acutilobus* (PP: 0.96/BS: 79%). Species and accessions in this group form a polytomy in most trees, with the exception of a few relationships. In the nuclear tree, the two accessions of *A. hypochondriacus* form a monophyletic group, but this is not the case in the chloroplast tree, where one accession is placed with *A. spinosus*. The remaining accession of *A. hypochondriacus* is highly supported as belonging to another clade with *A. hybridus* and *A. cruentus* (PP: 1.0/BS: 85%). Also, the two accessions of *A. caudatus* are monophyletic in the nuclear tree (PP: 0.99/BS: 63%), but not in the chloroplast tree, where *A. caudatus* ARG is strongly supported as the sister lineage to *A. quitensis* EC (PP: 1.0/BS: 64%).

DIOECIOUS/PUMILUS CLADE(S)—All dioecious species of *Amaranthus* are included in this group, except for *A. palmeri* and *A. watsonii* in the nuclear trees (see Hybridus Clade details above). The group is supported as monophyletic, with the inclusion of the monoecious species *A. pumilus*, by the concatenated nuclear dataset (PP: 1.0/BS: 69%), but not by the chloroplast dataset, which supports two separate clades of dioecious species plus *A. pumilus* and places *A. palmeri* and *A. watsonii* into the larger of these (Figs. 1A, B). The smaller dioecious clade in the chloroplast tree is made up of only *A. australis* and *A. cannabinus*. A single Dioecious/Pumilus clade is supported by ITS (PP: 1.0/BS: 68%) and *A36* (PP: 0.98/BS: –), but in the *Waxy* and *G3PDH* trees, these species appear as two or three phylogenetically disparate clades, respectively (Fig. S2A–D).

The dioecious species were all cloned for each nuclear gene, and alleles of a single accession often appear as non-monophyletic in patterns consistent with incomplete lineage sorting (Fig. S2A–D). This ambiguity obscures the relationships among species in this group, although some relationships between individual species are well resolved. *Amaranthus pumilus*, a monoecious species, is clearly closely related to the dioecious species, as its inclusion in a clade with some or all dioecious species is highly supported by every gene. The three accessions of *A. pumilus* are similar genetically and form a clade in all trees. Also, the sister-species relationship between *A. australis* and *A. cannabinus* is supported by both nuclear and chloroplast datasets (PP: 1.0/BS: 100%; PP: 1.0/BS: 95%, respectively).

GALÁPAGOS CLADE(S): ANDERSSONII, SCLERANTOIDES, AND SQUAMULATUS CLADES—The remaining species, all from the Americas, appear in various combinations in trees based on different genes. Only one gene, *G3PDH*, recovers these species as a monophyletic group (PP: 0.97/BS: 88%), which we call the Galápagos clade (because all Galápagos species occur in this clade) (Fig. S2D). The other datasets do not support this clade, but several analyses recover smaller "Galápagos clades" that each contains one of the Galápagos species and its close relatives. The Galápagos endemic *A. anderssonii* is closely related to the Caribbean species *A. polygonoides*; in fact, the sequences are identical for several genes, and every gene and concatenated dataset places the two in a highly-supported monophyletic group. This small clade and the Caribbean species *A. crassipes* are placed together along with the Mexican/Caribbean species *A. tamaulipensis* in the Anderssonii clade in the concatenated nuclear and chloroplast trees, albeit not with high support (PP: 0.63/BS: 88% and PP: 0.84/BS: –, respectively).

The western North American species *A. albus* and *A. californicus* are closely related, and occur together in a highly supported monophyletic group in every tree. In most trees, these species plus *A. blitoides* and *A. sclerantoides* (a Galápagos endemic) form what we have designated the Sclerantoides clade: the latter two species are a separate clade from *A. albus* and *A. californicus* in the chloroplast tree, and the *A36* nuclear gene supports *A. blitoides* in a clade with *A. albus* and *A. californicus* but excludes *A. sclerantoides* (PP: 0.97/BS: –). Finally, the Galápagos species *A. squamulatus* is closely related to two other species, the southwestern USA species *A. fimbriatus* and the South American species *A. urceolatus*, which form a group we call the Squamulatus clade. Somewhat surprisingly, *A. fimbriatus* and *A. urceolatus* appear to be each others' closest relatives (PP: 1.0/BS: 99% in the nuclear tree, PP: 1.0/BS: 89% in the chloroplast tree), with *A. squamulatus* being their sister taxon (PP: 1.0/BS: 100% in the nuclear tree, PP: 0.78/BS: – in the chloroplast tree).

TREE BACKBONE—There is some evidence for a monophyletic group consisting of the Hybridus clade and the Dioecious/Pumilus clade(s), as shown in the concatenated nuclear tree (PP: 1.0/BS: 99%) (Fig. 1A). This relationship is supported by *A36* (PP: 1.0/BS: 66%) and *G3PDH* (PP: 0.98/BS: 62%) and very weakly by ITS (PP: 0.57/BS: –), but is not supported by *Waxy* (which includes the Sclerantoides and Squamulatus Galápagos clades in the same clade as these two large groups [PP: 0.93/BS: –]); the relationship between the clades is unresolved in the concatenated chloroplast tree (Figs. 1B, and S2A–D).

The position of the other major clades of *Amaranthus* relative to each other is uncertain, especially the position of the Galápagos clade(s) relative to the ESA + South American clade and the Hybridus + Dioecious/Pumilus clade, and the position of these two latter clades relative to each other. A polytomy of these clades within a monophyletic *Amaranthus* appears to be the best-supported representation of evolutionary relationships based on this study. The major clades are shown as colored boxes in Figs. 1A and 1B.

Topology Tests—We used Templeton's (1983) nonparametric test implemented in PAUP* to test several hypotheses derived from the phylogenetic analyses. First, we wanted to test whether incomplete lineage sorting in the dioecious species was highly supported in the individual gene trees, by comparing the length of the shortest tree constrained to keep

alleles from the same dioecious accessions monophyletic to the length of the shortest unconstrained tree for each gene. We also constrained the trees to keep each dioecious species monophyletic, which allowed alleles from a single accession to be nonmonophyletic.

Results of these tests depended on the gene. ITS trees were not significantly longer with dioecious alleles or species constrained to be monophyletic (390 and 389 steps respectively, compared to 388 steps in the unconstrained tree). But *A36* trees were significantly longer (198 steps ($p = 0.02$) and 200 steps ($p = 0.01$) respectively, compared to 191 steps in the unconstrained tree), and *G3PDH* trees were significantly longer than the basic tree (566 steps) with alleles constrained as monophyletic (617 steps, $p < 0.0001$) or species constrained as monophyletic (600 steps, $p = 0.0001$). *Waxy* trees were also significantly longer than the unconstrained tree (929 steps) with alleles constrained as monophyletic (1000 steps, $p < 0.0001$) or species constrained as monophyletic (981 steps, $p = 0.0001$).

Next, we wanted to test whether the placement of *A. palmeri* and *A. watsonii* was highly supported by the nuclear dataset and the chloroplast dataset. When the nuclear tree was constrained to place *A. palmeri* and *A. watsonii* in a monophyletic group with *A. pumilus* (as in the chloroplast tree), the constrained tree was significantly longer (1952 vs. 1881 steps in the unconstrained tree, $p < 0.0001$). When the nuclear tree was constrained to place the two species in the broader monophyletic group of *A. acanthochiton, A. arenicola, A. floridanus, A. greggii, A. tuberculatus,* and *A. pumilus*, the constrained tree was still significantly longer (1966 steps, $p < 0.0001$). When the chloroplast tree was constrained to put the two species in a monophyletic group with *A. spinosus* (as in the nuclear tree), the tree was significantly longer (622 vs. 604 steps in the unconstrained tree, $p = 0.0001$). However, when the constrained tree contained a broader monophyletic group with the two species, *A. spinosus* and the rest of the Hybridus clade, the tree was not significantly longer (609 steps).

Finally, we wished to test the monophyly of the Galápagos clade, given that all of the Galápagos species and their close relatives occur in a single clade in the *G3PDH* tree, and the placement of various Galápagos clades within the genus is not highly supported in most other trees. When the nuclear tree or the chloroplast tree was constrained to keep all the Galápagos species and their close relatives in a single monophyletic group, the constrained trees were not significantly longer (1891 vs. 1881 steps, and 605 vs. 604 steps, respectively). However, a *Waxy* tree constrained to create a single Galápagos clade was significantly longer (959 vs. 929 steps, $p < 0.0001$).

DISCUSSION

Our phylogenetic analyses of relationships between species in the genus *Amaranthus* supports the suspected relationships between some species and clades based on morphology, and offers new insights into the relationships of other species where morphological data have been equivocal. Three of the major clades identified here roughly correspond to the three morphologically-defined subgenera of *Amaranthus* recognized by Mosyakin and Robertson (1996), Costea et al. (2001a), and Bayón (2015): the Eurasian/South African/Australian (ESA) + South American clade corresponds to *Amaranthus* subgenus *Albersia*, the Hybridus clade corresponds to *Amaranthus* subgenus *Amaranthus*, and the Dioecious/Pumilus clade(s) correspond(s) to *Amaranthus* subgenus *Acnida* (Figs. 1A, B).

But there are species in all three of these clades that were not predicted based on morphology, and no taxonomic authority has ever placed the Galápagos species and their relatives into a separate taxon or several separate taxa, instead lumping them into subgenus *Albersia*.

Genus Age and Origin of the ESA Clade—Biogeographical relationships among the species are also interesting, as the tree suggests that the genus probably originated and radiated first in the Americas, with only one clade giving rise to Old World species (Figs. 1A, B). We chose not to date our *Amaranthus* phylogeny (following the example of Sage et al. 2007) for several reasons: 1) No reliably identified fossils are available for the root of Amaranthaceae s. s., or for any clades within the group (Kadereit et al. 2003, 2012); 2) The age of the Galápagos Islands is not a useful calibration point (in contrast to the origin of Mauritius, in Thiv et al. 2006), because the age of various submerged and extant islands in the archipelago ranges between 9 and 1 MYA (Geist et al. 2014). Unfortunately, the Galápagos endemic species of *Amaranthus* do not compose an intra-archipelago radiation, nor is either species restricted to a single island (which could be confidently dated). However, we can roughly extrapolate the age of the genus from Kadereit et al. (2003), where the authors used fossils to calibrate estimates of the age of clades in the Amaranthaceae s. l. (including the Chenopodiaceae; stem age estimated at 87–47 MYA in Kadereit et al. 2012). That study dated the root of the Chenopodioideae at 65–56.5 MYA using two fossils, and with these plus another fossil at the crown of the Chenopodieae I clade, estimated a substitution rate of $2.8–4.1 \times 10^{-9}$ synonymous substitutions per site per year for the chloroplast *rbcL* gene. Since there are 51 substitutions among the 1124 synonymous sites of *rbcL* along the branches from the point of the Chenopodioideae root to the genus *Amaranthus* (included in their tree), we can estimate the stem age of *Amaranthus* at 16.2–11.1 MYA from Kadereit et al.'s (2012) analysis.

Even if this estimate is inaccurate, the genus *Amaranthus* is presumably younger than Amaranthaceae s. l., which implies that the Old World species of *Amaranthus* arose from dispersal, rather than vicariance. The exact sequence of continental separation during the break-up of the Southern Hemisphere supercontinent of Gondwana is still disputed, but the latest date for the end of the break-up is 80 MYA (Upchurch 2008). Furthermore, it appears from our phylogenies that a single dispersal event out of South America could have given rise to the entire ESA clade. Whether *Amaranthus* first dispersed to South Africa, Australia, or even Eurasia is unclear, due to poor resolution within the ESA + South American clade; the chloroplast dataset weakly supports a South American-Australian connection, which has some precedent in the Amaranthaceae s. s. (the 19 MYA split between South American *Nitrophila* and Australian *Hemichroa* in the Polycnemoideae; Masson and Kadereit 2013). The taxonomic diversity of the genus in Australia also lends some credence to this hypothesis. Whichever continent was colonized first, the subsequent arrival of *Amaranthus* on the other two Old World continents is consistent with at least two long-dispersal events.

Precedence for Phylogenetic Relationships Among Monoecious Species—Relationships among species in the ESA + South American clade are generally poorly resolved in our phylogenies, but the few well-resolved relationships have some precedent in the taxonomic and phylogenetic literature on *Amaranthus*. Hunziker (1951) considered the South American species *A. persimilis, A. standleyanus, A. crispus,*

and *A. cardenasianus* similar morphologically, although he also thought *A. squamulatus* resembled *A. cardenasianus*, and believed that *A. kloosianus* was related to *A. urceolatus* rather than the former group. Bayón (2015) noted the close morphological similarity between *A. crispus* and *A. standleyanus*, and noted that *A. vulgatissimus* was similar to *A. deflexus*, although he placed *A. cardenasianus* into *Amaranthus* subgenus *Amaranthus* (which roughly corresponds to our Hybridus clade) rather than *Amaranthus* subgenus *Albersia*. Brenan (1981), contemplating introduced *Amaranthus* species in southern Africa, noticed that *A. deflexus*, *A. viridis*, and *A. muricatus* were similar, and Mosyakin and Robertson (2003) mentioned that *A. deflexus* and *A. muricatus* hybridize naturally.

Previous analyses have not predicted the apparent relationship between the Australian, Eurasian, and South African species. Because of nomenclatural confusion surrounding *A. graecizans*, it has been frequently mentioned in the taxonomic literature as similar to *A. albus* and *A. blitoides* (Costea et al. 2001b); however, Mosyakin and Robertson (2003) proposed that it was more closely related to Old World taxa with trimerous flowers, which is consistent with our results. Similarly, *A. blitum* and *A. viridis* were considered very similar by Eliasson (1987), and Coons (1981) noted that these species hybridize in South America. In our nuclear tree, the basal node in the ESA clade separates *A. blitum* from the remaining members. Within the Australian species, Palmer (2009) concluded that *A. centralis* was most similar to *A. induratus*, which was echoed by Bayón (2015), but these species are not closely related in our trees, although the nuclear and chloroplast trees disagree on the placement of *A. centralis*. We did not include several South African species in our study (*A. schinzianus, A. dinteri,* and *A. capensis*), so the close relationship between the African species *A. thunbergii* and *A. praetermissus* in our trees may be an artifact of sampling.

The Hybridus clade, on the other hand, has been the subject of many studies because of great interest in the origin of the pseudograin amaranth species, *A. hypochondriacus* (from Central Mexico), *A. cruentus* (from southern Mexico/ Guatemala), and *A. caudatus* (from the Andes) (Sauer 1950). Costea et al. (2001a) completed a taxonomic treatment on the "*Amaranthus hybridus* species complex," including the grain amaranths, *A. hybridus*, *A. hybridus* subsp. *quitensis*, *A. powellii*, and *A. retroflexus*, supporting the recognition of the domesticated species as taxonomic entities separate from *A. hybridus*. Sauer (1967) hypothesized that *A. powellii*, *A. hybridus*, and *A. quitensis* were the respective progenitors of *A. hypochondriacus*, *A. cruentus*, and *A. caudatus*, but subsequent authors found support for Sauer's alternative hypothesis of a single or multiple origins of the grain amaranths from *A. hybridus,* with possible involvement of *A. quitensis* in the domestication of *A. caudatus* (Coons 1977, 1978; Hauptli and Jain 1984; Chan and Sun 1997; Xu and Sun 2001). The most recent studies support the hypothesis that *A. hybridus* was domesticated independently at least twice, possibly three times, in different geographical areas to create the three grain species (Mallory et al. 2008; Maughan et al. 2011; Kietlinski et al. 2014; Clouse et al. 2016; Stetter and Schmid 2017). The role of *A. quitensis* is still debated, as this species is a closely associated weed of *A. caudatus* fields, and subsequent gene flow could explain the genetic similarity of these taxa (Sauer 1967; Kietlinski et al. 2014; Stetter et al. 2017).

Our study was not focused on identifying the relationships between domesticated grain species and their wild ancestors, and our results can contribute little to this discussion, given our minimal sampling of these taxa. Several studies based on rapidly-evolving markers such as isozymes, RAPDs, and microsatellites have produced polytomies of *A. hybridus* and the grain amaranths, with *A. quitensis* either inside or the sister taxon to this group if it was included in the study (e.g. Chan and Sun 1997; Sun et al. 1999; Xu and Sun 2001; Mallory et al. 2008); this pattern is essentially what our trees show. For a better-resolved picture of grain species relationships and review of current knowledge in this area, see Kietlinski et al. (2014). Many of these same domestication-focused studies recovered the sister-lineage relationship of *A. powellii* and/or *A. retroflexus* (shown in our trees) to this core *A. hybridus* group, as did Song et al. (2000). Mosyakin and Robertson (2003) noted that *A. wrightii* was closely related to *A. retroflexus*, which is borne out in our study. Chan and Sun's (1997) isozyme/RAPD phylogenies and Stetter and Schmid's (2017) GBS phylogenies both included *A. acutilobus* and inferred that it fell within the *A. hybridus* clade, which is also consistent with our results.

Sauer (1967) hypothesized from the cytological work of Grant (1959b) that *A. spinosus* probably hybridized with a species of the *A. hybridus* complex to create the allotetraploid *A. dubius*. Our chloroplast tree strongly supports *A. dubius* as the sister lineage to *A. spinosus*, which leads us to believe that *A. spinosus* or the lineage that led to *A. spinosus* was its maternal parent. In the nuclear tree, *A. dubius* is supported as belonging to the core Hybridus group, although we cannot tell which of the species in the complex was its paternal parent; single nuclear genes support one allele of each accession with *A. spinosus* and the other allele in the core Hybridus group. The placement of *A. dubius* in the Hybridus clade was anticipated genetically by Chan and Sun (1997) and Stetter and Schmid (2017).

Chloroplast Capture and ILS in the Dioecious Species—The placement of *A. palmeri* and *A. watsonii* has been unclear in the previous literature. Their sister relationship was strongly suggested based on morphology (Standley 1914; Brenan 1961). Mosyakin and Robertson's (1996) taxonomic treatment included all the dioecious *Amaranthus* species in *Amaranthus* subgenus *Acnida*, even though the authors recognized that the group was "artificial and polyphyletic" (Mosyakin and Robertson 2003). There were several previous indications that *A. palmeri* might be related to the Hybridus clade: Franssen et al. (2001) noticed that the pollen morphology of *A. palmeri* was unlike that of the other dioecious *Amaranthus* species sampled and more closely resembled that of the monoecious species. Chan and Sun (1997) placed *A. palmeri* as the sister lineage to their *A. hybridus* clade with isozyme and RAPD data, Wassom and Tranel (2005) placed *A. palmeri* and *A. spinosus* together based on AFLP data, and Riggins et al. (2010) placed *A. palmeri* and *A. spinosus* together and as the sister group to the Hybridus clade based on the *ALS* gene. It is also noteworthy that *A. spinosus* is one of only two diploid monoecious species of *Amaranthus* with spatial separation of male and female flowers: in this species, only female flowers are found in leaf axils and at the base of the inflorescence spike, while male flowers are found at the spike apex (rather than the typical mixed-sex glomerules found in monoecious species) (Bayón 2015). (The Cuban endemic species *A. minimus* (not sampled in this study) has male and female flowers in separate leaf axils, but its overall morphology is different from *Amaranthus*

subgenus *Amaranthus*.) To some authors, this separation within the plant suggested a trend toward dioecy (Mosyakin and Robertson 1996); the same authors anticipated the placement of *A. spinosus* as sister to the remainder of *Amaranthus* subgenus *Amaranthus* in our nuclear tree (Mosyakin and Robertson 2003).

The present study found strongly-supported incongruence between the nuclear and chloroplast datasets in the placement of *A. palmeri* + *A. watsonii* (further upheld by Templeton tests). This pattern suggests a possible ancient chloroplast capture event from the lineage leading to the other dioecious species (see Rieseberg and Soltis 1991; Rieseberg et al. 1996; and Tsitrone et al. 2003 for reviews of chloroplast capture in plant phylogenies and conditions that promote capture). This type of plastid introgression event appears more likely than an event in which nuclear material from both hybridizing species was retained, as none of the four nuclear genes support the chloroplast tree's placement of *A. palmeri* + *A. watsonii* in the Dioecious/Pumilus clade. It is curious that *A. pumilus* is the most closely related species to *A. palmeri* + *A. watsonii* according to the chloroplast tree, because they are native to opposite ends of a continent: *A. pumilus* is an endangered beach specialist endemic to the Atlantic coast of the USA, and *A. palmeri* and *A. watsonii* are both from the southwestern USA. The fact that *A. palmeri* and *A. watsonii* are the only dioecious species placed outside of the Dioecious/Pumilus clade by the nuclear tree also suggests that dioecy in *Amaranthus* could be a trait encoded or influenced strongly by the chloroplast. However, dioecy is dominant over the monoecious condition in crosses of monoecious species with *A. tuberculatus*, regardless of the direction of the cross, which implies a nuclear element in breeding-system determination (Murray 1940; Trucco et al. 2006). Alternatively, perhaps the ancestor of *A. palmeri* + *A. watsonii* was already dioecious before the chloroplast capture event, extending the trend toward spatial separation of flowers of different sexes observed in *A. spinosus*.

The apparent inclusion of *A. pumilus* in the Dioecious/Pumilus clade was anticipated by one previous study. Nolan et al. (2010) studied the population genetics and phylogenetic relationships of *A. pumilus* using ISSRs, and found that *A. arenicola* was weakly grouped with *A. pumilus* by neighbor-joining and Bayesian inference, although these analyses did not group the other sampled dioecious species with this clade. No other authors have put forward hypotheses about the evolutionary affinities of *A. pumilus*, because of its morphological distinctiveness in the genus. This federally threatened monoecious species has larger seeds than any other *Amaranthus* species, and the entire plant is fleshy (Mosyakin and Robertson 2003).

Incomplete lineage sorting (ILS) leads to problems with recovering the species tree from single-gene trees (Degnan and Rosenberg 2009). In our analyses, the topology of the Dioecious/Pumilus clade is different in our phylogenies based on different nuclear genes. Concatenation of genes may lead to an incorrect species-tree phylogeny when gene trees differ and molecular models of evolution are different for each gene (Degnan and Rosenberg 2009); however, our concatenated nuclear dataset is partitioned to account for this. Increased within-species sampling can improve the likelihood of estimating the true species tree for shallower phylogenies (Maddison and Knowles 2006). A number of new methods for estimating species' trees in the presence of incomplete lineage sorting are becoming available (e.g. Mirarab and Warnow 2015; Vachaspati and Warnow 2015); an in-depth study of

relationships in the Dioecious/Pumilus clade would ideally include more within-species sampling and would test several of the new methods for congruence.

It should be noted that the strongly supported non-monophyly of *A. tuberculatus* in the concatenated nuclear tree (as opposed to possible monophyly in the chloroplast tree) may not be an artifact of incomplete lineage sorting, but a correct reflection of evolutionary history. *Amaranthus tuberculatus* was previously considered to be two largely allopatric species based on morphology (Sauer 1967; Pratt and Clark 2001), and the sample of *A. tuberculatus* from west of the Mississippi River is placed phylogenetically with other western dioecious species, whereas the sample from east of the Mississippi River is grouped with eastern North American dioecious species. The two "species" are now considered varieties by some authors (Costea and Tardif 2003), and it is possible that they or their ancestral taxa might have originated separately from different dioecious groups and subsequently coalesced into one species through hybridization.

Biogeography of Galápagos Clade(s)—The Galápagos clades in our trees support the relationships of the three endemic or native Galápagos *Amaranthus* species with North and South American species. Some of these relationships were predicted based on morphology: Eliasson (1985, 1987) noted that the Galápagos species *A. anderssonii* and the Caribbean *A. berlandieri* (= *A. polygonoides*) were virtually indistinguishable morphologically. However, Eliasson also proposed that *A. anderssonii* and *A. squamulatus*, another Galápagos native that also occurs in the coastal province of Guayas in mainland Ecuador, were closely related, which is not supported in our trees. Instead, *A. squamulatus* forms a separate clade with a pair of species from North America (*A. fimbriatus*) and South America (*A. urceolatus*), and oddly enough, it appears to be the sister lineage to this group, rather than being more closely related to the South American species. This observation raises the possibility that the presence of *A. squamulatus* in mainland Ecuador is due to a dispersal event from the Galápagos, rather than the other way around. Its relationship to *A. urceolatus* was predicted by Eliasson (1987), but no author has previously linked the North American desert species *A. fimbriatus* to this group.

Several researchers recognized the similarity of *A. albus*, *A. blitoides*, and *A. californicus* (Mosyakin and Robertson 2003; Bayón 2015). *Amaranthus albus* and *A. blitoides* were placed together in a neighbor-joining tree based on *ALS* gene sequence data by Riggins et al. (2010), and based on ITS data by Song et al. (2000). The only author to connect the Galápagos species *A. sclerantoides* to this group was Hunziker (1965), who placed nine species in a group based on their axillary inflorescences, 1–5 tepals, and 1–5 stamens, and included all four of the species in this clade. However, he also included *A. looseri* and *A. acutilobus*, which are supported in our study as belonging to the paraphyletic South American group subtending the ESA clade, and the Hybridus clade, respectively. Finally, the Caribbean species *A. crassipes* was discovered for the first time in the Galápagos Islands during the course of this study: it was observed as a single population on Isla Santiago in both 2011 and 2013, and thus represents a persistent (but not necessarily anthropogenic) introduction.

Templeton tests indicate that we cannot rule out the possibility that all of the Galápagos species and their close relatives, which appear in three separate clades in the nuclear tree and four clades in the chloroplast tree, are actually a single

monophyletic group. Three Galápagos colonization events from a single group of *Amaranthus* and none from any of the other three major clades in the genus would imply that successful colonization of the Galápagos Islands involves a non-random set of ancestral traits. More phylogenetic work to resolve the relationships among the major clades of the genus might settle this point. Furthermore, the connection of the Galápagos species to Caribbean and southwestern North American relatives is congruent with the discovery that a number of endemic Galápagos vascular plant species previously assumed to be closely tied to nearby South America (Porter 1979) in fact originated in the Caribbean, Central America, "Tropical Americas," or southwestern North America (Tye and Francisco-Ortega 2011). The endemic Galápagos *Amaranthus* species are probably dispersed internal or externally (in mud) by birds, and many Galápagos birds are migratory and travel thousands of miles each year (Porter 1983). Rare bird dispersal of *Amaranthus* to the archipelago could explain the Galápagos biogeography seen in this genus.

Comparison with Stetter and Schmid (2017)—With regard to overall phylogenetic patterns, it is very interesting to compare the recent phylogenetic results of Stetter and Schmid (2017) to our results here, given that entirely different methods were used to reconstruct phylogenies in each case. Stetter and Schmid (2017) used biallelic SNPs gleaned from analysis of GBS data to reconstruct neighbor-joining and SNAPP (species tree) phylogenies, whereas we used single Sanger-sequenced loci to construct gene trees, concatenated loci from the nuclear or chloroplast genome to obtain better support for relationships, and used maximum parsimony or Bayesian inference for tree building. Both their neighbor joining tree (Stetter and Schmid 2017 figure 2) and their SNAPP consensus tree (Stetter and Schmid 2017 figure 5) are comparable to our concatenated nuclear tree in Fig. 1A, because genotyping-by-sequencing primarily captures nuclear SNPs (since the nuclear genome is so much larger than the chloroplast genome). Additionally, the authors' de novo assembly (which conceivably could include cpDNA SNPs) produced the same phylogeny as when they aligned to reference genomes (see Stetter and Schmid's (2017) figure S1).

Several accessions were misidentified in Stetter and Schmid's (2017) analyses (based on visual inspection of their voucher photos in Supplementary Material 2), including ID1 *Amaranthus acanthochiton*, ID4 *A. arenicola*, ID5 *A. asplundii*, ID123 *A. hybridus*, and probably ID211 and ID216 *A. graecizans*. *Amaranthus* species are notoriously morphologically confusing, and even curated seedbanks such as USDA GRIN and IPK Gatersleben can have mistakes in germplasm labeling, which means that researchers working in taxonomically complex groups should not have too much confidence in the listed taxonomic designations. In the current study, all species identifications were double-checked morphologically, either against a voucher (if field collected) or by growing the seeds to reproductive maturity to key out.

If these misidentified accessions in the analyses of Stetter and Schmid (2017) are ignored, the nuclear trees from both analyses show a large amount of concordance. Both our Fig. 1A and Stetter and Schmid's (2017) figure 2 show what we call the core Hybridus group and the other authors call the "Hybridus complex" of *A. hybridus, A. quitensis, A. caudatus, A. hypochondriacus*, and *A. cruentus*, although in our analysis *A. dubius* accessions appear nested inside of this clade. Since the genome-wide data from Stetter and Schmid's (2017) GBS method captured a larger amount of the nuclear genome than our methods, it is probable that *A. dubius* was being pulled in two directions by nuclear alleles from its two parents in their analysis, with the result that it was placed between its parent in the "Hybridus complex" and its other parent, *A. spinosus*. Stetter and Schmid (2017) included a much larger number of accessions of the pseudograin domesticates and their close relatives than our analyses, leading to a better resolution of the phylogenetic origin of these species. Also placed in their *Amaranthus* subgenus *Amaranthus* (our Hybridus clade) is the clade composed of *A. acutilobus, A. retroflexus, A. powellii*, and *A. wrightii*.

In Stetter and Schmid's (2017) *Amaranthus* subgenus *Albersia* (plus *A. fimbriatus* and *A. tucsonensis*) are placed all the same species that are placed into our ESA + South American clade and Galápagos clades. This subgenus appears to be monophyletic in some of Stetter and Schmid's (2017) trees, unlike in our trees, in which the backbone of the generic phylogeny is less resolved. Notably, in their SNAPP tree, *A. crassipes, A. tamaulipensis*, and *A. polygonoides* are placed in a clade with *A. albus, A. blitoides*, and *A. californicus*, lending further credence to the hypothesis that at least two of the Galápagos species (*A. anderssonii* and *A. sclerantoides*), sister to different species in this group, may have originated from the same small clade within the genus (supported by our *G3PDH* trees and Templeton tests; see above). We did not include *A. × tucsonensis* in our final analyses, as this taxon is a suspected recently-arisen hybrid (Henrickson 1999); in the process of our phylogenetic analyses, the same USDA accession of this species was included and then removed from all trees to clarify results, and it appeared in the Squamulatus clade based on nuclear genes (K. Waselkov unpubl. data). The consensus on *A. × tucsonensis'* close relationship to *A. fimbriatus* is noteworthy, as its authority Henrickson (1999) eliminated *A. fimbriatus* as a parent based on morphology. Relationships within Stetter and Schmid's (2017) *Amaranthus* subgenus *Albersia* also largely agree with our results, with the qualification that their geographical sampling omitted Australia and South Africa.

In *Amaranthus* subgenus *Acnida*, Stetter and Schmid (2017) recovered two distinct clades that correspond to our nuclear Dioecious/Pumilus clade (but with sampling of only *A. australis, A. tuberculatus*, and *A. floridanus*) and the *A. palmeri/A. watsonii/A. spinosus* clade in our Hybridus clade. Indeed, the latter clade was supported as sister to *Amaranthus* subgenus *Amaranthus* in Stetter and Schmid's (2017) figure 5 phylogeny. It is remarkable that even with their much greater amount of nuclear data, Stetter and Schmid's (2017) analyses placed *A. palmeri* in a clade with *A. spinosus* with a high posterior probability, and never in a clade with the other dioecious species they sampled. Based on our chloroplast phylogeny, a hybridization event took place involving the ancestor of *A. palmeri* and *A. watsonii*. Consequently, these species are now phylogenetically placed in the Dioecious/Pumilus clade using chloroplast DNA, and in the Hybridus clade by at least some nuclear DNA; if there were any nuclear introgression from the Dioecious/Pumilus clade into these two species, Stetter and Schmid's (2017) GBS methods would have been more likely to detect it than our methods. Either a small amount of nuclear genomic material must have been introgressed during this ancestral hybridization event, or a chloroplast capture event occurred, as hypothesized above.

Evolution of Weediness in Amaranthus—There is much interest in *Amaranthus* in the weed science community from the perspective of agricultural invasion and especially herbicide resistance evolution (Tranel and Trucco 2009). The nine species listed as "introduced, invasive, and noxious weeds" by the USDA are scattered through the phylogeny: in the nuclear tree, *A. tuberculatus* is found in the Dioecious/ Pumilus clade, *A. hybridus, A. retroflexus, A. powellii, A. spinosus,* and *A. palmeri* in the Hybridus clade, *A. albus* and *A. blitoides* in the Sclerantoides clade, and *A. viridis* in the ESA clade. Phylogenetic signal for agricultural invasiveness was explicitly tested in R using several different metrics of "weediness" and no signal was found (Waselkov 2013); this supports the idea of a lack of phylogenetic constraint in the evolution of weeds in *Amaranthus*, and/or homoplasy in weedy traits. This study was not designed to explore weed evolution in depth, but should be used as a starting point for further phylogenetically informed research on agricultural invasiveness. A phylogenetic approach is seldom applied to researching the evolution of "weediness" (but see Daehler 1998; Lososová et al. 2008), despite its proven usefulness in understanding both traits correlated with invasion success and the potential interaction of the invasive with other community members (e.g. Burns 2004; Parker and Gilbert 2004; Van Kleunen et al. 2008; Fenesi et al. 2011). Furthermore, agriculturally adaptive traits other than herbicide resistance have been somewhat neglected in *Amaranthus* research (Tranel and Trucco 2009). Studies to identify morphological and physiological traits involved in "weediness" should involve carefully designed common garden studies of congeneric pairs of invasive and non-invasive *Amaranthus* species, and/or phylogenetically independent contrasts.

At the genus level, the relatedness of various *Amaranthus* species could affect their ability to hybridize, and potentially to exchange important "weedy" alleles such as herbicide resistance alleles (e.g. Molin et al. 2016). However, previous studies on hybridization between *Amaranthus* weeds have demonstrated that the level of reproductive compatibility between two species can be hard to predict based on their degree of relationship. *Amaranthus tuberculatus* and *A. palmeri* are as related as *A. tuberculatus* and *A. hybridus* (or more related, at least at chloroplast loci), based on our phylogenetic results. However, stronger pre- and postzygotic barriers between *A. tuberculatus* and *A. palmeri* seem to exist: of the 69 offspring from an experimental cross of these two species, 60 were the result of agamospermy in *A. palmeri*, eight were nonviable, and only one was a true, fertile hybrid (Trucco et al. 2007). On the other hand, *A. tuberculatus* and *A. hybridus* frequently hybridize in nature (Pratt 1999) and can be successfully crossed in a controlled setting. Although fertility is greatly reduced in the hybrids, backcrosses with *A. tuberculatus* can transfer a number of *A. hybridus* alleles into this species (the same is not true for the reciprocal backcross) (Trucco et al. 2009). The phylogeny could be helpful for generating hypotheses about reproductive compatibility between weed species in the genus, but these should be carefully tested with greenhouse experiments.

Species for Future Phylogenetics—Our species-level sampling was the most complete of any phylogenetic study of the genus *Amaranthus* to date, but there remain at least 17–24 species to be placed phylogenetically (Henrickson 1999, 2004; Mosyakin and Robertson 2003; Bayón 2015). Two island

endemics would be particularly important to include in future studies: *A. brownii*, an endangered (possibly extinct) species endemic to Nihoa island in Hawai'i; and *A. minimus*, a species with highly derived reproductive morphology endemic to Cuba. A substantial effort was made to relocate *A. furcatus*, a localized species endemic to Isla Santa Cruz in the Galápagos, without success; this species is similar morphologically to *A. sclerantoides*. Also noteworthy is that new species of *Amaranthus* are still being described from the Americas (*A. hunzikeri* by Bayón (2007); *A. pedersenianus* by Bayón and Pelaez (2012); *A. neei* by Sánchez-del Pino et al. (2017)), and more undoubtedly remain undiscovered in herbaria (K. Waselkov pers. obs.).

We reconstructed the *Amaranthus* generic phylogeny using six molecular markers to answer questions about the biogeographic relationships and monophyly of the subgenera in the group. We found that the monophyly of the three subgenera, *Amaranthus* subgenera *Acnida, Albersia,* and *Amaranthus* (as defined in Mosyakin and Robertson 1996), is partially supported. The dioecious species of *Amaranthus* subgenus *Acnida* are closely related to a monoecious species, *A. pumilus*, which has never been included in this subgenus. The Eurasian/South African/Australian clade plus its subtending South American species contain most of the species from *Amaranthus* subgenus *Albersia*, but the position of the Galápagos species and their close relatives, which have been included in *Amaranthus* subgenus *Albersia*, is uncertain. Lastly, the Hybridus Clade includes all of the species usually included in *Amaranthus* subgenus *Amaranthus*, but also includes *A. palmeri* and *A. watsonii* (according to the nuclear gene trees), which are dioecious and usually placed into *Amaranthus* subgenus *Acnida*. The substantial disagreement between nuclear and chloroplast-based gene trees in the genus *Amaranthus* is another significant finding of our work: both chloroplast capture in the lineage leading to *A. palmeri/A. watsonii* and incomplete lineage sorting are invoked as explanations for this disagreement. The biogeographic relationships in *Amaranthus* are also interesting. Although the root of the tree is poorly resolved, the genus appears to have originated in the Americas, and only one major clade in the genus gave rise to Old World species, possibly via a single long-distance dispersal event from South America. Furthermore, the genus colonized the Galápagos Islands in three separate events, rather than radiating within the islands. The closest relatives of the Galápagos species are found in western North America or the Caribbean, rather than mainland South America, which fits well with a recent revision of the biogeographic relationships of many other Galápagos plants (Tye and Francisco-Ortega 2011). This study will be the starting point for more in-depth investigations into relationships between and within subgenera of *Amaranthus*, further testing of biogeographic hypotheses within the genus, and the study of the evolution and underlying genetics of weedy traits in the group.

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APPENDIX 1. Specimens examined with scientific name and authority, voucher information, locality of original collection (abbreviation ID in phylogenetic trees) and GenBank accession numbers for sequence data from *A36, G3PDH, ITS, Waxy, trnL5′-trnL3′*, and *matK/trnK*. A dash (—) indicates that data was not obtained for that gene. Voucher information: If seeds were obtained from the USDA GRIN Database (Agricultural Research Service in Ames, IA), a PI number or Ames number is listed. If material was obtained from another source, the collector and collection number, as well as the herbarium and herbarium accession number (if available), are listed instead.

Ingroup: *Amaranthus acanthochiton* J.D.Sauer, USDA PI 632238, USA: Texas, MG684892, MG684995, MG685198, MG685301, MG685402, MG685094. *Amaranthus acutilobus* Uline & W.L.Bray, USDA PI 633579, Germany, MG684893, MG684996, MG685199, MG685302, MG685403, MG685095. *Amaranthus albus* L., USDA PI 633580, Canada: Saskatchewan (CA), MG684894, MG684997, MG685200, MG685303, MG685404, MG685096. *Amaranthus albus* L., Le Roux sub Boatwright 528, NBG 0268533-0, South Africa (SA), MG684895, MG684998, MG685201, MG685304, MG685405, MG685097. *Amaranthus anderssonii* J.T.Howell, H. Jäger s.n., CDS 13607, Ecuador: Galápagos Islands, MG684896, MG684999, MG685202, MG685392, MG685406, MG685098. *Amaranthus arenicola* I.M. Johnson, USDA PI 607459, USA: Kansas, MG684897, MG685000, MG685203, MG685305, MG685407, MG685099. *Amaranthus australis* (A. Gray) J.D.Sauer, USDA PI 553076, USA: Florida (FL1), MG684898, MG685001, MG685204, MG685306, MG685408, MG685100. *Amaranthus australis* (A.Gray) J.D.Sauer, J. Richard Abbott 25276, FLAS 232341, USA: Florida (FL2), MG684899, MG685002, MG685205, MG685307, MG685409, MG685101. *Amaranthus blitoides* S.Watson, USDA PI 553079, USA: Iowa (IA), MG684900, —, MG685206, MG685308, MG685410, MG685102. *Amaranthus blitoides* S.Watson, USDA Ames 27956, USA: New Mexico (NM), MG684901, MG685003, MG685207, MG685309, MG685411, MG685103. *Amaranthus blitum* L. subsp. *blitum*, USDA PI 606751, Switzerland, MG684902, MG685004, MG685208, MG685310, MG685412, MG685104. *Amaranthus blitum* L. subsp. *emarginatus* (Moq. ex Uline & W.L. Bray) Carretero, Muñoz Garm. & Pedrol, J. Richard Abbott 24900, FLAS 226902, USA: Florida (FL), MG684903, MG685005, MG685209, MG685311, MG685413, MG685105. *Amaranthus blitum* L. subsp. *emarginatus* var. *pseudogracilis* (Thell.) Costea, USDA PI 632245, USA: North Carolina (NC), MG684905, MG685007, MG685211, MG685313, MG685415, MG685107. *Amaranthus blitum* L. subsp. *oleraceus* (L.) Costea, USDA PI 606282, Bangladesh, MG684904, MG685006, MG685210, MG685312, MG685414, MG685106. *Amaranthus californicus* (Moq.) S.Watson, USDA PI 595319, USA: California, MG684906, MG685008, MG685212, MG685314, MG685416, MG685108. *Amaranthus cannabinus* (L.) J.D.Sauer, USDA PI 568124, USA: Virginia, MG684907, MG685009, MG685213, MG685315, MG685417, MG685109. *Amaranthus cardenasianus* Hunz., D. Rocabado et al. 499, MO 4787435, Bolivia, MG684908, MG685010, MG685214, MG685316, MG685418, MG685110. *Amaranthus caudatus* L., USDA Ames 15178, Argentina (ARG), MG684909, MG685011, MG685215, MG685317, MG685419, MG685111. *Amaranthus caudatus* L., USDA PI 166045, India (IND), MG684910, MG685012, MG685216, MG685318, MG685420, MG685112. *Amaranthus centralis* J.Palmer & Mowatt, D. E. Albrecht 8892, CANB 527441, Australia: Northern Territory, MG684911, MG685013, MG685217, MG685397, MG685421, MG685113. *Amaranthus clementii* Domin, R. Cranfield 9595, CANB 496410, Australia: Western Australia (AU1), MG684912, MG685014, MG685218, —, MG685422, MG685114. *Amaranthus clementii* Domin, I. D. Cresswell 97VI-OP-03, CANB 497238, Australia: Western Australia (AU2), MG684913, MG685015, MG685219, MG685319, MG685423, MG685115. *Amaranthus cochleitepalus* Domin, D.E. Albrecht 9153, CANB 577421, Australia: Northern Territory, MG684914,

MG685016, MG685220, MG685393, MG685424, MG685116. *Amaranthus crassipes* Schltdl., K. Waselkov 222, CDS, Ecuador: Galápagos Islands (EC), MG684917, MG685019, MG685223, MG685322, MG685427, MG685119. *Amaranthus crassipes* Schltdl., USDA PI 642743, USA: Texas (TX1), MG684915, MG685017, MG685221, MG685320, MG685425, MG685117. *Amaranthus crassipes* Schltdl., USDA PI 649302, USA: Texas (TX2), MG684916, MG685018, MG685222, MG685321, MG685426, MG685118. *Amaranthus crispus* (Lesp. & Thévenau) A.Braun ex J. M.Coult. & S.Watson, USDA PI 633582, Hungary, MG684918, MG685020, MG685224, MG685323, MG685428, MG685120. *Amaranthus cruentus* L., USDA PI 566897, India (IND), MG684919, MG685021, MG685225, MG685324, MG685429, MG685121. *Amaranthus cruentus* L., USDA PI 477913, Mexico (MX), MG684920, MG685022, MG685226, MG685325, MG685430, MG685122. *Amaranthus* aff. *cuspidifolius*, R. Bates 50387, CANB 689602, Australia: South Australia, MG684921, MG685023, MG685227, MG685326, MG685431, MG685123. *Amaranthus cuspidifolius* Domin, J. Palmer 605, CANB 599739, Australia: Western Australia (AU1), MG684922, MG685024, MG685228, MG685327, MG685432, MG685124. *Amaranthus cuspidifolius* Domin, J. Palmer 699, CANB 775595, Australia: Western Australia (AU2), MG684923, MG685025, MG685228, MG685328, MG685433, MG685125. *Amaranthus deflexus* L., USDA PI 667169, Argentina (ARG1), MG684924, MG685026, MG685230, MG685329, MG685434, MG685126. *Amaranthus deflexus* L., S. Torres Robles 400, LP, Argentina: Buenos Aires (ARG2), MG684925, MG685027, MG685231, MG685330, MG685435, MG685127. *Amaranthus deflexus* L., USDA PI 633576, Portugal (PT), MG684926, MG685028, MG685232, MG685331, MG685436, MG685128. *Amaranthus dubius* Mart. ex Thell., USDA PI 642739, Cuba (CU), MG684927, MG685029, MG685233, MG685332, MG685437, MG685129. *Amaranthus dubius* Mart. ex Thell., USDA Ames 15320, Venezuela (VZ), MG684928, MG685030, MG685234, MG685333, MG685438, MG685130. *Amaranthus fimbriatus* (Torr.) Benth. ex S.Watson, USDA PI 612855, USA: Arizona (AZ1), MG684929, MG685031, MG685235, MG685334, MG685439, MG685131. *Amaranthus fimbriatus* (Torr.) Benth. ex S.Watson, USDA PI 662285, USA: Arizona (AZ2), MG684930, MG685032, MG685236, MG685335, MG685440, MG685132. *Amaranthus floridanus* (S.Watson) J.D.Sauer, USDA PI 553078, USA: Florida, MG684931, MG685033, MG685237, MG685336, MG685441, MG685133. *Amaranthus graecizans* L. subsp. *aschersonianus* (Thell.) Costea, Brenner, & Tardif, USDA PI 288277, India, MG684932, MG685034, MG685238, MG685337, MG685442, MG685134. *Amaranthus graecizans* L. subsp. *silvestris* (Villiers) Brenan, USDA PI 604196, Ecuador (EC), MG684934, MG685036, MG685240, MG685339, MG685444, MG685136. *Amaranthus graecizans* L. subsp. *silvestris* (Villiers) Brenan, USDA PI 658732, Portugal (PT), MG684933, MG685035, MG685239, MG685338, MG685443, MG685135. *Amaranthus graecizans* L. subsp. *thellugianus* (Nevski) Gusev, USDA PI 549157, Mauritania, MG684935, MG685037, MG685241, MG685340, MG685445, MG685137. *Amaranthus greggii* S.Watson, USDA PI 667170, USA: Louisiana (LA), MG684936, MG685038, MG685242, MG685341, MG685446, MG685138. *Amaranthus greggii* S.Watson, USDA PI 632240, USA: Texas (TX), MG684937, MG685039, MG685243, MG685342, MG685447, MG685139. *Amaranthus hybridus* L., USDA PI 677074, Guatemala (GT), MG684939, MG685041, MG685245, MG685344, MG685449, MG685141. *Amaranthus hybridus* L., K. Waselkov 38, MO, USA: Missouri (MO1), MG684938, MG685040, MG685244, MG685343, MG685448, MG685140. *Amaranthus hybridus* L., K. Waselkov 39, MO, USA: Missouri (MO2), MG684940, MG685042, MG685246, MG685345, MG685450, MG685142. *Amaranthus hypochondriacus* L., USDA PI 477915, India (IND), MG684941, MG685043, MG685247, MG685400, MG685451, MG685143. *Amaranthus hypochondriacus* L., USDA PI 477917, Mexico (MX), MG684942, MG685044, MG685248, MG685401, MG685452, MG685144. *Amaranthus induratus* C.A.Gardner ex J.Palmer & Mowatt, A.A. Mitchell 5749, CANB 556042, Australia: Western Australia, MG684943, MG685045, MG685249, MG685346, MG685453, MG685145. *Amaranthus interruptus* R.Br., L. A. Craven et al. 9659, CANB 498997, Australia: Northern Territory, MG684944, MG685046, MG685250, MG685347, MG685454, MG685146. *Amaranthus kloosianus* Hunz., A. Plos and P. Simon 133, MO 6455604, Argentina: Tucumán, MG684945, MG685047, MG685251, MG685348, MG685455, MG685147. *Amaranthus looseri* Suess., M. Muñoz 5103, MO 6219670, Chile, MG684946, MG685048, MG685252, MG685349, MG685456, MG685148. *Amaranthus macrocarpus* Benth. var. *macrocarpus*, J. Hosking 3238, TARCH 7630, Australia, MG684947, MG685049, MG685253, MG685350, MG685457, MG685149. *Amaranthus mitchellii* Benth., A. A. Mitchell 8726 B, CANB 711440, Australia: Western Australia, MG684948, MG685050, MG685254, MG685351, MG685458, MG685150. *Amaranthus muricatus* (Moq.) Hieronymus, J. Hurrell et al. 3881, LP, Argentina: Buenos Aires (ARG1), MG684949, MG685051, MG685300, MG685352, MG685459, MG685151. *Amaranthus muricatus* (Moq.) Hieronymus, A. Plos and P.

Simon 158, MO, Argentina: Salta (ARG2), MG684950, MG685052, MG685255, MG685353, MG685460, MG685152. *Amaranthus palmeri* S. Watson, USDA PI 633593, Mexico (MX), MG684953, MG685055, MG685258, MG685356, MG685463, MG685155. *Amaranthus palmeri* S. Watson, USDA PI 632235, USA: Arizona (AZ1), MG684951, MG685053, MG685256, MG685354, MG685461, MG685153. *Amaranthus palmeri* S. Watson, USDA PI 612856, USA: Arizona (AZ2), MG684952, MG685054, MG685257, MG685355, MG685462, MG685154. *Amaranthus persimilis* Hunz., A. Plos and P. Simon 135, MO 6455564, Argentina, MG684954, MG685056, MG685259, MG685357, MG685464, MG685156. *Amaranthus polygonoides* L., USDA PI 658733, USA: Texas, MG684955, MG685057, MG685260, MG685358, MG685465, MG685157. *Amaranthus powellii* S. Watson subsp. *bouchonii* (Thell.) Costea & Carretero, USDA PI 572261, Germany, MG684956, MG685058, MG685261, MG685359, MG685466, MG685158. *Amaranthus powellii* S.Watson subsp. *powellii*, USDA PI 604671, USA: Washington, MG684957, MG685059, MG685262, MG685360, MG685467, MG685159. *Amaranthus praetermissus* Brenan, J. Manning s.n., NBG, South Africa, MG684958, MG685060, MG685263, MG685361, MG685468, MG685160. *Amaranthus pumilus* Raf., Mt. Cuba Center 2000211*A, USA: New Jersey (NJ), MG684960, MG685062, MG685265, MG685363, MG685470, MG685162. *Amaranthus pumilus* Raf., USDA PI 553083, USA: North Carolina (NC), MG684959, MG685061, MG685264, MG685362, MG685469, MG685161. *Amaranthus pumilus* Raf., USDA PI 553085, USA: South Carolina (SC), MG684961, MG685063, MG685266, MG685364, MG685471, MG685163. *Amaranthus quitensis* Kunth, F. Zuloaga 11496, MO 6293643, Argentina: Jujuy (ARG), MG684963, MG685065, MG685268, MG685366, MG685473, MG685165. *Amaranthus quitensis* Kunth, USDA PI 511745, Ecuador (EC), MG684962, MG685064, MG685267, MG685365, MG685472, MG685164. *Amaranthus retroflexus* L., USDA PI 603852, US: Iowa, MG684964, MG685066, MG685269, MG685367, MG685474, MG685166. *Amaranthus rhombeus* R.Br., B. Gray 7948, CANB 670451, Australia: Queensland (AU1), MG684965, MG685197, MG685270, MG685394, MG685475, MG685167. *Amaranthus rhombeus* R.Br., A. P. Roberts et al. 804, CANB 693250, Australia: Northern Territory (AU2), MG684966, MG685067, MG685271, MG685398, MG685476, MG685168. *Amaranthus scariosus* Benth., I. Coronado G. and R. M. Rueda 3570, MO 6180339, Nicaragua, MG684967, MG685068, MG685272, MG685368, MG685477, MG685169. *Amaranthus sclerantoides* (Andersson) Andersson, K. Waselkov 206, CDS, Ecuador: Galápagos Islands, MG684968, MG685069, MG685273, MG685369, MG685478, MG685170. *Amaranthus spinosus* L., B. Summers 6179, MO, USA: Missouri (MO), MG684970, MG685071, MG685275, MG685371, MG685480, MG685172. *Amaranthus spinosus* L., USDA PI 632248, USA: North Carolina (NC), MG684969, MG685070, MG685274, MG685370, MG685479, MG685171 *Amaranthus squamulatus* (Andersson) B.L.Rob., K. Waselkov 205, CDS 48780, Ecuador: Galápagos Islands (EC1), MG684971, MG685072, MG685276, MG685372, MG685481, MG685173. *Amaranthus squamulatus* (Andersson) B.L.Rob., K. Waselkov 204, CDS 48779, Ecuador: Galápagos Islands (EC2), MG684972, MG685073, MG685277, MG685373, MG685482, MG685174. *Amaranthus standleyanus* Parodi ex Covas, A. Plos and P. Simon 132, MO, Argentina: Tucumán (ARG1), MG684973, MG685074, MG685278, MG685395, MG685483, MG685175. *Amaranthus standleyanus* Parodi ex Covas, F. Zuloaga 11559, MO 6293640, Argentina (ARG2), MG684974, MG685075, MG685279, MG685374, MG685484, MG685176. *Amaranthus tamaulipensis* Henrickson, USDA PI 642738, Cuba, MG684975, MG685076, MG685280, MG685375, MG685485, MG685177. *Amaranthus thunbergii* Moq., HK 871, NPGRC 1889, Namibia (NA1), MG684976, MG685077, MG685281, MG685376, MG685486, MG685178. *Amaranthus thunbergii* Moq., HK 1038, NPGRC 2111, Namibia (NA2), MG684977, MG685078, MG685282, MG685377, MG685487, MG685179. *Amaranthus tricolor* L., USDA PI 599683, India: Madhya Pradesh (IND1), MG684978, MG685079, MG685283, MG685378, MG685488, MG685180. *Amaranthus tricolor* L., USDA PI 566899, India: Tamil Nadu (IND2), MG684979, MG685080, MG685284, MG685379, MG685489, MG685181. *Amaranthus tuberculatus* (Moq.) J.D.Sauer, K. Waselkov 56, MO, USA: Illinois (IL), MG684981, MG685082, MG685286, MG685381, MG685491, MG685183. *Amaranthus tuberculatus* (Moq.) J.D.Sauer, K. Waselkov 74, MO, USA: Kansas (KS), MG684980, MG685081, MG685285, MG685380, MG685490, MG685182. *Amaranthus undulatus* R.Br., J. Palmer 580, CANB 599392, Australia: Western Australia (AU1), MG684982, MG685083, MG685287, MG685382, MG685492, MG685184. *Amaranthus undulatus* R.Br., J. Palmer 652, CANB 686336, Australia: Western Australia (AU2), MG684983, MG685084, MG685288, MG685383, MG685493, MG685185. *Amaranthus urceolatus* Benth., S. Llatas Quiroz 3057, MO 3318704, Peru, MG684984, MG685085, MG685289, MG685396, MG685494, MG685186. *Amaranthus viridis* L., USDA PI 652434, Brazil (BR), MG684985, MG685086, MG685290,

MG685384, MG685495, MG685187. *Amaranthus viridis* L., USDA PI 540445, Indonesia: Java (INA), MG684986, MG685087, MG685291, MG685385, MG685496, MG685188. *Amaranthus vulgatissimus* Speg., J. A. Tolaba and R. Alacón 3427, LP, Argentina: Salta (ARG1), MG684987, MG685088, MG685292, MG685386, MG685497, MG685189. *Amaranthus vulgatissimus* Speg., A. Plos and P. Simon 108, MO, Argentina: Tucumán (ARG2), MG684988, MG685089, MG685293, MG685387, MG685498, MG685190. *Amaranthus watsonii* Standley, A. C. Sanders et al. 8768, MO 4919874, Mexico: Sonora, MG684989, MG685090, MG685294, MG685388, MG685499, MG685191. *Amaranthus wrightii* S.Watson, USDA PI 632242, USA: Texas (TX1), MG684990, MG685091, MG685295, MG685389, MG685500, MG685192. *Amaranthus wrightii* S.Watson, USDA PI 632243, USA: Texas (TX2), MG684991, MG685092, MG685296, MG685390, MG685501, MG685193.

Outgroup: *Chamissoa altissima* (Jacq.) Kunth, Carrasco et al. 272, MO 4821722, Bolivia: Santa Cruz (BO), MG684994, —, MG685299, —, MG685504, MG685196. *Chamissoa altissima* (Jacq.) Kunth, L. Alvarado-Cárdenas et al. 1182, MO 6327402, Mexico: Chiapas (MX), MG684993, —, MG685298, MG685399, MG685503, MG685195. *Pleuropterantha revoilii* Franch., M. Thulin 10831, UPS BOT:V-122975, Somalia, MG684992, MG685093, MG685297, MG685391, MG685502, MG685194.