Phylogenetics and evolution of *Euphorbia* subgenus *Chamaesyce*

by

Ya Yang

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Ecology and Evolutionary Biology)
in The University of Michigan
2012

Doctoral Committee:

Professor Paul E. Berry, Chair
Professor Laura J. Olsen
Associate Professor Christopher W. Dick
Associate Professor Yin-Long Qiu

DEDICATION

To my parents: Yuqi Zhang, and Bangjie Yang

ACKNOWLEDGMENTS

I would like to thank my advisor Paul Berry for his guidance, patience and encouragement throughout the lengthy course of my Ph.D., and all the previous and present members on my dissertation committee, Victor Steinmann, Yin-Long Qiu, Chris Dick, Mark Hunter and Laura Olsen for their insights and thoughtful critiques of my work. I also thank the past and present members of the Berry Lab for all the valuable advice and inspiring discussions, especially postdoc scholars Benjamin van Ee, Ricarda Riina, Jeff Morawetz, and Jess Peirson; lab technicians Susana Pereira and Hope Draheim; fellow graduate students Brian Dorsey and Elizabeth Haber; and REU student Becky Povilus. I would not have made it through graduate school without help and support from friends and colleagues, especially Bin Wang, Yaowu Yuan, Huateng Huang, Libo Li, Malini Sridharan, Luis Chaves, Shalene Jha, Tory Hendry, and Sandra Yap. I would like to thank present and former staff members of the Department of Ecology and Evolutionary Biology and the University of Michigan Herbarium, especially Sonja Botes, Julia Eussen, Gail Kuhnlein, Rich Rabeler, and Jane Sullivan for their help over the years. I also acknowledge the curators of the following herbaria for the use of their specimens and assistance: MICH, SRSC, TEX, MO and FTG.

Much of my work involved field collections, and I am grateful for people who helped me during these trips, especially Jay Horn, Christian Torres-Santana, Lauren Raz, Kristie Wendelberger, Micheal Powell, Wendy Weckesser, Cliff Morden, Keith Bradley, Jennifer Possley, Alice Warren, and Terry Glancy; and the authorities of Miami-Dade County Department of Parks and Recreation, the Institute for Regional Conservation (Miami, FL), Everglades National Park, Big Bend National Park, and the Nature Conservancy for collection permits and providing detailed information of collecting sites.

Generous financial assistance for my study was provided by the following sources: the University of Michigan (Rackham Graduate School, Matthaei Botanical

Gardens, Department of Ecology and Evolutionary Biology, University of Michigan Herbarium), and the National Science Foundation.

Lastly and most importantly I am indebted to my parents, Yuqi Zhang and Bangjie Yang, and my boyfriend Top Chea, for all the love and patience.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ABSTRACT

Euphorbia subg. *Chamaesyce* Raf. contains about 600 species and includes the largest New World radiation within the Old World-centered *Euphorbia* (Euphorbiaceae). It is one of the few plant lineages to include members with C_3, C_4 and CAM photosynthesis, showing multiple adaptations to warm and dry habitats. The subgenus includes North American-centered groups that were previously treated at various taxonomic ranks under the names of "Agaloma", "Poinsettia," and "Chamaesyce". Here we provide a well-resolved phylogeny of *Euphorbia* subg. *Chamaesyce* using nuclear ribosomal ITS and chloroplast *ndhF* sequences, with substantially increased taxon sampling compared to previous studies. Based on the molecular phylogeny, we discuss the Old World origin of the subgenus, the evolution of cyathial morphology and growth forms, and then we provide a formal subgeneric classification, with descriptions and species lists for each section or subsection we recognize.

Among the fifteen sections we recognized within subg. *Chamaesyce*, sect. *Anisophyllum* is the largest lineage of C_4 plants among the eudicots, with 350 species including both narrow endemics and cosmopolitan weeds. We sampled this group worldwide with 138 ingroup species, using two nuclear (ITS and exon 9 of *EMB2765*) and three chloroplast markers (*matK*, *rpl16,* and *trnL-F*). Three major clades were recovered within the section [1(2,3)]: (1) the Acuta clade, containing three North American species with C_3 photosynthesis and C_3-C_4 intermediates; (2) the Peplis clade, mostly North American and entirely C_4; and (3) the Hypericifolia clade, all C_4, with both New World and Old World groups. Incongruence between chloroplast and ITS phylogenies and divergent cloned copies of *EMB2765* exon 9 suggest extensive hybridization.

Woody members of sect. *Anisophyllum* originated once from herbaceous members in the New World, probably through allopolyploidy, and diversified into 16 species that occupy all habitat types on the major Hawaiian islands. We further increased

taxon sampling within the Hawaiian radiation to 104 ingroup accessions including 15 of the 16 species. Chloroplast data including more than 8 kb of non-coding regions support old to young island dispersal along the Hawaiian island chain. Nuclear ITS, *LEAFY* and *G3pdhC* markers further support the hybrid origin of Hawaiian *Anisophyllum* with recent interspecific hybridizations.

CHAPTER I

INTRODUCTION

Euphorbia (Euphorbiaceae) comprises over 2000 species and is probably the second largest genus of flowering plants. It is best known for its great diversity of cactus-like succulents, particularly from Africa and Madagascar, but also for leafy ornamentals such as Poinsettias (from Mexico). From an ecological standpoint, *Euphorbia* exhibits multiple adaptations to aridity, and it is unique among plants in having species that include the three main types of photosynthetic systems (C_3, C_4, and CAM). All species share a unique flowering structure called the cyathium, which shows features intermediate between a flower and an inflorescence (Prenner and Rudall, 2007). The sheer size, geographical range, and widespread convergence in succulent growth forms make *Euphorbia* a difficult group to be comprehensively studied by a single research group.

The development of two kinds of molecular markers has made it feasible to reconstruct phylogenetic relationships in large and complex plant groups like *Euphorbia*. The first one is to compare sequences of the generally maternally inherited and relatively slowly evolving chloroplast genes. This idea was initially developed nearly three decades ago (Palmer, 1985; Palmer et al., 1988), and this approach has been greatly expanded thanks to the development of universal primers for amplifying chloroplast non-coding regions (Shaw et al., 2005; Shaw et al., 2007). A second kind of marker is sequences of the nuclear ribosomal intergenic spacer (ITS; Baldwin et al., 1995). ITS is biparentally inherited and is relatively fast evolving, with universal primers readily available, and therefore has become the standard "go to" region for an initial survey of relationships among closely related species. With these two sets of molecular markers to start with, we are able to untangle relationships in some of the groups that are economically and

ecologically important, but are large, complex and understudied, at the scale and depth that no one has been able to achieve before (Frodin, 2004).

The approach I took for my dissertation is more exploratory rather than hypothesis driven at the beginning. Among the four subgenera of *Euphorbia*, subg. *Chamaesyce* is the second largest, with around 600 species. It has a New World centered distribution, as opposed to being predominantly Old World for the other three subgenera. By being New World, both wild populations and background information for subg. *Chamaesyce* are relatively easily accessible, and I have been able to greatly benefit from the extensive collaboration network built by my advisor Paul Berry from his previous work in the New World. Subgenus *Chamaesyce* is not only relatively accessible, but also very interesting in being one of the few plant lineages that have C_3, C_4 and CAM photosynthetic systems, and it includes some very unusual C_4 species in the Hawaiian Islands. Coming from an ecophysiology-molecular systematics background from my undergraduate training, I was especially attracted to the divergent evolutionary trajectories leading to C_4 and CAM among closely related species within *Euphorbia* subg. *Chamaesyce*.

In Chapter II, I explored the relationships within *Euphorbia* subg. *Chamaesyce* with two molecular markers that have been successfully used previously to reconstruct phylogenetic relationships within the genus (Steinmann and Porter, 2002): the nuclear ITS and chloroplast *NADH dehydrogenase* subunit *F (ndhF*) region. By substantially expanding taxon sampling compared to all previous studies added (Steinmann and Porter, 2002; Park and Jansen, 2007; Zimmermann et al., 2010; Horn et al., in review), I reconstructed a well-resolved phylogeny and comprehensively revised the taxonomic framework within the subgenus. Fifteen sections were described, majority of which are either newly designated, or modified from previous sectional circumscriptions. In the framework of an updated taxonomic system, evolutionary trends of growth forms, cyathial appendages, and photosynthetic systems were discussed.

Among these fifteen sections, sect. *Anisophyllum* is especially interesting because it contains the largest C_4 lineage in eudicots (Sage et al., 2011; Yang and Berry, 2011). C_4 photosynthesis evolved at least 62 times in angiosperms, and it is one of the most notable convergences in plants (Sage et al., 2011). Previous studies on C_4 plants have been

mostly focused on monocots, especially in the economically and ecologically important grass family (summarized by Sinha and Kellogg, 1996; Edwards et al., 2010). More recently, physiological and comparative studies on C_4 photosynthesis have expanded to the eudicots (summarized by Brown et al., 2005), and now we know that C_4 eudicots are as old and species-rich as C_4 monocots (Christin et al., 2011). Among all these C_4 eudicots, around 20% of them belong to a single lineage, *Euphorbia* section *Anisophyllum* subsection *Chamaesyce*. This lineage contains some very successful weeds, and its members can be found on sidewalk cracks in cities all over the world. Through frequent long-distance dispersals and extensive interspecific hybridizations, followed by local adaptation, subsect. *Chamaesyce* has achieved a worldwide distribution with around 350 species. We sampled around half of the total species using two nuclear and three chloroplast markers, and we discuss the origin and diversification of this prominent C_4 lineage (Chapter III).

Although the majority of species in subsect. *Chamaesyce* are herbaceous and more or less weedy, there is a clade of 16 species that are all woody perennials that radiated on the Hawaiian Islands. They are found in habitats from coastal vegetation to dry forests, wet forests and even bogs, ranging in habit from subshrubs and shrubs to trees up to ten meters tall. Many taxa are endemic to a single island (Koutnik, 1987; Lorence and Wagner, 1996; Wagner et al., 1999). Some of them are the only C_4 plants that adapted to wet forest understory environments. Taxon sampling covering 15 of the total 16 species, using nuclear ITS, eight chloroplast non-coding regions, and two additional nuclear low-copy non-coding regions, provides evidence for the complex reticulate history in this island radiation (Chapter IV).

Chapters II, III and IV are the data chapters. Among them, Chapter III has been published (Yang and Berry, 2011). For Chapter II, I contributed 5% of the field collections, 20% of the lab work, 85% of the data analysis, and 80% of the writing; for Chapter III, I carried out 80% of the field collections, 98% of the lab work, 98% of the data analysis, and 80% of the writing; for Chapter IV, I designed the study with the help of my advisor, conducted 5% of the field work, 100% of the lab work, 100% of the data analysis, and wrote 100% of the chapter.

LITERATURE CITED

BALDWIN, B. G., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA - a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247-277.

BROWN, N. J., K. PARSLEY, AND J. M. HIBBERD. 2005. The futured C_4 research - Maize, *Flaveria* or *Cleome*? *Trends in Plant Science* 10: 215-221.

CHRISTIN, P.-A., C. P. OSBORNE, R. F. SAGE, M. ARAKAKI, AND E. J. EDWARDS. 2011. C_4 eudicots are not younger than C_4 monocots. *Journal of Experimental Botany* 62: 3171-3181.

EDWARDS, E. J., C. P. OSBORNE, C. A. E. STROMBERG, S. A. SMITH, W. J. BOND, P. A. CHRISTIN, A. B. COUSINS, et al. 2010. The origins of C_4 grasslands: integrating evolutionary and ecosystem science. *Science* 328: 587-591.

FRODIN, D. G. 2004. History and concepts of big plant genera. *Taxon* 53: 753-776.

KOUTNIK, D. L. 1987. A taxonomic revision of the Hawaiian species of the genus *Chamaesyce* (Euphorbiaceae). *Allertonia* 4: 331-388.

LORENCE, D. H., AND W. L. WAGNER. 1996. A new species of *Chamaesyce* section *Sclerophyllae* (Euphorbiaceae) from Kaua'i. *Novon* 6: 68-71.

PALMER, J. D. 1985. Chloroplast DNA and molecular phylogeny. *Bioessays* 2: 263-267.

PALMER, J. D., R. K. JANSEN, H. J. MICHAELS, M. W. CHASE, AND J. R. MANHART. 1988. Chloroplast DNA variation and plant phylogeny. *Annals of the Missouri Botanical Garden* 75: 1180-1206.

PARK, K. R., AND R. K. JANSEN. 2007. A phylogeny of Euphorbieae subtribe Euphorbiinae (Euphorbiaceae) based on molecular data. *Journal of Plant Biology* 50: 644-649.

SAGE, R. F., P.-A. CHRISTIN, AND E. J. EDWARDS. 2011. The C_4 plant lineages of planet earth. *Journal of Experimental Botany* 62: 3155-3169.

SHAW, J., E. B. LICKEY, E. E. SCHILLING, AND R. L. SMALL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94: 275-288.

SHAW, J., E. B. LICKEY, J. T. BECK, S. B. FARMER, W. S. LIU, J. MILLER, K. C. SIRIPUN, et al. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

SINHA, N. R., AND E. A. KELLOGG. 1996. Parallelism and diversity in multiple origins of C_4 photosynthesis in the grass family *American Journal of Botany* 83: 1458-1470.

STEINMANN, V. W., AND J. M. PORTER. 2002. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* 89: 453-490.

WAGNER, W. L., D. R. HERBST, AND S. H. SOHMER. 1999. Manual of the flowering plants of Hawaii, revised edition with supplement by W. L. Wagner and D. R. Herbst. Bishop Museum Special Publication, vol. 97. University of Hawaii Press, Honolulu, Hawaii, USA.

YANG, Y., AND P. E. BERRY. 2011. Phylogenetics of the Chamaesyce clade (*Euphorbia*, Euphorbiaceae): Reticulate evolution and long-distance dispersal in a prominent C_4 lineage. *American Journal of Botany* 98: 1486-1503.

ZIMMERMANN, N. F. A., C. M. RITZ, AND F. H. HELLWIG. 2010. Further support for the phylogenetic relationships within *Euphorbia* L. (Euphorbiaceae) from nrITS and *trnL–trnF* IGS sequence data. *Plant Systematics and Evolution* 286: 39-58.

CHAPTER II

MOLECULAR PHYLOGENETICS AND CLASSIFICATION OF *EUPHORBIA* SUBGENUS *CHAMAESYCE*: A GROUP WITH EXTRAORDINARY DIVERSITY IN PHOTOSYNTHETIC SYSTEMS AND GROWTH FORMS

ABSTRACT

Euphorbia subg. *Chamaesyce* Raf. contains about 600 species and includes the largest New World radiation within the Old World-centered *Euphorbia*. It is one of the few plant lineages to include members with C_3, C_4 and CAM photosynthesis, showing multiple adaptations to warm and dry habitats. The subgenus includes North American-centered groups that were previously treated at various taxonomic ranks under the names of "Agaloma", "Poinsettia," and "Chamaesyce". Here we provide a well-resolved phylogeny of *Euphorbia* subg. *Chamaesyce* using nuclear ribosomal ITS and chloroplast *ndhF* sequences, with substantially increased taxon sampling compared to previous studies. Based on the molecular phylogeny, we discuss the Old World origin of the subgenus, the evolution of cyathial morphology and growth forms, and then provide a formal subgeneric classification, with descriptions and species lists for each section or subsection we recognize.

INTRODUCTION

Euphorbia L. (Euphorbiaceae) has about 2000 species worldwide and is well known for the remarkable diversity of succulent plants that are avidly grown by horticultural enthusiasts. *Euphorbia* is characterized by the presence of a cyathium, which is a highly compact flowering structure intermediate in some ways between a flower and an inflorescence (Prenner and Rudall, 2007; Prenner et al., 2011). The genus appears to have originated in the Old World (Steinmann and Porter, 2002), with multiple New World groups embedded in it. The largest New World lineage consists of around 500 species that are characterized by petaloid appendages subtending the cyathial glands,

although this feature has been subsequently lost a number of times. This petaloid appendage-bearing New World group is deeply nested within an Old World grade, and together they constitute *Euphorbia* subgenus *Chamaesyce* Raf. (Steinmann and Porter, 2002; Bruyns et al., 2006; Horn et al., in review). Subgenus *Chamaesyce* is best known for its leafy, non-succulent ornamental species, such as the Christmas Poinsettia (*E. pulcherrima* Willd. ex Klotzsch), one of the most profitable potted plants in the world (Mayfield 1997). Other widely cultivated members are "snow-on-the-mountain" (*E. marginata* Pursh.) and "Diamond Frost" (a cultivar of *E. graminea* Jacq.). It also includes a large number of cosmopolitan weedy species such as the spotted spurge (*E. maculata* L., Fig. 2.1E).

Among the four subgenera of *Euphorbia*, *Euphorbia* subg. *Chamaesyce* is the second most species-rich one, encompassing around 600 recognized species worldwide. It is highly diverse in growth forms, including annual or perennial herbs, shrubs, trees, and pencil-stem succulents (Fig. 2.1, A–E). Notably it is the only plant lineage at or below the level of genus that has all known photosynthetic types: C_3, C_4 and CAM, plus a C_2 system that represents an early stage of C_3 to C_4 transition (Webster et al., 1975; Sage et al., 2011). C_4 photosynthesis evolved once within subg. *Chamaesyce* sect. *Anisophyllum* and this C_4 group subsequently diversified into around 350 species worldwide (Yang and Berry, 2011); C_2 photosynthesis also evolved once, again in sect. *Anisophyllum*, and is present in two species restricted to southwestern United States and northern Mexico (Sage et al., 2011). CAM photosynthesis, in contrast, evolved multiple times in subg. *Chamaesyce* in both the Old World and the New World (Horn et al., 2011; in prep.).

Due to widespread convergence in growth forms and cyathial characters, subgeneric classification within *Euphorbia* has been notoriously contentious. The current scheme of four subgenera is based on molecular evidence. All molecular phylogenetic studies to date support that all cyathium-bearing species form a monophyletic *Euphorbia* s.l. (Steinmann and Porter, 2002; Bruyns et al., 2006; Park and Jansen, 2007; Zimmermann et al., 2010; Horn et al., in review). These studies also support the monophyly of four major clades within *Euphorbia*, which were informally named by Steinmann and Porter (2002) as clades A, B, C and D. Among them, clade D was later

recognized by Bruyns et al. (2006) as subg. *Chamaesyce* Raf. However, all of these studies either have limited taxon sampling within subg. *Chamaesyce*, or else rely mainly on a single marker, or they have low statistical support within subg. *Chamaesyce*. Steinmann and Porter (2002) sampled 82 of the 600 species in subg. *Chamaesyce* for ITS, among which 40 also had *ndhF* sequences. Using maximum parsimony, they found that the majority of deep nodes within the genus received low statistical support. Three subsequent molecular studies added some additional Old World taxa and further supported the four-clade scheme in *Euphorbia* (Bruyns et al., 2006; Park and Jansen, 2007; Zimmermann et al., 2010), although each only added a few taxa within subg. *Chamaesyce*, and relationships among major groups still remained poorly supported. This problem was specifically addressed by the "backbone" phylogeny of Horn et al. (in review), which sampled 176 species across *Euphorbia* using ten loci, including nuclear, mitochondrial and chloroplast regions, with 31 species representing all major lineages within subg. *Chamaesyce*. This study highly supports the monophyly of subg. *Chamaesyce*, as well as its sister relationship to *Euphorbia* subg. *Euphorbia*. Of all five genus-wide molecular studies, three of them support a basal Old World grade within subg. *Chamaesyce*, with New World groups being monophyletic and deeply nested in the Old World grade (Steinmann and Porter, 2002; Zimmermann et al., 2010; Horn et al., in review). The other two studies both lack statistical support for deep nodes within subg. *Chamaesyce* (Bruyns et al., 2006; Park and Jansen, 2007). In addition to these studies, Yang and Berry (2011) constructed a robust phylogeny of *Euphorbia* subg. *Chamaesyce* sect. *Anisophyllum*, which corresponds to the previously segregate genus *Chamaesyce* S.F. Gray. In their analysis, 138 ingroup species were sequenced with two nuclear loci and three chloroplast loci, and the monophyly of sect. *Anisophyllum* was well supported. Taking all six molecular studies together, until now only a third of the species in subg. *Chamaesyce* have been sampled, and most species outside of sect. *Anisophyllum* have only ITS data available.

With the international collaboration network established by the *Euphorbia* Planetary Biodiversity Inventory (*Euphorbia* PBI) project, we have been able to greatly expand our worldwide taxon sampling to reconstruct a well-sampled molecular phylogeny, and now we can begin to answer questions about biogeography,

morphological evolution, and evolutionary transitions between C_3 and C_4, and C_3 and CAM. The main purpose of this paper is to propose a revised sectional and subsectional classification within *Euphorbia* subg. *Chamaesyce* in light of the updated phylogeny we have produced. This will provide a stable nomenclatural base for subsequent research works that will address a number of evolutionary questions mentioned above.

MATERIALS AND METHODS

***Taxon sampling*—**Silica-preserved leaf samples were collected in all major areas where *Euphorbia* occurs during 2006–2009. Additional samples were taken from recently collected herbarium sheets in MICH. To include as many taxa as possible belonging to subgenus *Chamaesyce*, we conducted a preliminary maximum parsimony analysis in PAUP using *ndhF* sequences to assign each taxon to one of the four subgenera in *Euphorbia*. In addition, all sequences in GenBank that belong to subg. *Chamaesyce* were included. In total, our taxon sampling covered 290 out of the total 600 species in the subgenus, with all previously recognized sections and most subsections represented. Since the monophyly and subclade structure within sect. *Anisophyllum* has been well-established in an earlier study (Yang and Berry, 2011), we reduced taxon sampling within sect. *Anisophyllum* to 15 species representing all major subclades. Duplicated DNA accessions that grouped together with conspecific sequences during analysis were excluded. In total, our final matrices include 167 ingroup species; additionally, 11 species representing the other three subgenera of *Euphorbia* were selected as outgroups.

***DNA extraction, amplification, and sequencing*—**DNA extraction and PCR amplification of the ITS region were carried out following Yang and Berry (2011). The chloroplast *NADH dehydrogenase F* (*ndhF*) coding region was PCR amplified in two pieces: the 5' half was amplified using primer 536 and 1318R (Olmstead and Sweere, 1994), and the 3' half using 972 (Olmstead and Sweere, 1994) and 2110Ri (Steinmann and Porter, 2002). The PCR mixture contained 0.15 μL of 5 units/μL *Ex Taq*™ (Takara Bio Inc., Otsu, Shiga, Japan), 2.5 μL 10×Ex Taq Buffer, 2.0 μL dNTP (2.5 mM), 1.0 μL of each primer (10 μM), 2 μL of diluted template DNA (dilution varies between 1/20 to 1/80), and ddH_2O to bring the final volume to 25 μL. The PCR profile consisted of an

initial 4 min denaturing step at 95°C followed by 40 cycles of 45 s denaturing at 95°C, 45 s annealing at 53.6°C, and 2 min "slow and cold" extension at 65°C (Shaw et al., 2007).

PCR products were purified with ExoSap-IT® (USB Corporation, Cleveland, Ohio, USA), or QIAquick PCR Purification Kit (Qiagen, Valencia, California, USA) for weak PCR products. Cleaned PCR products were sequenced at the University of Michigan DNA Sequencing Core using the respective PCR primers.

***Phylogenetic analyses*—**Chromatograms were assembled and edited in the program Sequencher® v. 4.10.1 (Gene Codes, Ann Arbor, Michigan, USA). Sequence alignments were performed in the program MUSCLE v. 4 (Edgar, 2004) using the default parameters, and manually adjusted in the program MacClade v. 4.08 (Simmons, 2004; Maddison and Maddison, 2005).

Phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) were conducted on the ITS and *ndhF* matrices separately, with gaps treated as missing data. Congruence between the resulting ITS and *ndhF* trees were visually inspected before concatenating them into a combined matrix. ITS, *ndhF* and the combined matrices were each subjected to the analyses described below.

Maximum likelihood analyses were carried out in the program RAxML v. 7.0.3 (Stamatakis, 2006), partitioning ITS vs. *ndhF* regions. The nucleotide substitution model was set to GTR + γ as recommended by the RAxML manual. 500 ML bootstrap replicates were performed, followed by a thorough search for the best tree. Bayesian Inference was conducted in the program MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Two independent runs (four for the combined dataset) of four chains each (three heated, one cold), starting from random trees, using the default temperature of 0.2, were run for 15 000 000 generations. Trees were sampled every 1000 generations. Each analysis was conducted using the nucleotide substitution model GTR + I + γ as selected by AIC in the program MrModeltest v. 2.3 (Nylander, 2004). "brlenspr=unconstrained:exponential(100.0)" was applied to prevent unrealistically long branches (Brown et al., 2010; Marshall, 2010). All parameters were visually examined in the program Tracer v. 1.5 (Rambaut and Drummond, 2007) to verify stationary status. Trees from the first 2 500 000 generations were discarded as burn-in; the remaining trees were used to compute the majority rule consensus.

***Criteria for circumscription sections and subsections*—**Lengthy debate has been devoted to whether paraphyletic taxonomic units should be recognized (for example, Ebach et al., 2006; Horandl, 2006; Abouheif, 2008). Here we argue that monophyly, morphological similarities, and evolutionary processes should be the basis of circumscribing taxa at the level of subsection and above. First, each of these taxa should be monophyletic and would ideally be supported by both nuclear and organellar markers. Second, each taxon should be definable by a set of morphological characters. However, frequently there is a transitional grade at the base of a taxon exhibiting intermediate character states, and representing serial transition on the way to character integration. In this case we would take evolutionary processes into consideration and include these intermediate species into a broadly defined higher taxon. In cases where conflicting signals occur between nuclear and chloroplast markers, morphological and biogeographical considerations should be taken into account in favoring one phylogenetic hypothesis or another.

RESULTS

Overall statistics of the gene regions sequenced for this study are summarized in Table 2.1. Results of phylogenetic analyses are shown in Figs. 2.2 and 2.3 and summarized in Fig. 2.4. For each of the three analyses (ITS, *ndhF* and combined ITS + *ndhF*), BI and ML results are congruent for most nodes when moderately or highly supported (ML bootstrap support is ≥ 50 and Bayesian PP is ≥ 0.80); monophyly of subg. *Chamaesyce* and its sister relationship to *Euphorbia* subg. *Euphorbia* are both highly supported. Groups are numbered consistently across Figs. 2.2–2.4, and labels for sections, subsections, and subclades are shown on the combined tree (Fig. 2.3).

***ITS dataset*—**The ITS dataset has a relatively high proportion of variable sites compared to *ndhF* (71.1% vs. 45.1%, Table 2.1). Maximum likelihood analysis is congruent with BI except for the placement of sect. *Eremophyton* + sect. *Cheirolepidium*. ML moderately supports sect. *Eremophyton* + sect. *Cheirolepidium* as sister to the Madagascar clade (Fig. 2.2A), but BI moderately supports these sections in a grade sister to all species shown in Fig. 2.2B. Monophyly of each section is strongly supported by both ML and BI analyses.

***ndhF dataset*—**Compared to ITS, the *ndhF* coding region is unambiguously aligned with relatively fewer variable sites (Table 2.1). Maximum likelihood analysis is congruent with BI in all moderately to well-supported clades. Monophyly of each section is moderately to strongly supported, except for sect. *Poinsettia* (Fig. 2.2B), where *E. jaliscensis* is moderately supported to be sister to sect. *Poinsettia* + sect. *Alectoroctonum*.

***Combined ITS + ndhF dataset*—**Analysis of the combined dataset produced a well-resolved phylogeny (Fig. 2.3). Monophyly of each section is strongly supported, except for sect. *Crossadenia*, which has sect. *Gueinziae* nested within it. Relationships within each clade are well resolved in general, except for certain nodes in sect. *Alectoroctonum* (Fig. 2.3B) and sect. *Articulofruticosae* (Fig. 2.3A). There are five instances of moderate to strongly supported incongruence between the ITS and *ndhF* datasets, and such incongruences affect the combined analysis in different manners (Figs. 2.2 and 2.3). 1) In sect. *Alectoroctonum*, the two clades marked with "*" are strongly supported to be sister to each other in the combined analysis and in the *ndhF* phylogeny, while ITS strongly supports them to form a grade. 2) *Euphorbia jaliscensis* is strongly supported as nested within sect. *Poinsettia* in the combined analysis and with ITS, but not with the *ndhF* data alone. 3) *Euphorbia gueinzii* is nested within sect. *Crossadenia* in the ITS analysis with moderate support, while *ndhF* provides low support for *E. gueinzii* being sister to sect. *Crossadenia*; the combined analysis is congruent with the ITS topology in this case but with weaker support values. 4) *Euphorbia salota* is strongly supported by the combined and *ndhF* analyses to be nested within the Madagascar clade, while ITS places it sister to sect. *Cheirolepidium* + sect. *Eremophyton* with moderate support, separated from the rest of the Madagascan species. 5) Within sect. *Articulofruticosae*, results from ITS either conflict extensively with *ndhF* or are poorly resolved, and the combined tree is also poorly resolved.

DISCUSSION

Our results are consistent with the previous genus-wide molecular phylogenetic studies that show subg. *Chamaesyce* to be monophyletic and sister to subg. *Euphorbia* (Steinmann and Porter, 2002; Bruyns et al., 2006; Park and Jansen, 2007; Zimmermann et al., 2010; Horn et al., in review). Three of these five previous studies supported a

single origin of New World taxa from an Old World grade within subg. *Chamaesyce*, with the eastern Brazilian clade sect. *Crossadenia* being sister to the rest of the New World group (Zimmermann et al., 2010; Horn et al., in review). Our results differ somewhat in that they support an eastern Brazilian-Old World clade sister to a New World clade, and together these are nested in an Old World grade (Fig. 2.4).

Prior to this study, Bruyns et al. (2006) made an attempt to provide a sectional classification of subg. *Chamaesyce* based on molecular data. They recognized four sections within the subgenus: 1) "sect. *Chamaesyce*", which included the New World clade + Old World-eastern Brazil clade + sect. *Tenellae*, sharing petaloid appendages (the "petaloid appendage clade", Fig. 2.4; Horn et al., in review); 2) sect. *Frondosae*, a diverse Old World group; 3) sect. *Articulofruticosae*, a group of pencil-stem succulents from southern Africa that is both molecularly and morphologically distinct; and 4) sect. *Espinosae*, a clade of two shrubby species from Africa; they left *E. tannensis* unplaced. Among the four sections that they recognized, we agree with their naming and circumscription for all but the first one. In that case, we propose that their "sect. *Chamaesyce*" needs to be divided into six sections: sect. *Tenellae*, sect. *Gueinziae*, sect. *Crossadenia*, sect. *Anisophyllum*, sect. *Poinsettia*, and sect. *Alectoroctonum*. In addition, we designate six additional Old World sections to accommodate species that were either unsampled or unplaced by Bruyns et al. (2006), namely, sect. *Cheirolepidium*, sect. *Eremophyton*, sect. *Scatorhizae*, sect. *Denisiae*, sect. *Bosseriae*, and sect. *Plagianthae*. In the following discussion, we focus on comparing our results to the marker-rich but relatively taxon-poor "backbone" analysis of Horn et al. (in review).

***The Old World grade*—**On Fig. 2.4, clades from sect. *Espinosae* up to sect. *Tenellae* are entirely Old World, forming the early-diverging Old World grade in subg. *Chamaesyce*. Within this grade, BI analysis of ITS places sect. *Eremophyton* + sect. *Cheirolepidium* as sister to the petaloid appendage clade, similar to the placement of Bruyns et al. (2006), based on BI of ITS alone (Fig. 2.2A). However, ML analysis of ITS, and both BI and ML analyses of *ndhF,* all support this clade as sister to the Madagascar clade. The cause of this incongruence between BI and ML is unknown, but it could be because BI is more prone to long branch attraction (Kolaczkowski and Thornton, 2009).

Our results disagree with Horn et al. (in review) as to which is the earliest

diverging clade in the Old World (Fig. 2.4). Our combined analysis recovered sect. *Articulofruticosae* + sect. *Espinosae* as sister to the rest of the subgenus with moderate support. However, Horn et al. (in review) recovered sect. *Cheirolepidium* + sect. *Eremophyton* + sect. *Scatorhizae* + the Madagascar clade + sect. *Frondosae* as sister to the rest of subg. *Chamaesyce*, and this relationship received strong statistical support and may be more accurate because of their better molecular sampling.

Park and Jansen's (2007) *ndhF* sequence of *E. cuneata* retrieved from GenBank is nested in our sect. *Scatorhizae* (Fig. 2.2A). However, our own field-collected sample of *E. cuneata* is placed squarely within *Euphorbia* subg. *Rhizanthium* (Riina et al., in prep). Also, Park and Jansen's *E. cuneata* has the same sequence as the *E. polyantha ndhF* sequence by Steinmann and Porter (2002), except that Park's *ndhF* sequence has a 9-bp deletion in the middle. Because we are confident of the identification of our own sample, which was compared with the type and taxonomic treatments of *E. cuneata*, we believe that Park and Jansen (2007) misidentified *E. polyantha* as *E. cuneata* in their paper. Because they did not provide voucher information for their accessions, we were not able to verify the identification of their sample.

Among the ten sections we recognize in the Old World grade, sect. *Tenellae* is of particular interest because it shares petaloid gland appendages with the Old World-eastern Brazilian clade + the New World clade. The petaloid appendages (Fig. 2.1, H–J) likely evolved in the common ancestor of sect. *Tenellae* + Old World-eastern Brazilian clade + the New World clade, and together they form the "petaloid appendage clade" (Horn et al., in review), which also corresponds to the "*Agaloma* alliance" of Steinmann and Porter (2002).

***The Old World - eastern Brazilian clade*—**With substantially increased taxon sampling, our analyses recovered two Old World species, *E. hainanensis* Croizat and *E. gueinzii* Boiss., grouped with the Brazilian sect. *Crossadenia* rather than with any other Old World group (Figs. 2.2 and 2.3). However, we still consider the position of *E. hainanensis* to be doubtful. It is a shrub endemic to Hainan Island of southern China, and it is distinctive in having three cyathial glands and has been postulated to be closely related to species from tropical Australasia that belong to *Euphorbia* subg. *Euphorbia* (Croizat, 1940; Dorsey & al., in prep). This is a very rare species, and we were only able

to obtain a single ITS sequence. On the other hand, the placement of *E. gueinzii* is more reliable, since both ITS and *ndhF* sequences place it close to sect. *Crossadenia*. In addition to our sequence data, a separate accession of *E. gueinzii* was sequenced for ITS at the Smithsonian Institution, and it resulted in the same phylogenetic placement (K. Wurdack, pers. comm.).

Although ITS data places *E. gueinzii* within sect. *Crossadenia* with moderate support, *ndhF* data moderately supports its sister relationship to sect. *Crossadenia*. The combined analysis places it within sect. *Crossadenia*, but the support for this is weak. Because of its distinctive morphology and widely disjunct South African distribution compared to the otherwise entirely Brazilian sect. *Crossadenia*, we believe that the *ndhF* placement is more likely to reflect the relationships of *E. gueinzii*. Consequently, we propose a new section for *E. gueinzii*, based on its position as sister to sect. *Crossadenia* in the *ndhF* tree. In the case of the enigmatic *E. hainanensis*, we leave it unplaced until more data is available.

Given the pattern of distribution summarized in Fig. 2.4, it is possible that New World groups in subg. *Chamaesyce* did not have a single origin from the Old World, but that sect. *Crossadenia* became established in Brazil first, and then there was a separate introduction accounting for the three North American-centered New World groups discussed below: sect. *Anisophyllum*, sect. *Poinsettia* and sect. *Alectoroctonum*. Alternatively, there could have been a long-distance dispersal from the Old World to the New World, followed by dispersal back to the Old World. It is unclear which scenario is more likely until further information on *E. gueinzii*, *E. hainanensis* and their close relatives is available.

The (largely) New World clade—Sister to the Old World-eastern Brazilian clade is a largely New World clade of around 500 species. This New World clade consists of three major subclades [1(2,3)]: 1) sect. *Anisophyllum* is distinctive in being mostly C_4 and having a specialized growth form with early abortion of the main shoot. It is most prevalent in warm semi-desert regions and disturbed areas worldwide, with its greatest diversity in the New World. 2) Sect. *Poinsettia* is characterized by a serial loss of petaloid gland appendages and the development of brightly colored leafy bracts subtending densely clustered terminal cymes, with the whole structure resembling a large

blossom. It occurs mainly in forests and desert scrub of subtropical North America. 3) Sect. *Alectoroctonum* largely corresponds to the former subg. *Agaloma* (Raf.) House and is predominantly composed of herbs and shrubs, but also has pencil-stem succulents with CAM photosynthesis. All species in this group have petaloid cyathial gland appendages, and sometimes they are quite showy. This group occurs in desert scrub to moist montane forests and prairies in subtropical to temperate areas of North and South America.

Within this New World clade there has been a considerable divergence of classification schemes. Bruyns et al. (2006) opted to lump the entire "petaloid appendage clade" into a single section "Chamaesyce"; however, their study had an Old World focus, with a very sparse sampling of New World taxa. Our denser sampling of New World species shows that there are three well supported groups within this clade, and each or part of each of them, has been treated previously at the rank of subgenus or even genus (e.g., "Chamaescye", "Poinsettia", and "Agaloma"; see Dressler, 1961; Koutnik, 1984; Ward, 2001). If we were to follow that tendency and recognize genera or subgenera here, we would have to break up groups in the Old World grade into separate genera or subgenera as well in order to preserve monophyly. Instead, we choose to recognize three sections within this part of the New World clade: sect. *Poinsettia*, sect. *Anisophyllum*, and sect. *Alectoroctonum*.

Section Poinsettia—In view of our molecular results and a reevaluation of the morphological characters of the group, we propose a broader circumscription of sect. *Poinsettia* compared to the previous treatments by Dressler (1961) and Mayfield (1997). These authors restricted the application of the name "Poinsettia" to what we recognize here as subsect. *Stormiae* Croizat, namely those species with deeply cup-shaped involucral glands often one or few in number that lack petaloid appendages (Fig. 2.1K). Other characteristic features commonly found in these species include colored bracts subtending the congested terminal cymes; pandurately lobed to linear, often heteromorphic leaves with at least some serration on the margins; glandular stipules; and coarsely tuberculate seeds, sometimes with a deeply sunken caruncular facet.

The three other subsections that we recognize in sect. *Poinsettia* are successively sister to subsect. *Stormiae*, beginning with subsect. *Exstipulatae*, followed by subsect. *Erianthae*, and then subsect. *Lacerae* (Fig. 2.3). These three subsections all have some

kind of involucral gland appendage, but that character is variable within the entire section, and two species nested in subsect. *Stormiae*, *E. chersonesa* and *E. cornastra*, can also have a rudimentary appendage on the outer lip of the glands (Huft, 1984; Mayfield, 1997). In the case of *E. bifurcata*, which is placed here in subsect. *Exstipulatae*, it would be an otherwise indistinguishable member of sect. *Stormiae* if not for the whitish appendage of its usually single cupular gland. Except for *E. eriantha*, which was placed by Boissier (1862) in sect. *Poinsettia*, the species in the three new subsections proposed here were previously included in sect. *Zygophyllidium* (Boissier, 1862; Dressler, 1961; Huft, 1984), which is included within sect. *Alectoroctonum* in this paper.

In addition to the molecular evidence, which strongly supports the monophyly of an expanded sect. *Poinsettia* (PP 1; MLB 100), there are morphological characters that support the inclusion of the additional species in sect. *Poinsettia*, and, conversely, their exclusion from sect. *Alectoroctonum*. First are the serrate leaf margins, which are evident in all species of sect. *Poinsettia*, except *E. pinetorum*, *E. colorata*, *E. restiacea* (all in subsect. *Stormiae*), and *E. eriantha* (subsect. *Erianthae*), although teeth can usually be seen in the latter two species with magnification. These four species all have linear leaves, and their teeth may have become inconspicuous or obscured as the leaves became narrower. On the other hand, serrate leaves are quite rare in sect. *Alectoroctonum*. Both species of sect. *Lacerae*, which is sister to the other three subsections of sect. *Poinsettia*, have serrate, heteromorphic and/or pandurate leaves, which are usually considered to be hallmarks of sect. *Poinsettia* sensu stricto. The gland appendages of subsect. *Erianthae* are unique in the genus and bear no resemblance to any of those in sect. *Alectoroctonum* (Fig. 2.1H). Likewise, the two-horned gland appendages in *E. lacera* appear to be different from the petaloid appendages in sect. *Alectoroctonum*.

Another factor that may have confounded recognizing an expanded sect. *Poinsettia* distinct from sect. *Alectoroctonum* in the past was the erroneous placement of *E. bilobata* in sect. *Poinsettia* in the molecular phylogeny of Steinmann and Porter (2002). *Euphorbia bilobata* is a true member of sect. *Alectoroctonum*, as shown by its placement in this study close to *E. hexagona* (the type species of Boissier's sect. *Zygophyllidium*; Fig. 2.3B); a review of its morphological features show that they are

fully consistent with its placement in sect. *Alectoroctonum*. The position of *E. bilobata* in Steinmann & Porter (2002) may have been due to a misidentification or to a lab error.

In summary, we see no overriding morphological conflicts with sect. *Alectoroctonum* in expanding the circumscription of sect. *Poinsettia* to include the five additional species in three subsections recognized here. Within the context of the "petaloid appendage clade" (Horn et al., in review) to which sect. *Poinsettia* belongs, it is not surprising that the earliest diverging subsections in section *Poinsettia* would have petaloid appendages and that these were subsequently lost in subsect. *Stormiae*.

Section Anisophyllum—Boissier (1862) proposed eight subdivisions within sect. *Anisophyllum*. Since then, due to the relatively homogenous morphology and wide distribution of this group, Boissier's classification scheme remain largely unchanged except for some minor modifications (Binojkumar and Balakrishnan, 2010). Yang and Berry's (2011) analyses of chloroplast markers highly support three major subclades within sect. *Anisophyllum* [1(2,3)]: 1) the Acuta clade, with only three species endemic to southwestern U.S.A. and northern Mexico that have C_2 and C_3 photosynthesis and glandular stipules; 2) the Peplis clade, consisting of mostly glabrous, perennial herbs with entire leaf margins that all have C_4 photosynthesis; and 3) the Hypericifolia clade, consisting of annual and perennial herbs to woody perennials, often with toothed leaf margins and usually with some kind of pubescence, and mostly endemic to the southwestern U.S.A. and northern Mexico, and also all C_4. Since no character or character sets can readily distinguish species in the later two clades, and nuclear markers indicate that there has been widespread reticulate evolution among members of these two clades, here we only recognize two subsections in subsect. *Anisophyllum*: the Acuta clade constituting subsect. *Acutae*, and all remaining species comprising subsect. *Chamaesyce*.

Section Alectoroctonum—Classification within sect. *Alectoroctonum* is extremely difficult due to its diversity of growth forms and the incompletely resolved phylogeny we obtained. Shrubs have evolved multiple times from herbaceous ancestors (Horn et al., in review), and morphologically similar species repeatedly turn out to belong to distinct groups in our molecular phylogeny. In addition, the majority of deep nodes in the section are very short and are poorly or only moderately supported (Figs. 2.2B and 2.3B); and some well-supported branches conflict among markers. Between our ITS and *ndhF*

results (Fig. 2.2B), the two clades marked with an asterisk are sister to each other in the *ndhF* analysis but form a grade in the ITS analysis, with each placement being well-supported. Additional gene regions such as nuclear ribosomal ETS, chloroplast *matK,* and the nuclear low-copy coding region exon 9 of *EMB2765* revealed even more extensive conflicts among well-supported clades (data not shown), similar to the patterns found in sect. *Anisophyllum* (Yang and Berry, 2011). Therefore, additional markers, expanded taxon sampling, and careful morphological studies are needed to better resolve relationships within sect. *Alectoroctonum* and to formally circumscribe subsections. Here we discuss informal species groups that are well supported by both morphological and molecular data, and then point out ambiguities that will require further investigation.

Clades 15-1 to 15-4 are successively sister to the rest of sect. *Alectoroctonum* (Fig. 2.3B). The majority of species in this grade occupy mid-elevation pine-oak forests in Mexico, while clade 15-1 and *E. acerensis* of clade 15-3 occur in the Caribbean and South America, and *E. graminea*, also of clade 15-3, is a widespread and variable species across tropical North and South America. Species in clade 15-1 are distinctive in having only two glands per cyathium, or sometimes three in *E. insulana*. Clade 15-2 largely corresponds to an as yet unpublished subsection in sect. *Tithymalopsis* (Klotzsch and Garcke) Boiss. (Fig. 2.1J; Huft, 1979). It is endemic to Mexico and has globose roots (Huft, 1979). However, *E. macropus*, which is also a Mexican species with globose roots, is placed in clade 15-4 in our analysis, and it indeed shares morphological characters of both clade 15-2 and 15-4. Clade 15-3 is distinctive in having stalked glands and four or five glands per cyathium (sometimes also two or three in *E. graminea*). Leaf shape in this clade is highly variable, and the leaf margin is sometimes sinuate, instead of being entire as in most of sect. *Alectoroctonum*. Within this clade, *E. graminea* and *E. ariensis* are supported as sister taxa in our analysis, and they share white, showy bracts. *Euphorbia graminea* is very similar to species of clade 15-1 in gross morphology, but it differs from clade 15-1 in having glabrous instead of densely pubescent capsules (Ward, 2001). *Euphorbia graminea* is the type of sect. *Cyttarospermum* Boiss., but other species that were placed in that section by Boissier (1862) are spread over many separate clades within sect. *Alectoroctonum*. Species in clade 15-4 are characterized by opposite leaves

and branches, four or five glands per cyathium, green gland appendages, and all are confined to Mexico.

Euphorbia misella is situated in an isolated position sister to clades 15-5 to 15-8. It is a slender fall annual herb of around ten cm tall native to high elevation pine-oak forests in Mexico. It is very similar to *E. sinaloensis* and *E. succedanea* of clade 15-5 in morphology, growth form, habitat and distribution. However, these three Mexican annuals do not form a monophyletic group. Clade 15-5 contains mostly shrubs; species from *E. misera* to *E. californica* have alternate branches, with spiral leaves on short shoots, and they occur in desert scrub of the Sonoran Desert. In contrast, species from *E. cotinifolia* to *E. macvaughii* in Fig. 2.3 have ternate leaves and branches and occupy tropical forests from Mexico to South America. Clade 15-6 corresponds to subsect. *Petaloma* Raf. ex Pax; both *E. marginata* and *E. bicolor* are annual herbs that are widely cultivated as ornamentals for their showy, white-margined bracts. Clade 15-7 corresponds to sect. *Arthrothamnus* § *Americanae* Boiss., and it is characterized by dioecious pencil-stem shrubs, with opposite or whorled, ridged branches; leaves are scale-like or caducous, and they lack stipules. Both *E. alata* and *E. cassythoides* occur in the Greater Antilles (Cuba and Jamaica, respectively). The closely related Galápagos endemic *E. equisetiformis* is also a leafless opposite-stemmed shrub, yet these three species are not monophyletic in our analysis. *Euphorbia innocua* occurs in an isolated position sister to clade 15-8; it was treated as the sole representative of sect. *Tithymalopsis* subsect. *Innocuae* G.L. Webster (Webster, 1967). It is a prostrate herb with four glands that superficially resembles sect. *Anisophyllum*. Webster (1967) considered it to be intermediate between sect. *Alectoroctonum* and sect. *Anisophyllum*, but such a relationship is not supported by our analyses. Clade 15-8 represents the northernmost distribution for sect. *Alectoroctonum*, extending from the eastern United States north to southern Canada. Species in this group are perennial herbs with leaves that are rounded at the apex, seeds that are ovoid, rounded in cross-section, smooth or shallowly pitted on the surface, and lacking a caruncle. Clade 15-8 largely corresponds to sect. *Tithymalopsis* subsect. *Ipecacuanhae* Boiss. (Huft, 1979), except for *E. aaron-rossii* (Holmgren and Holmgren, 1988), which belongs to a second unpublished subsection in sect. *Tithymalopsis* (Huft, 1979; Holmgren and Holmgren, 1988).

Clade 15-9 is composed of shrubs with ternate leaves and five involucral glands. They differ from other shrubs of sect. *Alectoroctonum* in having entirely white and showy bracts. Both species have carunculate seeds, which is otherwise rare in sect. *Alectoroctonum*. Clade 15-10 includes three species that are densely branched pencil-stem shrubs with a waxy surface, with cyathia in axillary cymes, five involucral glands with well-developed appendages; and seeds that are ovoid with a smooth surface. Between Clade 15-10 and 15-11, there are two species that form part of a polytomy and whose exact affinities are unclear. One of these, *E. fulgens* ("scarlet plume"), is a widely cultivated species for its large and showy gland appendages. In group 15-11, *E. bilobata* and *E. hexagona* both have entirely opposite leaves and branches and were treated as part of sect. *Zygophyllidium* Boiss. The remaining species of sect. *Zygophyllidium* as defined by Boissier (1862) are scattered in other parts of sect. *Alectoroctonum* and in sect. *Poinsettia* in our analyses. *Euphorbia soobyi* and *E. segovienesis* of clade 15-12 are morphologically consistent with clade 15-14 and may prove to belong to that clade with additional data. Clade 15-13 includes shrubs with ternate leaves and branches, deciduous leaves, and cyathia with five or six cyathial glands clustered in dense cymes. Between clade 15-13 and 15-14, *E. gumaroi* is a small succulent, decumbent species that was compared to *E. antisyphilitica* in its original description (García, 2000), but such an affinity is not supported by our molecular data, and cyathia in *E. gumaroi* are terminal rather than axillary in *E. antisyphilitica*. Finally, clade 15-14 is a large group of herbs and shrubs from tropical North and South America. Some species in this clade were treated as part of sect. *Cyttarospermum* Boiss. (Boissier, 1862), but the type of that section (*E. graminea*) belongs in clade 15-3. Species in Clade 15-14 are characterized by petioles that are longer than the leaves; five cyathial involucral glands; glandular appendages often deeply lobed; and ecarunculate seeds that are deeply pitted with protrusions on distinctive honeycomb ridges (Fig. 2.1Q).

***Evolution of growth forms and photosynthetic types*—**Figure 2.5A illustrates the basic structure of a *Euphorbia* plant (from Wheeler, 1941: plate 655). Annual members of the genus best exemplify this architecture, as various parts of the plant die back in perennials. After the cotyledonary node, the plant sometimes develops nodes with opposite leaves and side branches. Further up the main shoot, both leaves and branches

switch to being alternate. Either, neither, or both of the opposite/alternate sections can be absent. Later, the main shoot terminates with a whorl of two or more leaves and usually an equal number of branches, concomitant with a switch to reproductive growth, which is typically a pleiochasium of cymes. In this scheme, the synflorescence in *Euphorbia* typically has three levels of organization: 1) the cyathium itself bearing tightly packed male and/or female flowers, 2) the cyathia arranged in cymes, and 3) the cymes arranged in pleiochasia at the point of apical termination of the main stem (Fig. 2.5A). Despite various terminologies that have been applied to these three organization levels, here we call the leaves (or bracts when they are not green) that directly subtend a single cyathium "subcyathial bracts", leaves/bracts at lower nodes of the synflorescence "dichasial bracts", and the basalmost whorl of leaves associated with the inflorescence "pleiochasial bracts" (Molero and Rovira, 1992). This hierarchical three-level arrangement, plus the two optional vegetative stages below the inflorescence, accommodates opportunities for expanding, contracting, eliminating or rearranging different components of the plant (Fig. 2.5, B–E).

One notable modification of the basic plant structure in *Euphorbia* is the elimination of both of the opposite and alternate-leaved vegetative stages, and a complete lack of apical growth (Figs. 2.1E & 2.5B, Hayden, 1988). This growth form is a synapomorphy for sect. *Anisophyllum*, in which essentially the entire plant body is a synflorescence. With its prostrate or ascending growth habit, the plant grows to fill a two-dimensional space that maximizes its exposure to sunlight (Koontz et al., 2009; Horn et al., in review). Together with early flowering, copious fruiting and seed production, and C_4 photosynthesis, sect. *Anisophyllum* has been very successful in colonizing warm and semi-desert areas and disturbed habitats worldwide. However, both *E. remyi* and *E. halemanui*, two ascending shrubs endemic to the Hawaiian Archipelago, develop main shoots with continued apical growth carrying opposite leaves (Koutnik, 1987). In *E. potentilloides*, and occasionally in *E. angusta* and *E. viscoides*, main shoots terminate in a whorl of three or more leaves before producing the terminal pleiochasial cymes (Simmons and Hayden, 1997). Both cases probably represent partial reversal toward the basic growth form (Fig. 2.5, A and B).

Another notable example of modification in growth form is the continued elongation of main shoots with alternately arranged leaves and branches (Fig. 2.5B). In this growth form, cyathial cymes are all axillary instead of arranged in a terminal whorl. This type is seen in all members of clade 15-14 of sect. *Alectoroctonum* (Figs. 2.1C and 2.3B). A further modification of this growth form occurs when stems become fleshy and photosynthetic, and leaves deciduous or reduced; then the plants become stem succulents with alternate branching and axillary cyathia or cyathial cymes (Fig. 2.5D). This type of growth form is seen in sect. *Plagianthae* (*E. plagiantha*, Fig. 2.1A) and sect. *Alectoroctonum* (clade 15-10 and *E. gradyi* of clade 15-14).

Another type of stem succulent of separate origin occurs when the main shoot terminates with a pleiochasial cyme, and both the vegetative section and part of synflorescence becomes succulent. This way the plants have mostly dichotomous or whorled branching, with terminal cyathia or cyathial cymes (Fig. 2.5E). This growth form is found in all species of sect. *Articulofruticosae* (Fig. 2.1B), sect. *Bosseriae*, part of sect. *Crossadenia* (subsect. *Appariciana*e + *E. gymnoclada*), and part of sect. *Alectoroctonum* (clade 15-7 + *E. equisetiformis*). *Euphorbia gumaroi*, a stem succulent of sect. *Alectoroctonum* that is endemic to central Mexico (García, 2000), is unique in having alternate branches, with single, terminal cyathia (Fig. 2.1D) from extreme reduction of the synflorescence. All species of this type of stem succulents are densely branched and grow no more than 50 cm tall due to their determinate growth.

Euphorbia is extremely diverse in succulent growth forms, and most of the diversity in the genus is found in subg. *Euphorbia* and subg. *Rhizanthium*. In subg. *Chamaesyce*, stem succulents evolved at least six times, with multiple origins in both the Old World (southern Africa and Madagascar) and the New World (eastern Brazil and North America). Occurrences of stem succulents are usually associated with CAM photosynthesis. Stable isotope ratios (δ13C) have been tested in sect. *Articulofruticosae*, within which *E. ephedroides* has a value of typical CAM plants, while *E. rhombifolia* is intermediate between typical C_3 and CAM plants; in sect. *Bosseriae*, only *E. platyclada* was tested for δ13C, which indicates CAM photosynthesis; in sect. *Crossadenia*, *E. appariciana* has a C_3-CAM intermediate ratio; and in sect. *Alectoroctonum*, only *E.*

ceroderma (clade 15-10) has been tested, and the value is consistent with CAM photosynthesis (Horn et al., in prep).

Conclusions*—Euphorbia* subg. *Chamaesyce* has been recircumscribed here based on molecular data. With taxon sampling covering nearly half of the ingroup species and a well-resolved phylogeny, we are beginning to understand evolutionary trends in a worldwide lineage with diverse patterns of biogeography, growth forms and photosynthetic types. In addition, we are able to identify fifteen sections that are each morphologically and geographically distinctive. An updated sectional and subsectional classification is proposed below, with descriptions and lists of accepted species for each section or subsection.

TAXONOMIC TREATMENT

Species with molecular sequence data available in GenBank, published here or previously, are shown in bold in the species lists under each section or subsection. A searchable and downloadable full list of accepted names, their synonymies and type information is online at http://app.tolkin.org/projects/72/taxa.

Euphorbia* subg. *Chamaesyce Raf., Amer. Monthly Mag. 2: 119. 1817. – Type: *E. chamaesyce* L. (*ICBN* Art. 22.6).

Annual or perennial herbs, shrubs or trees. Stems and leaves sometimes more or less fleshy to succulent. Taproot slender or variously thickened, cylindrical to globose. Branches few to many, prostrate, decumbent, or upright; alternate, opposite and/or ternate; sometimes the apices become spinelike. Herbage glabrous or variously pubescent. Leaves alternate, opposite and/or ternate. Leaf shape varies; sometimes a dark-green vein can be seen on species with C_4 photosynthesis; stipules glandular, or linear, subulate to triangular. Cyathia bisexual, or rarely unisexual; solitary or in cymes, axillary or terminal, sometimes subtended by green or brightly colored bracts; glands [1--]4--5[--7], often with petaloid appendages, less often appendage horns, linear, or missing; ovary and glabrous or pubescent; styles 3, connate or free at the base, bifid or entire at the tip. Capsules 3-lobed, or less often subglobose. Seeds ovoid or oblong, more or less

quadrangular in cross-section, or less often rounded; surface variously sculptured or smooth; carunculate or ecarunculate.

Discussion. – Within *Euphorbia* subg. *Chamaesyce*, 588 species are recognized and distributed among 15 sections, with ***E. hainanensis*** Croizat sampled but left unplaced as to section. There are another 20 or so species in the process of being formally described, and there are still some unplaced species in *Euphorbia* that may prove to belong to this subgenus with further study.

1. *Euphorbia* [subg. *Chamaesyce*] sect. *Espinosae* Pax & K. Hoffm. in Engler, Pflanzenw. Afrikas [Veg. Erde 9] 3, 2: 149. 1921. *Euphorbia* sect. *Lyciopsis* subsect. *Espinosae* (Pax & K. Hoffm.) Pax & K. Hoffm., Natu¨rl. Pflanzenfam. 19c: 213. 1931. – Type: *E. espinosa* Pax.

Woody monoecious shrubs, stems with a shiny or papery bark, the stem apices often drying and becoming spinelike. Leaves alternate, shortly petiolate; stipules glandular, conspicuous. Cyathia bisexual; solitary, axillary, subsessile, or on lateral short shoots, surrounded at the base by a cluster of small leaf-like or scarious bracts; glands 5, entire, yellow-green; appendages absent (Fig. 2.1F); ovary subtended by an obvious 3-lobed perianth; styles joined at the base, with spreading bifid apices. Capsule well-exserted on a reflexed pedicel, deeply 3-lobed, glabrous. Seeds ovoid, slightly dorsiventrally compressed, smooth, with a cap-like caruncle.

Distribution and habitat. – Southern and eastern Africa (Angola, Botswana, Namibia, northern South Africa, Kenya, Malawi, Tanzania, Zambia, and Zimbabwe); hilly, deciduous woodlands, 300--1400 m.

Included species (2). – ***E. espinosa*** Pax and ***E. guerichiana*** Pax.

Discussion. – There are several other sections that resemble sect. *Espinosae* in their shrubby habit, coppery bark, and sometimes spinose branches. These include *E.* sect. *Somalica* S. Carter, *E.* sect. *Lyciopsis* Boiss., the *E. balsamifera* group (all in subg. *Rhizanthium*, also from Africa); and *E.* sect. *Plagianthae* (subg. *Chamaesyce*, from Madagascar).

2. *Euphorbia* [subg. *Chamaesyce*] sect. *Articulofruticosae* Bruyns, Taxon 55: 416. 2006. – Type: *E. aequoris* N.E. Br.

Generally dioecious, semi-woody to succulent shrubs; branches dichotomous or opposite, usually much-branched from the base; branches cylindrical or variously ridged, apices drying spinelike in some species. Leaves opposite, small and often scale-like, quickly deciduous leaving a calloused scar; stipules apparently absent or glandular and conspicuous. Cyathia or cyathial cymes terminal (sometimes appear to be axillary due to borne on apex of short shoots); cymes branch few to many times, internodes progressively shorter above; sub-cyathial bracts and dichasial bracts spathulate or similar to the leaves, deciduous. Cyathia small, unisexual, subsessile; glands 5, entire, appendage absent; ovary often subtended by an obvious 3-lobed perianth; styles free or connate at the base, bifid at the tip. Capsule subsessile or exerted and recurved, glabrous or pubescent. Seeds conical, obtusely 4-angled, surface finely tuberculate, ecarunculate (Fig. 2.1O).

Distribution and habitat. – Most diverse in the arid winter-rainfall region of western South Africa and southern Namibia, extending into southern Angola and Botswana and east to KwaZulu-Natal, South Africa. Grows in sandy soils or on rock outcrops, in low shrublands to deserts and consolidated dunes, from sea level to ca. 1000 m.

Included species (40 by Govaerts & al., 2000 [*19* by Bruyns & al., 2006]). – ***E. aequoris*** N.E. Br., *E. amarifontana* N.E. Br. [= *E. rhombifolia* sensu Bruyns], *E. angrae* N.E. Br., ***E. arceuthobioides*** Boiss., *E. aspericaulis* Pax [= *E. muricata* sensu Bruyns], *E. bayeri* L.C. Leach [=*E. rhombifolia* sensu Bruyns], ***E. brachiata*** E. Mey. ex Boiss. [=*E. rhombifolia* sensu Bruyns], ***E. burmannii*** E. Mey. ex Boiss., ***E. caterviflora*** N.E. Br. [=*E. rhombifolia* sensu Bruyns], ***E. chersina*** N.E. Br. [=*E. rhombifolia* sensu Bruyns], *E. cibdela* N.E. Br. [= *E. spartaria* sensu Bruyns], *E. corymbosa* N.E. Br. [= *E. burmannii* sensu Bruyns], ***E. einensis*** G. Will. [= *E. rhombifolia* sensu Bruyns], ***E. ephedroides*** E. Mey. ex Boiss., ***E. exilis*** L.C. Leach, ***E. gentilis*** N.E. Br., ***E. giessii*** L.C. Leach, ***E. glandularis*** L.C. Leach & G. Will. [= *E. exilis* sensu Bruyns], ***E. herrei*** A.C. White, R.A. Dyer, & B. Sloane, ***E. indecora*** N.E. Br. [= *E. rhombifolia* sensu Bruyns], ***E. juttae*** Dinter, ***E. karroensis*** (Boiss.) N.E. Br. [= *E. burmannii* sensu Bruyns], *E. lavranii* L.C. Leach, ***E. lumbricaulis*** L.C. Leach [= *E. stapeliodies* sensu Bruyns], *E. macella* N.E. Br. [= *E. burmannii* sensu Bruyns], ***E. mundii*** N.E. Br. [= *E. rhombifolia* sensu Bruyns], *E.*

muricata Thunb., *E. negromontana* N.E. Br., *E. perpera* N.E. Br. [= *E. rhombifolia* sensu Bruyns], *E. rectirama* N.E. Br. [= *E. spartaria* sensu Bruyns], ***E. rhombifolia*** Boiss., *E. rudolfii* N.E. Br. [= *E. rhombifolia* sensu Bruyns], ***E. spartaria*** N.E. Br., *E. spicata* E. Mey. ex Boiss. [= *E. muricata* sensu Bruyns], ***E. spinea*** N.E. Br., ***E. stapeliodes*** Boiss., ***E. suffulta*** Bruyns, *E. tenax* Burch. [= *E. arceuthobioides* sensu Bruyns], *E. vaalputziana* L.C. Leach [= *E. gentilis* sensu Bruyns], *E. verruculosa* N.E. Br.

Discussion. – This is a very well characterized group of pencil-stem succulents or wiry leafless bushes that are readily distinguished by their opposite or dichotomous branching, usually glandular stipules, and unisexual cyathia (Fig. 2.1B). They have undergone a recent substantial radiation in southern Africa, as seen from the extremely short terminal branches on phylograms (Figs. 2.2A & 2.3A). However, species limits are unclear, and the group is seriously in need of a systematic revision, as evidenced by the two alternative taxonomies alluded to above. Between them, Bruyns & al. (2006) probably excessively synonymized some of the names, but this will need to be examined on a case-by-case basis.

3. *Euphorbia* [subg. *Chamaesyce*] sect. *Cheirolepidium* Boiss. in DC., Prodr. 15(2): 9, 70. 1862. *Euphorbia* sect. *Cheirolepidium* Boiss. subsect. *Cheirolepidium* Boiss. ex Pax & K. Hoffm., Natu¨rl. Pflanzenfam. 19c: 213. 1931. *Euphorbia* subg. *Cystidospermum* (Prokh.) Prokh. in Komarov et al., Flora U.R.S.S. 14: 480. 1949. – Type: *E. cheirolepis* Fisch. & C.A. Mey.

Dematra Raf., Aut. Bot. 96. 1840. *Euphorbia* subg. *Esula* Pers. sect. *Dematra* (Raf.) Prokh. in Komarov et al., Flora U.R.S.S. 14: 476. 1949. Type: *D. sericea* Raf. (= *E. petiolata* Banks & Sol.).

Euphorbia 'ser.' *Exappendiculatae* Boiss. sect. *Tithymalus* (Scop.) Boiss. subsect. *Crotonopsideae* Boiss. in DC., Prodr. 15(2): 101. 1862. Type: *E. petiolata* Banks & Sol.

Ctenadenia Prokh., Consp. Syst. Tithymal. As. Med.: 28. 1933. – Type: *C. lanata* (Sieb.) Prokh. (= *E. petiolata* Banks & Sol.).

Cystidospermum Prokh., Consp. Syst. Tithymal. As. Med.: 25. 1933. *Euphorbia* subg. *Cystidospermum* (Prokh.) Prokh. in Komarov et al., Flora U.R.S.S. 14: 480. 1949. – Type: *E. cheirolepis* Fisch. & C.A. Mey.

Annual erect herbs, branches many. Herbage densely villous to subglabrous. Leaves and branches opposite at the base, alternate in the mid-section before the termination of apical growth and switch to dichotomous branching, with each fork subtended by dichasial bracts. Leaves linear-lanceolate to elliptic or ovate, margin distinctively spinulose-dentate; stipules subulate. Cyathia exserted, solitary between the forks of dichotomous branches, or few-clustered in axillary cymes; both dichasial and subcyathial bracts leaf-like but much reduced in size. Glands 4 per cyathium, with deep finger-like to linear lobes, stalked (*E. cheirolepsis*) or not (*E. petiolata*), yellow-green, sometimes turning red with age; gland appendages absent. Styles 3, free or connate at the base, tip entire. Ovary and capsule exserted at maturity, densely pubescent, 3-lobed. Seeds tetragonous in cross-section, surface tuberculate; caruncle large and stipitate in *E. petiolata* (Fig. 2.1M), or distinctively ligulate with two long flaps in *E. cheirolepis* (Fig. 2.1N).

Distribution and habitat. – From northern Africa through Central Asia; fallow fields and dry, open habitats, 500--1500 m.

Included species (2). – ***E. cheirolepis*** Fisch. & C.A. Mey., ***E. petiolata*** Banks & Sol.

Discussion. – These two species have been variously treated as members of subg. *Esula*, and they are certainly anomalous geographically for the remaining groups of subg. *Chamaesyce* (excluding sect. *Anisophyllum*). The presence of stipules and the pectinate cyathial glands distinguish both species from members of subg. *Esula*. The ligulate caruncle in *E. cheirolepsis* is unique in *Euphorbia* (Fig. 1N, Pahlevani & Akhani, 2011). However, it is deciduous and may appear to be ecarunculate on herbarium sheets.

The pectinate protrusions appear to directly extend from the rim of glands (Fig. 2.1G), unlike petaloid appendages in the "petaloid appendage clade" that appear to extend from the involucre and emerge from below the glands (Fig. 2.1, I & J). Steinmann & Porter (2002) interpreted these two species as possessing petaloid appendages. Interestingly Bayesian analysis of ITS places both sect. *Cheirolepidium* and sect. *Eremophyton* as sister to the petaloid appendage clade, although this relationship is not supported by any other analyses.

4. *Euphorbia* [subg. *Chamaesyce*] sect. *Eremophyton* Boiss. in DC., Prodr. 15(2): 9, 70. 1862. *Euphorbia* subg. *Eremophyton* (Boiss.) Wheeler, Amer. Midl. Nat. 30: 483. 1943. *Euphorbia* sect. *Eremophyton* subsect. *Eueremophyton* (Boiss.) Pax in Engl. & Prantl, Naturl. Pflanzenfam. 3(5): 107. 1891. – Lectotype: *E. eremophila* A. Cunn. (= *E. tannensis* subsp. *eremophila* (A. Cunn.) D.C. Hassall), designated by Wheeler, Amer. Midl. Nat. 30: 483. 1943.

Euphorbia sect. *Eremophila* Benth. & F. Mueller, Fl. Austral. 6: 45. 1873. – Type: *E. eremophila* A. Cunn. (= *E. tannensis* subsp. *eremophila* (A. Cunn.) D.C. Hassall).

Annual or perennial herbs to small shrubs, glabrous to sparsely pubescent. Stem erect, multibranched; leaves and branches opposite at the base, alternate in the mid-section before the termination of apical growth and then switching to dichotomous branching with each fork subtended by dichasial bracts. Leaves linear-lanceolate to ovate, margins serrate; stipules glandular or subulate. Cyathia solitary between the forks of dichotomous branches or few-clustered in axillary cymes, with dichasial and subcyathial bracts leaf-like but much reduced in size; glands 4, yellow, ovate, margins entire or crenate to palmatifid, gland appendages absent; ovary glabrous; styles 3, connate at the base, bifid at the tips. Capsules exserted, erect, 3-lobed. Seeds more or less tetragonous in cross-section, surface tuberculate to reticulate; caruncle present, variously shaped.

Distribution and habitat. – Australia, New Caledonia, Vanuatu; coastal sands to inland desert and scrub.

Included species (3) – ***E. parvicaruncula*** D.C. Hassall, ***E. planiticola*** D.C. Hassall, ***E. tannensis*** Spreng. (with two subspecies).

Discussion. – Boissier (1862) first established sect. *Eremophyton* to include three Old World species, but these now belong to three different sections in subg. *Chamaescyce*. In addition to the lectotype *E. eremophila* A. Cunn., the African *E. agowensis* is placed by our analyses in sect. *Scatorhizae*; while the third species, the South African *E. gueinzii*, is placed in sect. *Gueinziae*. Wheeler (1943) broadened the concept of sect. *Eremophyton* and elevated it to the rank of subgenus, but this was a heterogeneous assemblage that is not supported by molecular data.

Hassall (1977) treated five native Australian *Euphorbia* species as forming a natural group within *Euphorbia* subg. *Eremophyton*. However, our molecular data strongly reject monophyly of a clade containing all five species: *E. stevenii* and *E. boopthona* are both nested in *E.* subg. *Euphorbia* (Dorsey & al., in prep); the other three form a monophyletic group in subg. *Chamaesyce*, as treated here in the updated sect. *Eremophyton*.

5. *Euphorbia* [subg. *Chamaesyce*] sect. *Scatorhizae* Y. Yang & P.E. Berry, **sect. nov.** – Type: *E. scatorhiza* S. Carter.

Annual or perennial herbs, or shrubs; when woody often with peeling bark; with or without tubers. Leaves alternate basally, opposite distally, petiolate, margin entire, or undulate-margined, sometimes with gland-tipped marginal teeth at base; stipules glandular or subulate. Cymes in 2--3-branched umbels or cyathia solitary; subcyathial bracts small to well-developed. Cyathia sessile or subsessile, glands 4 or 5, elliptic to subcircular, exappendiculate; styles connate at the base, bifid at the tip. Ovary and capsule sessile or exserted on a recurved pedicel; 3-lobed, surface glabrous or pubescent. Seeds ovoid to oblong, more or less 4-angled in cross-section, dorsal-ventrally flattened, face smooth, wrinkled, or tuberculate; ecarunculate (*E. kabridarensis*), with a large cap-like caruncle (*E. applanata*, *E. agowensis*, *E. polyantha*, *E. trichiocyma*), or capsules and seeds unknown (*E. scatorhiza*).

Distribution and habitat. – Africa (Angola, Ethiopia, Kenya, Somalia, Tanzania), Arabia (Saudi Arabia, Yemen), India; rocky or sandy scrub or deserts, 200--1850 m.

Included species (7) – ***E. agowensis*** Hochst. ex Boiss., ***E. kabridarensis*** Thulin, ***E. polyantha*** Pax, ***E. scatorhiza*** S. Carter, *E. trichiocyma* S. Carter. Species that may also belong here: *E. applanata* Thulin & Gifri, *E. suborbicularis* Thulin.

Discussion. – Section *Scatorhizae* is characterized by non-succulents herbs to shrubs, sessile or subsessile cyathia, and flattened, 4-angled seeds. All four carunculate members plus *E. scatorhiza* has been treated in *Euphorbia* subg. *Eremophyton* (Boiss.) Wheeler (Carter & Radcliffe-Smith, 1988; Thulin & Al-Gifri, 1995) while the ecarunculate *E. kabridarensis* has been treated in sect. *Lyciopsis* Boiss. (Carter, 1992).

Species in sect. *Scatorhizae* have often been confused with sect. *Frondosae* Bruyns due to largely overlapping distribution, habit, and seed morphology.

6. *Euphorbia* [subg. *Chamaesyce*] sect. *Denisiae* T. Haevermans & X. Aubriot, **sect. nov.** – Type: *E. denisii* Oudejans.

Nonsucculent and prostrate shrubs to small trees, from stolons or tubers. Branches many, alternate, brown-green with transverse linear darker patches, twigs slightly succulent to ligneous. Leaves arranged spirally on short shoots, deciduous; blade obcordate to rounded, subpeltate with a cylindrical petiole, margin entire; stipules deciduous, small. Plants monoecious; cyathia subsessile, bisexual, subterminal, subcyathial bracts present but extremely reduced; glands 5, appendages absent. Styles connate at base, bifid at the tip. Capsule subsessile, erect, included in the cyathium cup at maturity. Seeds smooth, carunculate.

Distribution and habitat. – Southernmost Madagascar, in xerophytic vegetation.

Included species (2) – ***E. denisii*** Oudejans, *E. subpeltatophylla* Rauh.

Discussion. – Other species from southern Madagascar have converged morphologically with *E. denisii* and have been incorrectly identified as this species.

7. *Euphorbia* [subg. *Chamaesyce*] sect. *Bosseriae* T. Haevermans & X. Aubriot, **sect. nov.** – Type: *E. bosseri* Leandri.

Perennial herbs, stems succulent, with colored or dark blotches. Branching monochasial or dichasial. Leaves non-succulent, rounded and petiolate, or reduced to scales, alternate to subopposite; stipules glandular. Plants monoecious, cyathia solitary, terminal, subcyathial bracts apparently lacking; glands 4-5, margin entire, without appendages; ovary glabrous or sparsely pubescent, usually erect, although in *E. bemarahaensis* it can be recurved, impairing the development of the covered gland; styles 3, connate at the base, bifid almost to the base. Capsule 3-lobed, subsessile. Seeds tuberculate and pointed in *E. platyclada*; unclear in other two species. Caruncle unclear.

Distribution and habitat. –Southwestern and southernmost Madagascar, in generally xerophytic vegetation.

Included species (3) – ***E. bemarahaensis*** Rauh & R. Mangelsdorff, *E. bosseri* Leandri, ***E. platyclada*** Rauh.

Discussion. – These three species grow in remote areas and are locally rare microendemics. *Euphorbia bemarahaensis* is restricted to the Tsingy of Bemaraha (in the west), *E. platyclada* occurs south of Tulear and east to Fort Dauphin, and *E. bosseri* is restricted to the Betroka area (northwest of Fort Dauphin). Rauh and Mangelsdorff (1999) placed these three species in his informal "groupe d'*E. bosseri*" but without a clear idea of its affinities. Cremers (1984) confused *E. bosseri* and *E. platyclada* to be related to species like *E. enterophora* subsp. *enterophora* (subg. *Euphorbia* sect. *Tirucalli*) due to convergence in flattened twigs.

8. *Euphorbia* [subg. *Chamaesyce*] sect. *Plagianthae* T. Haevermans & X. Aubriot, **sect. nov.** – Type: *E. plagiantha* Drake.

Broom-like shrubs or trees with coppery-shiny bark peeling in papyraceous rings. Branches alternate, densely aggregated. Leaves scale-like and quickly deciduous (*E. plagiantha*) or developed and elongate (*E. salota*), distributed all along the twigs; stipules minute. Plants dioecious, pistillate cyathia usually single, staminate cyathia in few-cyathia cymes, on axillary branches (reduced in *E. plagiantha*, elongated in *E. salota*); subcyathial bracts green, inconspicuous in *E. plagiantha*, leaflike in *E. salota*; involucre rounded, with a sunken rim and [4--]5[--6] inconspicuous glands with an entire margin; gland appendage absent; ovary glabrous. Capsules dehiscent, 3-lobed, always oriented upward; surface smooth, green. Seeds surface smooth, ecarunculate.

Distribution and habitat. – Southern Madagascar, in xerophytic vegetation.

Included species (2) – ***E. plagiantha*** Drake, ***E. salota*** Leandri.

Discussion. – *Euphorbia plagiantha* is a striking tree with its coppery, peeling bark and leafless, photosynthetic stems (Fig. 2.1A), whereas *E. salota* is a broom-like shrub. *Euphorbia plagiantha* is widely distributed in semixerophytic forests and shrublands, whereas *E. salota* is restricted to a few ridges around Betroka, northwest of Fort Dauphin. Both species share the peeling bark, dioecy, and capsules oriented upwards regardless twig orientation. They also have cyathia with a shrunken rim bearing tiny reduced glands, and the cyathia are borne laterally on the distal part of the twigs, while

the vegetative branching occurs on the basal part of the twigs. *Euphorbia plagiantha* was previously grouped with *E. tirucalli* (Cremers, 1984), which is nested in subg. *Euphorbia* (Bruyns & al., 2006).

9. *Euphorbia* [subg. *Chamaesyce*] sect. *Frondosae* Bruyns, Taxon 55: 416. 2006. – Type: *E. goetzei* Pax.

Annual or perennial herbs, to shrubs, tuberous in perennial species. Stems terete and semisucculent to succulent, green and photosynthetic, branching sparingly to many (*E. leistneri*). Herbage glabrous or pubescent. Leaves alternate, ternate, and then opposite, deciduous, margin entire; stipules glandular, mainly on young growth (or absent). Inflorescence of 3–5-branched terminal umbels of cymes with internodes on primary rays up to 6--12 cm long, bracts similar to leaves in size and shape; cyathial glands 4[5], usually bilobed (sometimes entire), or with 2--4 suberect linear processes (*E. barbicollis* and *E. goetzei*); ovary glabrous or pubescent; styles free or connate at the base, bifid to 1/2 length at the tip. Capsules exserted on a reflexed pedicel; 3-lobed. Seeds ovoid to oblong, apex pointed, 4-angled on cross-section; surface wrinkled to tuberculate, with or without a caruncle; caruncle shape and size varies.

Distribution and habitat. –Eastern to southern Africa (Angola, Botswana, Ethiopia, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Uganda, Zambia, Zimbabwe) and the Arabian Peninsula (Oman, Saudi Arabia, Yemen); open to dense bushland, forest, 450-2700 m.

*Included species (11) –**E. barbicollis*** P.R.O. Bally, *E. engleri* Pax, ***E. goetzei*** Pax, ***E. leistneri*** R.H. Archer, ***E. pirottae*** N. Terrac., ***E. quaitensis*** S. Carter, and *E. transvaalensis* Schltr. These species may also belong here: *E. arrecta* N.E. Br., *E. dolichoceras* S. Carter, *E. radiifera* L.C. Leach, and *E. ruficeps* S. Carter.

Discussion – This group is characterized by being fleshy, stem photosynthetic and often tuberous herbs and shrubs; umbellate rays well-spaced with long internodes; glands variable, margin either entire or undulate to having processes, sometimes tips of these processes forked. Bruyns & al. (2006) placed *E. transvaalensis* in this clade, but also included it erroneously in subg. *Rhizanthium* in the same publication.

10. *Euphorbia* [subg. *Chamaesyce*] sect. *Tenellae* Pax & K. Hoffm. in Engler, Pflanzenw. Afrikas [Veg. Erde 9] 3, 2: 147. 1921. – Type: *E. glaucella* Pax (= *E. pfeilii* Pax).

Euphorbia sect. *Stachydium* § *Capensis* Boiss. in DC., Prodr. 15(2): 66. 1862. – Type: *E. phylloclada* Boiss.

Annual or perennial herbs, stems decumbent or erect, branches few to many; herbage glabrous. Leaves all opposite, narrow to subcordate, margin entire or denticulate; stipules subulate or inconspicuous. Cymes forking many times, bracts leaf-like. Cyathial glands 4, with petaloid appendages or apparently exappendiculate (may have a very thin rim on the glands); ovary glabrous or pubescent; styles free or connate at the base, tip bifid, 3-lobed. Capsule exserted on a recurved pedicel. Seeds oblong, 4-angled in cross-section, tuberculate to smooth, with a cap-like caruncle.

Distribution and habitat. – Southern Africa (Angola, Botswana, Namibia, South Africa, Zimbabwe); in open desert areas, exposed gravelly or sandy soils and rocky slopes, ca. 500--1100 m.

Included species (5) – E. claytonioides Pax, ***E. glanduligera*** Pax, *E. pfeilii* Pax, and ***E. phylloclada*** Boiss. Likely to belong here: *E. macra* Hiern.

Discussion – This is a small, but very significant section because it is sister to the rest of the petaloid appendage clade, which is otherwise mostly New World. Pax and Hoffman (1921: 147) recognized its similarities to sect. *Anisophyllum,* and Koutnik (1984) placed *E. glanduligera* in *Chamaesyce* S.F. Gray (= sect. *Anisophyllum*) and also asked whether *E. pfeilii* should belong there as well. *Euphorbia pfeilii* has sometimes been treated as a synonym of *E. glanduligera*, but Carter & Leach (2001) maintained it as distinct from that species, which they say has shorter internodes, swollen nodes, and denticulate floral bracts. *Euphorbia macra* appears to be similar to *E. pfeilii*, but it has a woody, perennial base.

Boissier (1862) placed *E. phylloclada* in *E.* [subg. *Euphorbia*] sect. *Stachydium*, perhaps because the subcyathial bracts can be congested, but they tend to be monochasial and enclose the cyathia more completely in sect. *Stachydium*. Later, Pax (in Pax & Hoffmann, 1921) placed *E. phylloclada* in *E.* sect. *Pseudacalypha*, presumably because of its axillary cyathia and herbaceous habit, but the rest of sect. *Pseudacalypha* is now

placed in subg. *Rhizanthium* (Steinmann & Porter, 2002; Horn et al., in press). Another species, *E. claytonioides,* is similar to *E. phylloclada*, both having subcordate leaves as opposed to the much narrower leaves of the other species in this section.

11. *Euphorbia* [subg. *Chamaesyce*] sect. *Gueinziae* Riina, **sect. nov.** – Type: *E. gueinzii* Boiss.

Perennial herbs with large tuberous roots, glabrous to densely pubescent; stems simple or multiple, branching dichotomously. Leaves usually alternate on lower portion of stem, opposite at the bifurcations of the upper branches, subsessile, lanceolate to ovate-lanceolate; stipules inconspicuous, glanduliform. Cyathia bisexual, sometimes unisexual; in terminal cymes or solitary at the bifurcation of branches; glands 5, trapezoid or oblong-ovate, the distal margin usually entire, rarely minutely crenulate, without appendages; ovary pubescent; styles 3, connate at the base, tips bifid and spreading. Capsule well-exserted, subglobose, 3-lobed. Seeds oblongoid, more or less tetragonal in cross-section, obscurely sculptured, pale grayish; without a caruncle.

Distribution and habitat. –South Africa (Mpumalanga, Free State, KwaZulu-Natal and Eastern Cape), Lesotho, Botswana, and Swaziland; dry grasslands on rocky slopes and sandstone cliffs, 600--1300 m.

Included species (1) – ***E. gueinzii*** Boiss.

Discussion. – In his treatment of *E. gueinzii* in Flora Capensis, Brown & al. (1913) characterized this species as dioecious, however, further observations suggest that it is monoecious, but sometimes it presents unisexual cyathia as well as bisexual ones (Hargreaves, 1992). There is no mention of dioecy in the protologue (Boissier 1862). Besides the relationship of *E. gueinzii* with the Brazilian *E.* sect. *Crossadenia* indicated by molecular data (Figs. 2.2, 2.3), there are no evident morphological affinities between *E. gueinzii* and other South African species. Hargreaves (1992) indicated that *E. gueinzii* was closely related to two other southern African geophytes, *E. trichadenia* Pax and *E. tuberosa* L., however recent phylogenetic analyses place these species within subg. *Rhizanthium* (Steinmann & Porter, 2002; Bruyns et al. 2006; Riina & al., in prep.). In the *Euphorbia* seed atlas (Morawetz & al., 2010) the seed shown of *E. gueinzii* was incorrectly identified as this species.

12. *Euphorbia* [subg. *Chamaesyce*] sect. *Crossadenia* Boiss., in DC., Prodr. 15(2): 9, 64. 1862. – Lectotype: *E. sarcodes* Boiss., designated by Wheeler in Amer. Midl. Nat. 30: 481. 1943.

Euphorbia sect. *Ephedropeplus* Müll. Arg. in Mart., Fl. Bras. 11(2): 668. 1874. *Ephedropeplus* (Müll. Arg.) Müll. Arg. ex Pax in Engl. & Prantl., Natu¨rl. Pflanzenfam. 3(5): 106. 1891. – Type: *E. gymnoclada* Boiss.

Perennial herbs, small leafy shrubs, or pencil-stem succulents, glabrous or pubescent, stems branching dichotomously or verticellately. Leaves opposite to alternate [spiral] on the lower stem, whorled at the base of umbellate rays, and opposite above, either rudimentary, minute, and soon deciduous, or well developed and persistent; stipules inconspicuous, glanduliform, rarely subulate. Cyathia terminal and axillary, arranged in short cymes or umbellate cymose rays, subtended by a pair of scale-like or foliose dichasial bracts. Involucres unisexual or bisexual, with 4 or 5 yellowish to green, appendiculate or exappendiculate glands; when present, gland appendages are short crenulate-dentate or long deeply cleft to fimbriate; ovary glabrous or pubescent; styles 3, basally connate, tips entire or bifid. Capsule well-exserted, subglobose to deeply 3-lobed. Seeds subglobose to ovoid, more or less tetragonal in cross-section, apex mucronate, surface shallowly to obscurely tuberculate, tubercles usually rounded (Fig. 2.1P), covered by a crustaceous, hydrophylic layer; ecarunculate.

Discussion. – The characteristic ornamentation of the seed coat, with shallow and rounded tubercles, may be a synapomorphy for this group. Boissier (1862) described the seeds of sect. *Crossadenia* as having a crustaceous caruncle, but our observations indicate that the apical part of the seed does not have a true caruncle; rather, the mucronate apex is an extension of the seed coat. The whitish layer on the outside of the seeds is hydrophilic and becomes mucilaginous when wet, much like seeds in sect. *Anisophyllum*. Both molecular data (Figs. 2.2 &2.3) and morphological characters support the division of this group into two subsections.

Key to the subsections of sect. *Crossadenia*

1. Involucral glands 5, lacking appendages or with crenulate to dentate appendages < 0.2 mm long …….. 12a. subsect. *Appariciannae*

1. Involucral glands 4 (5 in *E. gymnoclada*), with finger-like appendages 2--4 mm long with acute tips...…….. 12b. subsect. *Sarcodes*

12a. *Euphorbia* [subg. *Chamaesyce* sect. *Crossadenia*] subsect. *Appariciannae* Riina **subsect. nov.** – Type: *E. appariciana* Rizzini.

Stem leaves rudimentary and soon deciduous. Involucral glands 5, gland appendages crenate to dentate, teeth < 0.2 mm long, or appendages lacking (*E. flaviana*).

Distribution and habitat. –Endemic to the state of Bahia, Brazil, growing on granitic domes (inselbergs) or sandstone outcrops, 280-1200 m.

Included species (3). – ***E. appariciana*** Rizzini, ***E. flaviana*** Carn.-Torres & Cordeiro, *E. teres* M. Machado & Hofacker.

12b. *Euphorbia* [subg. *Chamaesyce* sect. *Crossadenia*] subsect. *Sarcodes* Riina, **subsect. nov.** – Type: *E. sarcodes* Boiss.

Stem leaves usually well developed and persistent (rudimentary and deciduous in *E. gymnoclada*). Involucral glands 4 (5 in *E. gymnoclada*) with white, finger-like appendages, teeth 2-4 mm long.

Distribution and habitat. – Endemic to eastern-central Brazil (Bahia, Goiás, Minas Gerais, Distrito Federal, and Piauí), in "campo rupestre" vegetation on sandy substrates and coastal "restinga", 30-1400 m.

Included species (6). – *E. crossadenia* Pax & K. Hoffm., ***E. goyazensis*** Boiss., ***E. gymnoclada*** Boiss., ***E. lycioides*** Boiss., ***E. sarcodes*** Boiss., ***E. sessilifolia*** Klotzsch ex Boiss.

13. *Euphorbia* [subg. *Chamaesyce*] sect. *Anisophyllum* Roeper in A. DC, Bot. Gall., ed. 2, 1: 412. 1828. – Lectotype: *E. peplis* L., designated by Wheeler, Rhodora 43: 111. 1941.

Anisophyllum Haw., Syn. Pl. Succ. 159. 1812. – Lectotype: *A. peplis* (L.) Haw. (= *E. peplis* L.), designated by Wheeler, Rhodora 43: 110. 1941.

Aplarina Raf., New Fl. 4: 99. 1838. – Lectotype: *A. prostrata* Raf. (= *E. prostrata* Aiton), designated by Wheeler, Rhodora 43: 111. 1941.

Chamaesyce S.F. Gray, Nat. Arr. Brit. Pl. 2: 260. 1821. *Euphorbia* subg. *Chamaesyce* (S.F. Gray) Caesalp. ex Reichenb., Deut. Bot. Herb.-Buch 193. 1841. – Type: *C. maritima* S.F. Gray (= *E. peplis* L.).

Ditritra Raf., Sylva Tellur. 115. 1838. – Lectotype: *D. hirta* (L.) Raf. (= *E. hirta* L.), designated by Wheeler, Amer. Midl. Nat. 30: 464. 1943.

Endoisila Raf., Sylva Tellur. 114. 1838. – Type: *Endoisila myrsinites* Raf. (= *E. myrtillifolia* L.). See Wheeler, Amer. Midl. Nat. 30: 465. 1943, for explanation of Rafinesque's confusion over the specific epithet of the type species.

Xamesike Raf., Fl. Tellur. 4: 115. 1838. – Lectotype: *X. vulgaris* Raf. (= *E. chamaesyce* L.), designated by Wheeler, Amer. Midl. Nat. 30: 476. 1943.

Annual or perennial herbs, rarely subshrubs to shrubs. Branches many, dichotomous, prostrate or ascending, rarely erect. Main shoot aborts above the cotyledon node (less often continues growing for a few more nodes) and lateral shoots branch dichotomously. Leaves opposite, glabrous or pubescent, often with an asymmetrical leaf base; sometimes dark-green veins are visible on leaf blades; margins entire or serrate; stipules interpetiolar, glandular, or linear, subulate to triangular. Cyathium solitary at the bifurcation of branches, or clustered in an axillary cyme. Glands 4, rarely 5--7; appendages present or absent, petaloid when present. Style 3, free or connate at the base, tip bifid, rarely entire. Ovary and capsule glabrous or pubescent. Seed surface with transverse ridges, smooth, or with irregular wrinkles; quadrangular (rarely triangular or elliptic) in cross section; ecarunculate except in *E. carunculata*. C_2, C_3 or C_4 photosynthesis.

Distribution and habitat. – Warm, arid and semi-arid vegetation or disturbed habitats, and summer annuals of temperate areas; worldwide, sea level to 2600 m.

Discussion. – Section *Anisophyllum* is characterized by its specialized growth form with early abortion of main shoot; lateral shoots producing exclusively opposite leaves; all but three species with C_4 photosynthesis.

Key to the subsections of sect. *Anisophyllum*

1. Glandular stipules, leaf cross-section without typical Kranz anatomy, cyathial gland appendages fan-shaped; Texas, New Mexico, and northern Mexico…..13a. subsect. *Acutae*

1. Subulate, triangular or ciliated, non-glandular stipules, Kranz anatomy, cyathial gland appendages absent or present, shape varies; widespread distribution.....13b. subsect. *Chamaesyce*

13a. *Euphorbia* [subg. *Chamaesyce* sect. *Anisophyllum*] subsection *Acutae* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 104. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Acutae* Boiss. in DC., Prodr. 15(2): 18. 1862. – Lectotype: *E. acuta* Engelm., designated by Wheeler in Rhodora 43: 111. 1941.

Perennial herbs with a thickened woody taproot. Herbage pubescent. Stems prostrate, arching, ascending to erect. Leaves opposite, or occasionally annual shoots terminate with a whorl in *E. angusta*; margin entire; stipules glandular. Cyathia solitary at the bifurcation of branches; glands 4, appendages fan-shaped, all 4 are equal in size; ovary and capsule pubescent, tri-lobed; styles bifid. Seeds ovoid, quadrangular in cross-sections; face smooth, with irregular shallow depressions, or obscurely transversely rugose. C_2 or C_3 photosynthesis.

Distribution and habitat. – U.S.A. (western Texas) and Mexican (Coahuila, Durango and Tamaulipas); grassland to desert scrub of Chihuahuan Desert on sandy or gravel limestone substrates, 200--1500 m.

Included species (3). – ***E. acuta*** Engelm., ***E. angusta*** Engelm., ***E. johnstonii*** Mayfield.

Discussion. – Subsection *Acutae* are all non-C_4, and is diagnosed within the section by having glandular stipules.

13b. *Euphorbia* [subg. *Chamaesyce* sect. *Anisophyllum*] subsection *Chamaesyce* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 104. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Chamaesyce* Boiss. in DC., Prodr. 15(2): 27. 1862. – Type: *E. chamaesyce* L. (ICBN Art. 22.6). The designation of *E. peplis* L. as lectotype by Wheeler (in Rhodora 43: 111. 1941) has no standing.

Euphorbia sect. *Anisophyllum* subsect. *Cheloneae* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 105. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Cheloneae* Boiss. in DC., Prodr. 15(2): 16. 1862. – Lectotype: *E. nummularia* Hook.f., designated by Wheeler in Rhodora 43: 111. 1941.

Euphorbia sect. *Anisophyllum* subsect. *Elegantes* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 104. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Elegantes* Boiss. in DC., Prodr. 15(2): 18. 1862. – Lectotype: *E. elegans* Spreng., designated by Wheeler, Amer. Midl. Nat. 30: 480. 1943.

Euphorbia sect. *Anisophyllum* subsect. *Gymnadeniae* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 105. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Gymnadeniae* Boiss. in DC., Prodr. 15(2): 11. 1862. *Chamaesyce* sect. *Sclerophyllae* subsect. *Gymnadeniae* (Boiss.) Koutnik in Allertonia 4: 338. 1987. – Lectotype: *E. clusiifolia* Hook. & Arn., designated by Wheeler, Amer. Midl. Nat. 30: 480. 1943.

Euphorbia sect. *Anisophyllum* subsect. *Hypericifoliae* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 104. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Hypericifoliae* Boiss. in DC., Prodr. 15(2): 20. 1862. – Lectotype: *E. hypericifolia* L., designated by Wheeler in Rhodora 43: 111. 1941.

Euphorbia sect. *Anisophyllum* subsect. *Pleiadeniae* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 105. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Pleiadeniae* Boiss. in DC., Prodr. 15(2): 50. 1862. – Lectotype: *E. selloi* (Klotzsch & Garcke) Boiss., designated by Wheeler, Amer. Midl. Nat. 30: 480. 1943.

Euphorbia sect. *Anisophyllum* subsect. *Sclerophyllae* Boiss. ex Pax, in Engl. & Prantl, Natu¨rl. Pflanzenfam. 3(5): 105. 1891. *Euphorbia* sect. *Anisophyllum* Roeper § *Sclerophyllae* Boiss. in DC., Prodr. 15(2): 12. 1862. *Euphorbia* subg. *Chamaesyce* Raf. sect. *Sclerophyllae* (Boiss.) Binojk. & N.P. Balakr., Genus *Euphorbia* in India: 201. 2010. *Chamaesyce* Gray sect. *Sclerophyllae* (Boiss.) Hurusawa in J. Fac. Sci. Univ. Tokyo Bot. 6: 275. 1954 – Lectotype: *E. atoto* G. Forst., designated by Wheeler, Amer. Midl. Nat. 30: 480. 1943.

Euphorbia subg. *Chamaesyce* Raf. sect. *Longistylae* Binojk. & N.P. Balakr., Genus *Euphorbia* in India: 178. 2010. – Type: *E. longistyla* Boiss.

Annual or perennial herbs, rarely subshrubs to shrubs. Branches many, dichotomous, prostrate or ascending, rarely erect. Main shoot aborts above the cotyledon node (less often continues elongation) and lateral shoots branch dichotomously. Leaves opposite, glabrous or pubescent, often with an asymmetrical leaf base; sometimes dark-green veins are visible on leaf blades; margins entire or serrate; stipules interpetiolar, linear, subulate to triangular. Cyathium solitary at the bifurcation of branches, or clustered in an axillary cyme. Glands 4, rarely 5--7; appendages present or absent, petaloid when present. Style 3, free or connate at the base, tip bifid, rarely entire. Ovary and capsule glabrous or pubescent. Seeds ecarunculate; surface with transverse ridges, smooth, or with irregular wrinkles; quadrangular (rarely triangular or elliptic) in cross section. C_4 photosynthesis.

Distribution and habitat. – Warm, arid and semi-arid vegetation or disturbed habitats, and summer annuals in temperate areas; worldwide, from sea level to approx. 2,600 m.

Included species (354). – *E. abdita* (D.G. Burch) Radcl.-Sm., *E. abdulghafooriana* Abedin, ***E. abramsiana*** L.C. Wheeler, ***E. adenoptera*** Bertol., *E. alainii* Oudejans, *E. alatocaulis* V.W. Steinm. & Felger, ***E. albomarginata*** Torr. & A. Gray, *E. allocarpa* S. Carter, *E. alsiniflora* Baill., *E. alsinifolia* Boiss., *E. amandi* Oudejans, *E. amplexicaulis* Hook.f., *E. anisopetala* (Prokh.) Prokh., ***E. anthonyi*** Brandegee, ***E. anychioides*** Boiss., ***E. apatzingana*** McVaugh, *E. apicata* Wheeler, ***E. arabica*** Hochst. & Steud. ex Anderson, *E. arabicoides* N.E. Br., *E. arenarioides* Gagnep., *E. argillosa* Chodat & Hassl., ***E. arizonica*** Engelm., *E. armstrongiana* Boiss., *E. arnottiana* Endl., ***E. articulata*** Burm., ***E. astyla*** Engelm. ex Boiss., ***E. atoto*** G. Forst., *E. atrococca* A. Heller, ***E. australis*** Boiss., *E. austrooccidentalis* Thell., *E. bahiensis* (Klotzsch & Garcke) Boiss., *E. balakrishnanii* Binojk. & Gopalan, *E. balbisii* Boiss., *E. bartolomaei* Greene, *E. baueri* Engelm. ex Boiss., ***E. berteroana*** Balb. ex Spreng., *E. besseri* (Klotzsch & Garcke) Boiss., *E. biconvexa* Domin, *E. bifida* Hook. & Arn., *E. bindloensis* (Stewart) Y. Yang, ***E. blodgettii*** Engelm. ex Hitchc., *E. boliviana* Rusby, ***E. bombensis*** Jacq., *E. bracteolaris* Boiss., ***E. brandegeei*** Millsp., *E. bruntii* (Proctor) Oudejans, *E. burchellii* Müll. Arg., *E. burmanica* Hook.f., *E. calderoniae* V.W. Steinm., ***E. camagueyensis*** (Millsp.) Urb., *E. capillaries* Gagnep., ***E. capitellata*** Engelm., ***E.***

carissoides F.M. Bailey, *E. carunculata* Waterf., ***E. catamarcensis*** (Croizat) Subils, *E. cayensis* Millsp., ***E. celastroides*** Boiss., *E. centralis* B.G. Thomson, *E. centunculoides* Kunth, ***E. chaetocalyx*** (Boiss.) Tidestr., *E. chamaecaula* Weath., ***E. chamaerrhodos*** Boiss., ***E. chamaesyce*** L., *E. chamaesycoides* B. Nord., *E. chamberlinii* I.M. Johnst., ***E. chamissonis*** (Klotzsch & Garcke) Boiss., ***E. cinerascens*** Engelm., *E. clarkeana* Hook.f., *E. clavidigitata* Gage, ***E. clusiifolia*** Hook. & Arn., *E. coccinea* B. Heyne ex Roth, ***E. coghlanii*** F.M. Bailey, *E. compressa* Boiss., *E. concanensis* M.K. Janarth. & S.R. Yadav, ***E. conferta*** (Small) B.E. Sm., *E. convolvuloides* Hochst. ex Benth., *E. cordifolia* Elliott, *E. corrigioloides* Boiss., *E. cowellii* (Millsp. ex Britton) Oudejans, *E. cozumelensis* Millsp., *E. crassinodis* Urb., *E. crepitata* L.C. Wheeler, *E. crepuscula* (L.C. Wheeler) V.W. Steinm. & Felger, *E. cristata* B. Heyne ex Roth, ***E. cumbrae*** Boiss., *E. cumulicola* (Small) Oudejans, ***E. dallachyana*** Baill., *E. deccanensis* V.S. Raju, ***E. degeneri*** Sherff, *E. delicatissima* S. Carter, ***E. deltoidea*** Engelm. ex Chapm., ***E. densiflora*** (Klotzsch and Garcke) Klotzsch, ***E. dentosa*** I.M. Johnst., *E. deppeana* Boiss., *E. derickii* V.W. Steinm., *E. diminuta* S. Carter, ***E. dioeca*** Kunth, *E. drummondii* Boiss., *E. duckei* (Croizat) Oudejans, ***E. eichleri*** Müll. Arg., *E. eleanoriae* (D.H. Lorence & W.L. Wagner) Govaerts, *E. elegans* Spreng., *E. engelmannii* Boiss., *E. erythroclada* Boiss., ***E. eylesii*** Rendle, ***E. feddemae*** McVaugh, ***E. fendleri*** Torr. & A. Gray, *E. filicaulis* Urb., *E. fischeri* Pax, *E. flindersica* Halford & W.K. Harris, *E. floribunda* Engelm. ex Boiss., ***E. florida*** Engelm., *E. foliolosa* Boiss., *E. fosbergii* (J. Florence) Govaerts, *E. fruticulosa* Engelm. ex Boiss., *E. galapageia* B.L. Rob. & Greenm., *E. garanbiensis* Hayata, ***E. garberi*** Engelm. ex Chapm., *E. gaudichaudii* Boiss., ***E. geyeri*** Engelm. & A. Gray, ***E. glyptosperma*** Engelm., *E. goliana* Comm. ex Lam., ***E. golondrina*** L.C. Wheeler, ***E. gracillima*** S. Watson, ***E. grammata*** (McVaugh) Oudejans, *E. grandidieri* Baill., ***E. granulata*** Forssk., *E. guanarensis* Pittier, *E. hajhirensis* Radcl.-Sm., *E. halemanui* Sherff, *E. heleniana* Thell. & Stapf, *E. helwigii* Urb. & Ekman, *E. hepatica* Urb. & Ekman, *E. herbstii* (W.L. Wagner) Oudejans, *E. heyneana* Spreng., *E. hildebrandtii* Baill., ***E. hirta*** L., ***E. hirtella*** Boiss., *E. hispida* Boiss., ***E. hooveri*** Wheeler, *E. hsinchuensis* (S.C. Lin & S.M. Chaw) C.Y. Wu & J.S. Ma, *E. humbertii* Denis, ***E. humifusa*** Willd., *E. humistrata* Engelm. ex A.Gray, *E. hunzikeri* Subils, ***E. hypericifolia*** L., ***E. hyssopifolia*** L., ***E. inaequilatera*** Sond., *E. inaguaensis* Oudejans, *E.*

inappendiculata Domin, *E. incerta* Brandegee, ***E. indica*** Lam., ***E. indivisa*** (Engelm.) Tidestr., *E. infernidialis* V.W. Steinm., *E. jamesonii* Boiss., ***E. jejuna*** M.C. Johnst. & Warnock, *E. jodhpurensis* Blatt. & Hallb., *E. karibensis* S. Carter, *E. katrajensis* Gage, *E. kerstingii* Pax, *E. kilwana* N.E. Br., *E. kimberleyensis* B.G. Thomson, *E. kischenensis* Vierh., ***E. klotzschii*** Oudejans, *E. kuriensis* Vierh., ***E. kuwaleana*** O. Deg. & Sherff, *E. laciniata* Panigrahi, *E. laredana* Millsp., ***E. lasiocarpa*** Klotzsch, ***E. lata*** Engelm., *E. lawsonii* Binojkumar & Dwarakan, *E. lecheoides* Millsp., *E. leonardii* (D.G. Burch) Radcl.-Sm., *E. leptoclada* Balf.f., ***E. leucantha*** (Klotzsch & Garcke) Boiss., ***E. leucophylla*** Benth., *E. lineata* S. Watson, ***E. linguiformis*** McVaugh, *E. lissosperma* S. Carter, *E. liukiuensis* Hayata, *E. livida* E. Mey. ex Boiss., *E. loandensis* N.E. Br., *E. longinsulicola* S.R. Hill, *E. longistyla* Boiss., *E. lupatensis* N.E. Br., *E. lutulenta* (Croizat) Oudejans, *E. luzoniensis* Merr., *E. macgillivrayi* Boiss., *E. machrisiae* Steyerm., *E. maconochieana* B.G. Thomson, ***E. maculata*** L., ***E. magdalenae*** Benth., ***E. makinoi*** Hayata, *E. marayensis* Subils, ***E. meganaesos*** Featherman, *E. melanadenia* Torr. & A. Gray, ***E. mendezii*** Boiss., ***E. mertonii*** Fosberg, ***E. mesembryanthemifolia*** Jacq., *E. meyeniana* Klotzsch, *E. microcephala* Boiss., ***E. micromera*** Boiss., *E. minbuensis* Gage, *E. minutula* Boiss., ***E. missurica*** Raf., *E. mitchelliana* Boiss., ***E. mossambicensis*** (Klotzsch & Garcke) Boiss., ***E. mossamedensis*** N.E. Br., *E. muelleri* Boiss., ***E. multiformis*** Gaudich. ex Hook. & Arn., *E. multinodis* Urb., ***E. myrtillifolia*** L., *E. neocaledonica* Boiss., ***E. neopolycnemoides*** Pax & K. Hoffm., ***E. nocens*** (L.C. Wheeler) V.W. Steinm., *E. nodosa* Houtt., *E. notoptera* Boiss., *E. nummularia* Hook.f., ***E. nutans*** Lag., *E. obliqua* F.A. Bauer ex Endl., *E. occidentaustralica* Radcl.-Sm. & Govaerts, *E. ocellata* Durand & Hilg., ***E. olowaluana*** Sherff, *E. ophiolitica* (P.I. Forst.) Y. Yang, ***E. ophthalmica*** Pers., ***E. oranensis*** (Croizat) Subils, ***E. orbiculata*** Kunth, *E. orbifolia* (Alain) Oudejans, *E. origanoides* L., *E. oxycoccoides* Boiss., *E. pancheri* Baill., *E. parciflora* Urb., *E. paredonensis* (Millsp.) Oudejans, *E. parishii* Greene, *E. parkeri* Binojkumar & N.P. Balakr., ***E. parryi*** Engelm., *E. parva* N.E. Br., *E. parviflora* L., ***E. pediculifera*** Engelm., *E. pellegrinii* Leandri, ***E. peninsularis*** I.M. Johnst., ***E. peplis*** L., ***E. perennans*** (Shinners) Warnock & M.C. Johnst., *E. pergracilis* P.G. Mey., ***E. perlignea*** McVaugh, *E. peruviana* Wheeler, ***E. petrina*** S. Watson, *E. picachensis* Brandegee, *E. pilosissima* S. Carter, ***E. pionosperma*** V.W. Steinm. & Felger, ***E.***

platysperma Engelm. ex S. Watson, *E. podadenia* Boiss., ***E. polycarpa*** Benth., ***E. polycnemoides*** Hochst. ex Boiss., ***E. polygonifolia*** L., *E. pondii* Millsp., ***E. porteriana*** (Small) Oudejans, *E. portucasadiana* (Croizat) Subils, ***E. potentilloides*** Boiss., *E. potosina* Fernald, *E. proctorii* (D.G. Burch) Correll, ***E. prostrata*** Aiton, ***E. psammogeton*** P.S. Green, *E. pueblensis* Brandegee, *E. punctulata* Andersson, *E. pycnostegia* Boiss., *E. quitensis* Boiss., *E. radioloides* Boiss., *E. ramosa* Seaton, *E. reconciliationis* Radcl.-Sm., *E. recurva* Hook.f., ***E. remyi*** A. Gray ex Boiss., ***E. reniformis*** Blume, ***E. revoluta*** Engelm., *E. rhytisperma* (Klotzsch & Garcke) Boiss., ***E. riebeckii*** Pax, *E. rivae* Pax, *E. rochaensis* (Croizat) Alonso Paz & Marchesi, ***E. rockii*** C.N. Forbes, ***E. rosea*** Retz., *E. rubriflora* N.E. Br., *E. ruiziana* (Klotzsch & Garcke) Boiss., ***E. ruizlealii*** Subils, *E. rutilis* (Millsp.) Standl. & Steyerm., *E. sabulicola* Boiss., *E. sachetiana* (J. Florence) Govaerts, *E. salsicola* S. Carter, *E. salsuginosa* (McVaugh) Radcl.-Sm. & Govaerts, *E. sanmartensis* Rusby, *E. scabrifolia* Kurz, ***E. schizolepis*** F. Muell. ex Boiss., *E. schlechteri* Pax, ***E. schultzii*** Benth., *E. schumannii* Radcl.-Sm., *E. schweinfurthii* Balf.f., *E. scopulorum* Brandegee, *E. scordiifolia* Jacq., *E. sebastinei* Binojk. & N.P. Balakr., *E. seleri* Donn. Sm., ***E. selloi*** (Klotzsch & Garcke) Boiss., *E. selousiana* S. Carter, *E. senguptae* N.P. Balakr. & Subr., ***E. serpens*** Kunth, ***E. serpyllifolia*** Pers., *E. serratifolia* S. Carter, ***E. serrula*** Engelm., ***E. setiloba*** Engelm. ex Torr., ***E. setosa*** (Boiss.) Müll. Arg., *E. sharkoensis* Baill., ***E. simulans*** (L.C. Wheeler) Warnock & M.C. Johnst., *E. skottsbergii* Sherff, ***E. sparrmanii*** Boiss., *E. sparsiflora* A. Heller, *E. spellenbergiana* Mayfield & V.W. Steinm., *E. spissiflora* S. Carter, *E. standleyi* (Millsp.) Oudejans, ***E. stictospora*** Engelm., *E. stoddartii* Fosberg, *E. subterminalis* N.E. Br., *E. sumbawensis* Boiss., *E. taihsiensis* (Chaw & Koutnik) Oudejans, *E. taluticola* Wiggins, ***E. tamanduana*** Boiss., *E. tamaulipasana* (Millsp.) Oudejans, ***E. tettensis*** Klotzsch, ***E. theriaca*** L.C. Wheeler, ***E. thymifolia*** L., *E. tinianensis* Hosok., *E. tomentella* Engelm. ex Boiss., ***E. tomentulosa*** S. Watson, *E. torralbasii* Urb., ***E. trachysperma*** Engelm., *E. trancapatae* (Croizat) J.F. Macbr., ***E. trialata*** (Huft) V.W. Steinm., *E. trichophylla* Baker, *E. trinervia* Schumach. & Thonn., *E. tumistyla* (D.G. Burch) Radcl.-Sm., ***E. turpinii*** Boiss., ***E. umbellulata*** Engelm. ex Boiss., *E. vaginulata* Griseb., ***E. vallis-mortae*** (Millsp.) J.T. Howell, *E. vauthieriana* Boiss., ***E. velleriflora*** (Klotzsch & Garcke) Boiss., *E. velligera* Schauer, ***E. vermiculata*** Raf., *E. vestita* Boiss., *E. vezorum*

Leandri, *E. viatilis* Ule, ***E. villifera*** Scheele, *E. viminea* Hook.f., *E. viridula* Cordem. ex Radcl.-Sm., ***E. viscoides*** Boiss., *E. wheeleri* Baill., *E. yucatanensis* (Millsp.) Standl., ***E. zambesiana*** Benth.

Discussion. – Old World species in subsect. *Chamaesyce* are relatively understudied and there may be additional species to be described as well as to be placed in synonymy. Currently David Halford (BRI) is revising the group in Australia and has numerous new species he plans to name and publish as part of that revision.

The following names published under *Chamaesyce* are here formally transferred to *Euphorbia*:

Euphorbia bindloensis (Stewart) Y. Yang, **comb. & stat. nov.** *Chamaesyce bindloensis* (Stewart) D.G. Burch, Ann. Missouri Bot. Gard. 56: 176. 1969. – Type: Ecuador Galapagos: Bindloe Island, *Stewart 1968* (GH, holotype).

Euphorbia deltoidea subsp. *serpyllum* (Small) Y. Yang, **comb. & stat. nov.** *Chamaesyce serpyllum* Small, Fl. Florida Keys: 81. 1913. *Chamaesyce deltoidea* subsp. *serpyllum* (Small) D.G. Burch, Ann. Missouri Bot. Gard. 53: 99. 1966. *Euphorbia deltoidea* var. *serpyllum* (Small) Oudejans, Phytologia 67: 45. 1989. – Type: U.S.A. Florida: Big Pine Key, Monroe County, *J.K. Small 3811* (NY, holotype). New combination made to be consistent in recognizing four subspecies within the *Euphorbia deltoidea* complex.

Euphorbia ophiolitica (P.I. Forst.) Y. Yang, **comb. & stat. nov.** *Chamaesyce ophiolitica* P.I. Forst., Austrobaileya 5: 711. 2000. – Type: Australia Queensland: Port Curtis District, *P.I. Forster 15042* (BRI, holotype).

14. *Euphorbia* [subg. *Chamaesyce*] sect. *Poinsettia* (Graham) Baill., Étude Gén. Euphorb. 284. 1858. *Poinsettia* Graham, Edinburgh New Philos. J. 20: 412. 1836. *Euphorbia* subg. *Poinsettia* (Graham) House, Bull. New York State Mus. 254: 473. 1924. *Euphorbia* sect. *Poinsettia* (Graham) Boiss. in D.C., Prodr. 15 (2): 71. 1862. – Type: *Poinsettia pulcherrima* (Willd.) Graham (= *E. pulcherrima* Willd.).

Cyathophora Raf., Fl. Tellur. 4: 117. 1838. – Type: *C. heterophylla* (Raf.) L. (= *E. heterophylla* L.).

Pleuradena Raf., Atlantic J. 1: 182. 1833. *Euphorbia* subg. *Pleuradena* (Raf.) Croizat, Rev. Sudamer. Bot. 6: 10. 1939. – Type: *Pleuradena coccinea* Raf. (= *E. pulcherrima* Willd.).

Annual or perennial herbs, shrubs, or small trees, from a taproot or tuber(s). Earliest developing leaves and branches opposite, middle to upper nodes alternate or opposite/whorled, blades usually markedly to finely serrate, rarely entire, sometimes markedly heteromorphic; stipules minute, glanduliform, often inconspicuous. Cyathia few to many in usually congested, terminal cymes, sometimes appearing corymbiform, bracts sometimes whitish to bright red at the base or on the entire blade; cyathial glands 1--5(--8), slightly concave to deeply cupped, bilabiate, or circular, appendages lacking or present and variously shaped; styles 3, free or connate at the base, tip entire or bifid. Ovary and capsule glabrous or pubescent, 3-lobed. Seeds tetragonal to ovoid, sharply 4-angled to somewhat rounded in cross-section, variously tuberculate, with or without a caruncle.

Discussion – Species of the first two diverging subsections (subsects. *Lacerae* and *Erianthae*) are distinct in their large caruncles and sharply tetragonal seeds. The seeds of subsect. *Exstipulatae*, in contrast, are similar to many of those in subsect. *Stormiae*, which have a coarsely tuberculate surface, more ovoid shape, and are either carunculate or ecarunculate. See additional discussion in the main text for the expanded circumscription of sect. *Poinsettia*.

Key to the subsections of sect. *Poinsettia*

1. Leaves linear, mostly entire; involucral glands densely appressed-pubescent, with laciniate, pubescent appendages that arch upwards and inwards over the gland... subsect. *Erianthae*
1. Leaves linear to pandurate and usually toothed (entire in *E. colorata*); involucral glands either lacking appendages or the appendages do not cover the glands... 2

2. Involucral glands 4, appendages horned, or petaloid and green with crenate margins; styles entire; seeds sharply tetragonal, finely tuberculate, with a prominent stipitate caruncle nearly as wide as the seed itself... subsect. *Lacerae*

2. Involucral glands 1--5(--8), lacking appendages or, if present, then appendages whitish (green and obsolete in *E. chersonesa*), petaloid and not horned; styles bifid; seeds not sharply tetragonal, apically depressed on the ventral side, coarsely tuberculate, either ecarunculate or with a small caruncle much narrower than the seed...3

3. Involucral glands with whitish appendages... subsect. *Exstipulatae*

3. Involucral glands without appendages (or obsolete greenish appendages in *E. chersonesa*)... subsect. *Stormiae*

14a. *Euphorbia* [subg. *Chamaesyce* sect. *Poinsettia*] subsect. *Lacerae* Y. Yang & P.E. Berry, **subsect. nov**. – Type: *Euphorbia lacera* Boiss.

Annual herbs from a taproot. Leaves linear-lanceolate to pandurate, often heteromorphic, noticeably serrate, opposite at lowest nodes, then alternate in the mid-section, the shoot terminating with a whorl of leaves below the fertile branches. Cyathia in terminal, congested, few-cyathiate cymes, subtended by opposite leaves; glands 4, stipitate, laterally compressed and concave; appendages horned, or petaloid and green with crenate margins; styles entire. Seeds tetragonal, apically angled, finely tuberculate, with a prominent stipitate caruncle nearly as wide as the seed itself.

Distribution and habitat. – Xerophytic scrub, wooded ravines; central and western Mexico, 900--2500 m.

Included species (2) – ***E. jaliscensis*** B.L. Rob. & Greenm., ***E. lacera*** Boiss.

Discussion – The pandurate, serrate leaves of *E. jaliscencis* are remarkably similar to those found in *E. heterophylla* or *E. cyathophora*, and the leaves of *E. lacera* are polymorphic. Both species in this section have cupped, stipitate involucral glands. In both cases, the appendages are green and somewhat fleshy. *Euphorbia lacera* was initially placed by Boissier (1862) in *E.* sect. *Zygophyllidium*, together with *E. exstipulata* in an undesignated subcategory of § *Carunculatae* Boiss. The rest of the section consists of § *Ecarunculatae* Boiss., with two species that belong to sect. *Alectoroctonum*, *E. bilobata* and *E. hexagona*.

14b. *Euphorbia* [subg. *Chamaesyce* sect. *Poinsettia*] subsect. *Erianthae* Y. Yang & P.E. Berry, **subsect. nov**. – Type: *Euphorbia eriantha* Benth.

Annual or short-lived perennial herbs from a taproot. Branches few to many from the base. Herbage pubescent. Leaves linear, appearing entire but usually with a few inconspicuous teeth towards the apex, opposite at basal-most node, alternate in the midsection; stipules inconspicuous, minute and glanduliform, at the base of the petiole. Cyathia in terminal, congested, few-cyathia cymes, subtended by opposite or ternate bracts that are leaf-like; glands (2--)4--5, protruding from the outer, upper edge of the involucre, shallowly concave; glandular appendages elongate, laciniate, densely covered with white appressed trichomes, arching over and concealing the glands; styles free at the base, apex entire, purple. Ovary and capsule canescent, obloid or ovoid. Seeds tetragonal, coarsely tuberculate, covered with a white, crustaceous coating, with a circular caruncle about half as wide as the seed.

Distribution and habitat. – Southeastern U.S.A. (Arizona, California, New Mexico, Texas) and northern Mexico (Baja California, Baja California Sur, Chihuahua, Coahuila, Durango, Sonora); desert scrub and thorn scrub on rocky slopes and along washes, sea level to 900 m.

Included species (*1*) –***E. eriantha*** Benth.

Discussion – This subsection contains a single species that is unique because of its unusual cyathial gland appendages that curl over the gland towards the inside of the cyathium.

14c. *Euphorbia* [subg. *Chamaesyce* sect. *Poinsettia*] subsect. *Exstipulatae* Y. Yang & P.E. Berry, **subsect. nov**. – Type: *E. exstipulata* Engelm.

Small, annual herbs from a slender taproot, with opposite, arcuate branching. Leaves linear to ovate, serrate, opposite throughout or with some alternate leaves in the mid-stem section in *E. bifurcata*. Cyathia in terminal, congested few-cyathiate cymes; glands 1--4(--5), oblong to circular, stipitate, laterally compressed and concave, appendages entire, undulate, or divided into triangular segments; styles bifid. Ovary and capsule glabrous or pubescent in the keels, 3-lobed. Seeds broadly ovoid, apically

depressed on the ventral side, quadrangular to rounded in cross-section, coarsely tuberculate with 2 transverse ridges and a tiny, reniform caruncle (in *E. exstipulata*), or warty-papillate without an evident caruncle (*E. bifurcata*).

Distribution and habitat. – Southwestern U.S.A. and northern Mexico; desert scrub, grasslands, riparian areas, 800--2300 m.

Included species (2) – ***E. bifurcata*** Engelm., ***E. exstipulata*** Engelm.

Discussion – *Euphorbia bifurcata* is very similar to many species in subsect. *Stormieae* in its normally single cupulate gland, some subcyathial bracts that are whitish at the base, and its ecarunculate, verrucose seeds. *Euphorbia exstipulata* was initially placed by Boissier (1862) in *E.* sect. *Zygophyllidium*, together with *E. lacera* in a subdivision named § *Carunculatae* Boiss.

14d. *Euphorbia* [subg. *Chamaesyce* sect. *Poinsettia*] subsect. *Stormieae* Croizat, Rev. Sudamer. Bot. 6: 13. 1939. – Type: *Euphorbia stormiae* Croizat (= *E. radians* Benth. var. *stormiae* (Croizat) Rzed. & Calderón).

Annual or perennial herbs, shrubs or small trees. Branches opposite; leaves opposite at the epicotyledonary node, leaves and branches often alternate in the mid-section of plant, and then opposite or whorled on the flowering branches; leaves subtending the inflorescence often brightly colored. Cyathia in terminal, usually dense, sometimes monochasial cymes; involucral glands 1--5(--8), deeply to shallowly cupped and stalked, lacking petaloid appendages (or appendages green and vestigial in *E. chersonesa* and *E. cornastra*); styles 3, free or connate at the base, bifid to about one half their length from the apex, rarely entire or with only the very apex forked. Ovary and capsule glabrous or pubescent, 3-lobed. Seeds ovoid, tetragonal or somewhat rounded in cross-section, usually coarsely and unevenly tuberculate, or the tubercles disposed in one or more transverse, dorsal rows; ecarunculate, or caruncle variously shaped.

Distribution and habitat. – Widespread in the New World, from Canada to Argentina, but with a center of distribution in Mexico; in a wide variety of habitats from desert scrub to moist montane forests, sea level to 2000 m.

Included species (21). – ***E. chersonesa*** Huft, ***E. colorata*** Engelm., ***E. cornastra*** (Dressler) Radcl.-Sm., ***E. cuphosperma*** (Engelm.) Boiss., ***E. cyathophora*** Murray, *E.*

davidii Subils, ***E. dentata*** Michx., *E. elliptica* Lam., ***E. heterophylla*** L., ***E. hormorrhiza*** (Dressler) Radcl.-Sm., *E. kurtzii* Subils, ***E. pentadactyla*** Griseb., ***E. pinetorum*** (Small) G.L. Webster, ***E. pulcherrima*** Willd., ***E. pumicicola*** Huft, ***E. radians*** Benth., *E. restiacea* Benth., *E. schiediana* (Klotzsch & Garcke) Mayfield, ***E. strigosa*** Hook. & Arn., *E. tubadenia* (Boiss.) Mayfield, ***E. zonosperma*** Müll. Arg.

Discussion. – This subsection is the most diverse in sect. *Poinsettia* in terms of distribution, habit, and species. It includes those species Dressler (1961) included in *Poinsettia*, and those in *Euphorbia* subg. *Poinsettia sensu* Mayfield (1997), with the addition of *E. chersonesa*, which Huft (1984) thought belonged to sect. *Alectoroctonum* because of its vestigial involucral gland appendages and relatively flat glands. Mayfield (1997) recognized two groups in subg. *Poinsettia* (the *Euphorbia dentata* alliance and "subgenus Poinsettia sens. str."); our combined molecular tree (Fig. 2.3) indicates that the *E. dentata* alliance may be monophyletic, but that it is nested within the rest of the subsection.

In addition to the species listed above, there are four other species described but not validly published in Mayfield (1997)'s thesis. Mayfield (1997) also proposed a transfer from *Poinsettia* to *Euphorbia* and a new name and status for another species he recognized in subg. *Poinsettia*. These names are validated below.

Euphorbia schiediana (Klotzsch & Garcke) Mayfield, **comb. nov.** *Poinsettia schiedeana* Klotzsch & Garcke, Abh. Königl. Akad. Wiss. Berlin 1859: 102. 1860. – Type: Mexico. Veracruz: Hacienda de la Laguna, *C.J.W. Schiede 53* (B, holotype [B100244213]).

Euphorbia tubadenia (Boiss.) Mayfield, **nom. et stat. nov.** *Euphorbia dentata* var. *lasiocarpa* Boiss. in DC., Prodr. 15(2): 72. 1862. – Type: Mexico. Nuevo León: Tanquesillos, Jul-Oct 1842, *W.F. von Karwinski s.n.* (LE, holotype).

15. *Euphorbia* [subg. *Chamaesyce*] sect. *Alectoroctonum* (Schltdl.) Baill., Étude Gén. Euphorb. 284. 1858. *Alectoroctonum* Schltdl., Linnaea 19: 252. 1847. – Lectotype: *A. scotanum* (Schltdl.) Schltdl. (= *E. scotanum* Schltdl.), designated by Wheeler, Amer. Midl. Nat. 30: 459. 1943.

Agaloma Raf., Fl. Tellur. 4: 116. 1838. *Euphorbia* subg. *Agaloma* (Raf.) House, Bull. New York State Mus. Nat. Hist. 254: 471. 1924. – Lectoype: *E. corollata* L., designated by Rafinesque in Autik. Bot: 95. 1840.

Tithymalopsis Klotzsch & Garcke, Monatsber. Königl. Preuss. Akad. Wiss. Berlin 1859: 249. 1859. *Euphorbia* sect. *Tithymalopsis* (Klotzsch & Garcke) Boiss. in DC. Prodr. 15(2): 9, 66. 1862. Lectotype: *Euphorbia corollata* L., designated by Small in Britton & Brown, Ill. Fl. N. U.S., ed. 2., 2: 469. 1913.

Zalitae Raf., New Fl. 4: 98. 1838. – Type: *Zalitea linearis* Raf. (= *E. hexagona* Nutt.).

Euphorbia sect. *Zygophyllidium* Boiss. in DC., Prodr. 15(2): 9, 52. 1862. *Zygophyllidium* (Boiss.) Small in Fl. S.E. U.S.: 714, 1334. 1903. – Lectotype: *Z. hexagonum* (=*E. hexagona* Nutt.), designated by Small in Britton & Brown, Ill. Fl. N. U.S., ed. 2., 2: 468. 1913.

Annual or perennial herbs, shrubs, rarely succulent or small trees. Stem erect to decumbent, rarely prostrate. Leaves and branches opposite and/or alternate before the termination of apical growth with a 2--8-rayed umbel and usually equal number of leaves (sometimes no apical termination and main shoots continue elongation), and then switch to dichotomous branching, with each fork subtended by a pair of dichasial bracts. Leaves elliptic, ovate, obovate to linear, margin entire, rarely crenulate; stipules mostly minute and glanduliform, rarely subulate-filiform. Cyathia solitary or in cymes, terminal or axillary; both dichasial and subcyathial bracts leaf-like, sometimes greatly reduced in size, or white and showy. Glands 5 per cyathium (rarely 2, 3, 4 or 6), flat or shallowly concave, appendages petaloid. Styles free at the base, tip bifid or rarely entire. Ovary and capsule glabrous or pubescent, capsule exserted at maturity; 3-lobed. Seed ovoid, subglobose, or oblong, more or less quadrangular to rounded in cross-section; surface smooth or with wart-like protrusions, sometimes distinctively shallowly to deeply pitted. Seeds ecarunculate, or rarely carunculate.

Distribution and habitat. – Widespread but restricted to the New World with the center of diversity in Mexico and Central America; tropical and subtropical forests, desert scrub, and disturbed areas, sea level to 3000 m.

Included species (117) – ***E. aaron-rossii*** A.H. Holmgren & N.H. Holmgren, ***E. acerensis*** Boiss., ***E. adiantoides*** Lam., ***E. alata*** Hook., ***E. antisyphilitica*** Zucc., *E. arenaria* Kunth, ***E. ariensis*** Kunth, *E. armourii* Millsp., ***E. arteagae*** W.R. Buck & Huft, *E. barnesii* (Millsp.) Oudejans, ***E. bicolor*** Engelm. & A. Gray, ***E. bifurcata*** Engelm., ***E. bilobata*** Engelm., *E. boerhaavioides* Rusby, ***E. calcicola*** Fernald, ***E. californica*** Benth., ***E. caperata*** McVaugh, ***E. cassythoides*** Boiss., ***E. ceroderma*** I.M. Johnst., *E. chenopodiifolia* Boiss., ***E. colletioides*** Benth., ***E. corollata*** L., ***E. cotinifolia*** L., *E. curtisii* Engelm., ***E. cymosa*** Poir., *E. defoliata* Urb., ***E. delicatula*** Boiss., ***E. dioscoreoides*** Boiss., *E. discoidalis* Chapm., *E. dugandiana* Croizat, *E. dwyeri* D.G. Burch, ***E. eglandulosa*** V.W. Steinm., *E. ellipsifolia* Gilli, *E. ephedromorpha* Bartlett ex B.L. Rob. & Bartlett, ***E. equisetiformis*** A. Stewart, *E. estevesii* N. Zimm. & P.J. Braun, ***E. exserta*** (Small) Coker, ***E. francoana*** Boiss., *E. fraseri* Boiss., ***E. fulgens*** Karw. ex Klotzsch, ***E. gentryi*** V.W. Steinm. & T.F. Daniel, ***E. gradyi*** V.W. Steinm. & Ram.-Roa, ***E. graminea*** Jacq., ***E. guadalajarana*** S. Watson, ***E. guatemalensis*** Standl. & Steyerm., ***E. guiengola*** W.R. Buck & Huft, ***E. gumaroi*** J. Meyrán, *E. haematantha* Boiss., *E. henricksonii* M.C. Johnst., ***E. hexagona*** Nutt. ex Spreng., *E. hexagonoides* S. Watson, ***E. hindsiana*** Benth., ***E. hintonii*** L.C. Wheeler, ***E. humayensis*** Brandegee, ***E. innocua*** L.C. Wheeler, ***E. insulana*** Vell., ***E. ipecacuanhae*** L., ***E. ixtlana*** Huft, *E. jablonskii* V.W. Steinm., ***E. lagunensis*** Huft, *E. lancifolia* Schltdl., ***E. leucocephala*** Lotsy, *E. lottiae* V.W. Steinm., *E. luciismithii* B.L. Rob. & Greenm., ***E. macropodoides*** B.L. Rob. & Greenm., ***E. macropus*** (Klotzsch) Boiss., ***E. macvaughii*** Carvajal & Lomelí, ***E. marginata*** Pursh, *E. mercurialina* Michx., ***E. mexiae*** Standl., ***E. misella*** S. Watson, ***E. misera*** Benth., *E. monantha* C. Wright ex Boiss., *E. montereyana* Millsp., ***E. multiseta*** Benth., ***E. muscicola*** Fernald, *E. nayarensis* V.W. Steinm., *E. nephradenia* Barneby, ***E. oaxacana*** B.L. Rob. & Greenm., ***E. ocymoidea*** L., ***E. oerstediana*** (Klotzsch & Garcke) Boiss., *E. oppositifolia* McVaugh, *E. petiolaris* Sims, ***E. poeppigii*** (Klotzsch & Garcke) Boiss., ***E. polyphylla*** Engelm. ex Holz., ***E. pubentissima*** Michx., ***E. rossiana*** Pax, ***E. rzedowskii*** McVaugh, *E. saccharata* Boiss., ***E. scandens*** Kunth, ***E. schlechtendalii*** Boiss., ***E. sciadophila*** Boiss., *E. scotanum* Schltdl., ***E. segoviensis*** (Klotzsch & Garcke) Boiss., ***E. sinaloensis*** Brandegee, ***E. sonorae*** Rose, ***E. soobyi*** McVaugh, ***E. sphaerorhiza*** Benth., *E. spruceana* Boiss., *E. strictior* Holz., ***E. subpeltata*** S. Watson, ***E.***

subreniformis S. Watson, *E. subtrifoliata* Rusby, ***E. succedanea*** L.C. Wheeler, *E. surinamensis* Lanj., ***E. tresmariae*** (Millsp.) Standl., *E. tricolor* Greenm., *E. umbrosa* Bertero ex Spreng., *E. verapazensis* Standl. & Steyerm., *E. violacea* Greenm., *E. viridis* (Klotzsch & Garcke) Boiss., ***E. whitei*** Wheeler, *E. wrightii* Torr. & A. Gray, ***E. xalapensis*** Kunth, ***E. xanti*** Engelm. ex Boiss., *E. xbacensis* Millsp., *E. zierioides* Boiss.

Discussion. – Sect. *Alectoroctonum* is characterized by tiny, mostly glanduliform stipules, petaloid gland appendages, and usually entire leaves. Schlechtendal (1847) coined the genus name *Alectoroctonum* after the Spanish common name of "rooster killer," presumably referring to the toxicity of the species he assigned to the group.

Due to limited resolution in our analysis, incongruence among markers, and frequent convergence in morphology, additional markers are required to resolve relationships within sect. *Alectoroctonum*. Therefore here we do not designate formal subsections, but discuss some of the well-defined clades in the main text.

Table 2.1. Summary statistics for the aligned molecular data matrices.

	ndhF	ITS	combined *ndhF* + ITS
No of accessions	147	172	182
Range of raw length* (bp)	762-1480	336-651	584-2123
Aligned length	1547	714	2261
Variable characters (proportion)	697 (45.1%)	508 (71.1%)	1205 (53.3%)

* Lower ends of raw lengths are from partial sequences that the full-length sequences failed to amplify or sequence.

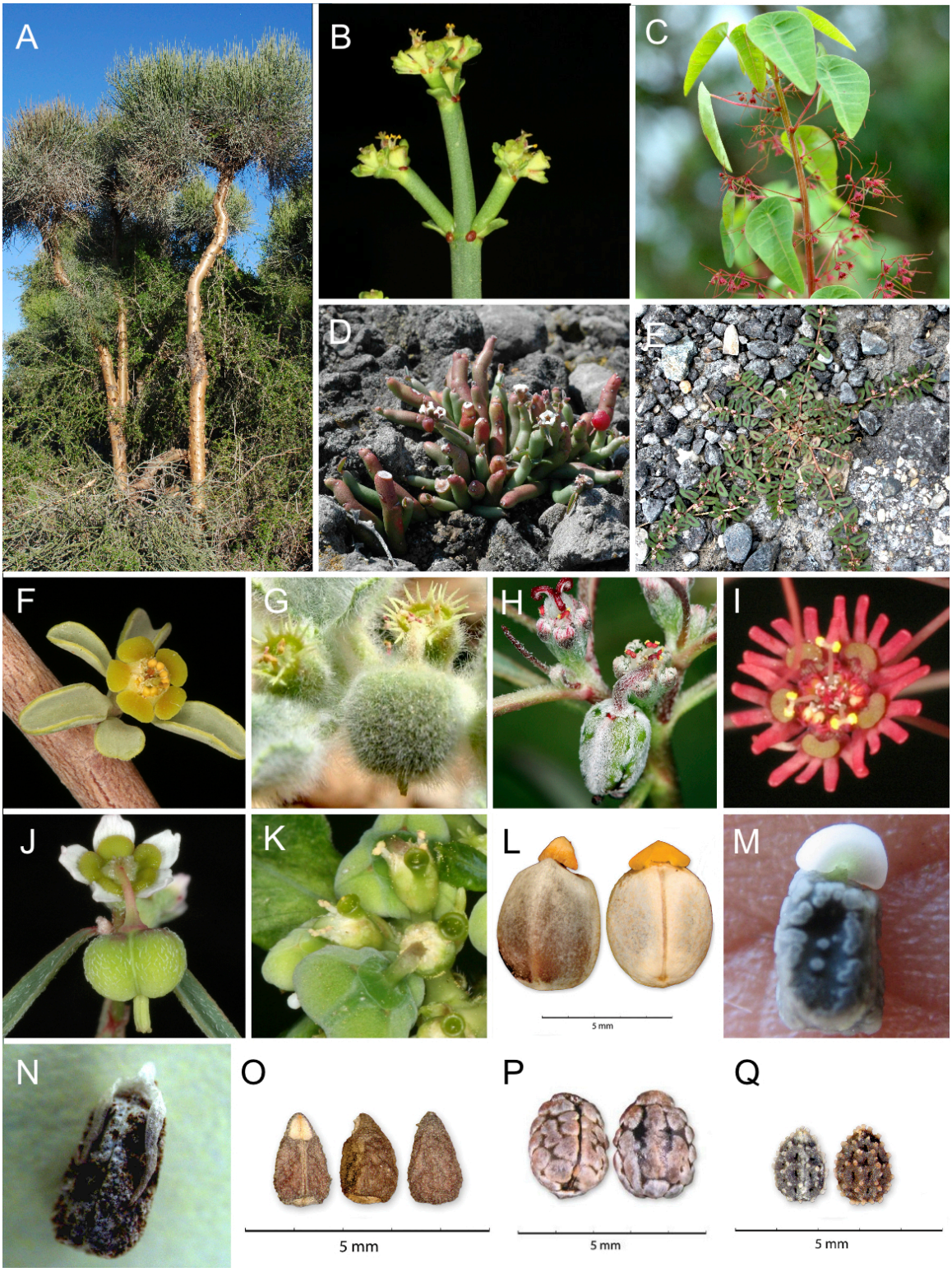
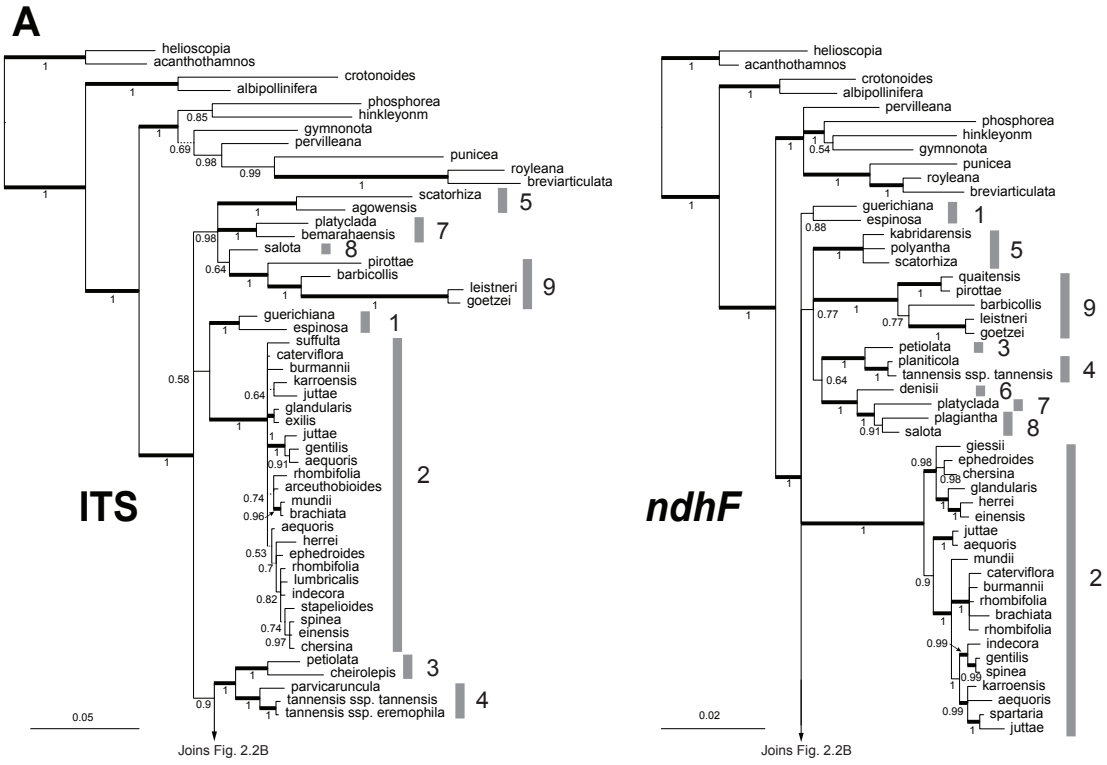

A
B
C
D
E
F
G
H
I
J
K
L
5 mm
M
N
O
5 mm
P
5 mm
Q
5 mm

Figure 2.1. Representative growth forms (A–E), cyathial morphologies (F–K), and seed morphologies (L–Q) in subg. *Chamaesyce*. (A) *E. plagiantha*, a broom-like tree (sect. *Plagianthae*; *Dorsey 164*, MICH). (B) *E. burmannii*, a stem-succulent shrub with opposite or dichotomous branching and terminal cymes (sect. *Articulofruticosae*; *Becker & Moller 1141*, UNIN). (C) *E. subpeltata*, an herb with indeterminate main shoots and axillary cymes (sect. *Alectoroctonum*; *Steinmann 5585*, IEB). (D) *E. gumaroi*, a stem-succulent herb with alternate branching and terminal cyathia (sect. *Alectoroctonum*; *Steinmann 5813*, MICH). (E) *E. maculata*, a prostrate herb with early termination of main shoots (sect. *Anisophyllum*). (F) *E. guerichiana*, showing glands that lack appendages (sect. *Espinosae*; *Becker & Moller 929*, UNIN). (G) *E. petiolata*, showing pectinate cyathial glands (sect. *Cheirolepidium*; *Zarre & Salmaki 39514*, TUH). (H) *E. eriantha*, showing gland appendages arching over and concealing the glands (sect. *Erianthae*). (I) *E. subpeltata*, showing cyathial appendages with many finger-like lobes (sect. *Alectoroctonum*; *Steinmann 5585*, IEB). (J) *E. sphaerorhiza*, showing cyathial morphology typical in sect. *Alectoroctonum* (*Yang 110*, MICH). (K) *E. heterophylla*, showing single, stalked and cupped glands typical of sect. *Poinsettia* subsect. *Stormieae* (Riina 1825, VEN). (L) *E. espinosa* (sect. *Espinosae*; *Leach 15938*, UNIN). (M) *E. petiolata* (sect. *Cheirolepidium*; *Zarre & Salmaki 39514*, TUH). (N) *E. cheirolepis* (sect. *Cheirolepidium*). (O) *E. mundii* (sect. *Articulofruticosae*; *Leach 17110*, UNIN). (P) *E. goyazensis* (sect. *Crossadenia*; *Caruzo 139*, HUEFS). (Q) *E. sonorae* (sect. *Alectoroctonum*; *Fishbein 2455*, RSA). *Photo credits*: (A), B. Dorsey; (B&F), A. Moller; (G), Y. Salmaki and S. Zarre; (H), S. Matson; (L, O&Q) B. Wagner; (N), D.V. Geltman.

Figure 2.2. Majority rule consensus tree recovered from Bayesian analyses of the nuclear ITS and the chloroplast *ndhF* coding region. Numbers below the branches indicate Bayesian posterior probabilities (PP). Thick branches indicate MLB ≥ 70 and PP ≥ 0.95, and branches in dashed lines have Bayesian MLB < 50 and PP < 0.80. Branch length scale on lower left. Numbers correspond to numbered sections on Fig. 2.3 and numbers in the taxonomic treatment. 1, sect. *Espinosae*; 2, sect. *Articulofruticosae*; 3. sect. *Cheirolepidium*; 4. sect. *Eremophyton*; 5. sect. *Scatorhizae*; 6. sect. *Denisiae*; 7, sect. *Bosseriae*; 8, sect. *Plagianthae*; 9, sect. *Frondosae*; 10, sect. *Tenellae*; 11. sect. *Gueinziae*; 12, sect. *Crossadenia*; 13, sect. *Anisophyllum*; 14, sect. *Poinsettia*; 15, sect. *Alectoroctonum*.



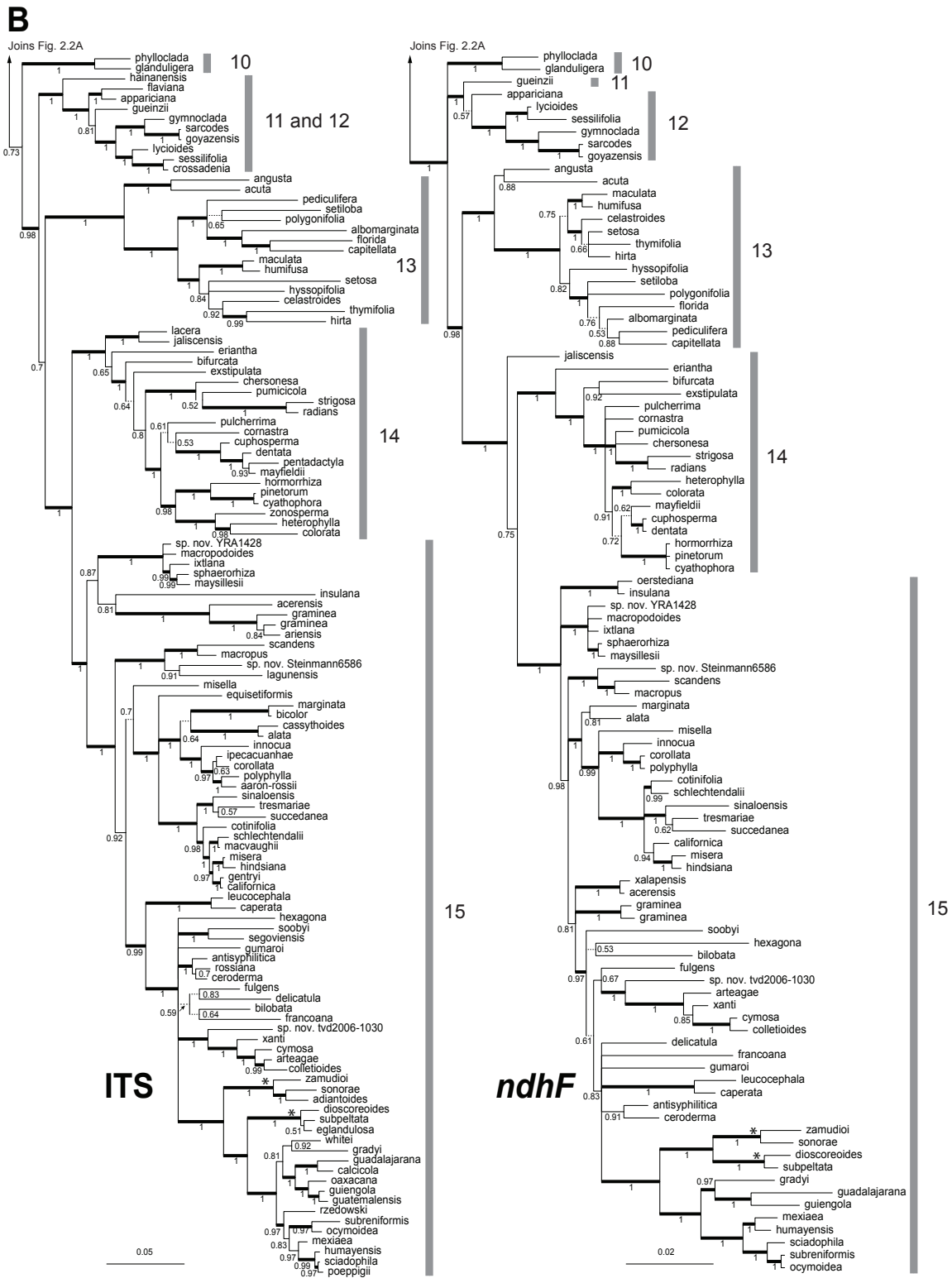
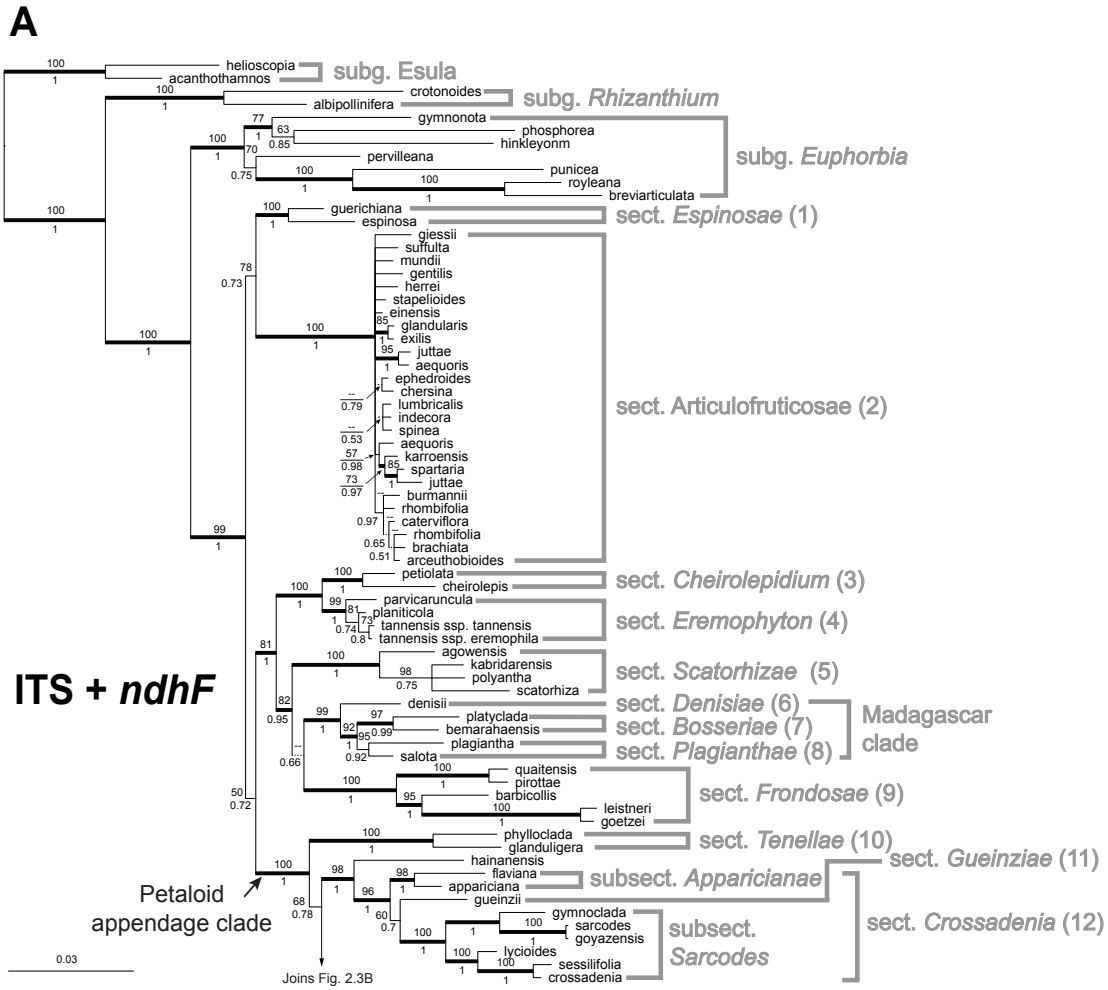

B
Joins Fig. 2.2A
phylloclada
glanduligera
hainanensis
flaviana
appariciana
gueinzii
gymnoclada
sarcodes
goyazensis
lycioides
sessilifolia
crossadenia
10
11 and 12
angusta
acuta
pediculifera
setiloba
polygonifolia
albomarginata
florida
capitellata
maculata
humifusa
setosa
hyssopifolia
celastroides
thymifolia
hirta
13
lacera
jaliscensis
eriantha
bifurcata
exstipulata
chersonesa
pumicicola
strigosa
radians
pulcherrima
cornastra
cuphosperma
dentata
pentadactyla
mayfieldii
hormorrhiza
pinetorum
cyathophora
zonosperma
heterophylla
colorata
14
sp. nov. YRA1428
macropodoides
ixtlana
sphaerorhiza
maysillesii
insulana
acerensis
graminea
graminea
ariensis
scandens
macropus
sp. nov. Steinmann6586
lagunensis
misella
equisetiformis
marginata
bicolor
cassythoides
alata
innocua
ipecacuanhae
corollata
polyphylla
aaron-rossii
sinaloensis
tresmariae
succedanea
cotinifolia
schlechtendalii
macvaughii
misera
hindsiana
gentryi
californica
leucocephala
caperata
hexagona
soobyi
segoviensis
gumaroi
antisyphilitica
rossiana
ceroderma
fulgens
delicatula
bilobata
francoana
sp. nov. tvd2006-1030
xanti
cymosa
arteagae
colletioides
zamudioi
sonorae
adiantoides
dioscoreoides
subpeltata
eglandulosa
whitei
gradyi
guadalajarana
calcicola
oaxacana
guiengola
guatemalensis
rzedowski
subreniformis
ocymoidea
mexiaea
humayensis
sciadophila
poeppigii
15
ITS
0.05
Joins Fig. 2.2A
phylloclada
glanduligera
10
gueinzii
11
appariciana
lycioides
sessilifolia
gymnoclada
sarcodes
goyazensis
12
angusta
acuta
maculata
humifusa
celastroides
setosa
thymifolia
hirta
hyssopifolia
setiloba
polygonifolia
florida
albomarginata
pediculifera
capitellata
13
jaliscensis
eriantha
bifurcata
exstipulata
pulcherrima
cornastra
pumicicola
chersonesa
strigosa
radians
heterophylla
colorata
mayfieldii
cuphosperma
dentata
hormorrhiza
pinetorum
cyathophora
14
oerstediana
insulana
sp. nov. YRA1428
macropodoides
ixtlana
sphaerorhiza
maysillesii
sp. nov. Steinmann6586
scandens
macropus
marginata
alata
misella
innocua
corollata
polyphylla
cotinifolia
schlechtendalii
sinaloensis
tresmariae
succedanea
californica
misera
hindsiana
xalapensis
acerensis
graminea
graminea
soobyi
hexagona
bilobata
fulgens
sp. nov. tvd2006-1030
arteagae
xanti
cymosa
colletioides
delicatula
francoana
gumaroi
leucocephala
caperata
antisyphilitica
ceroderma
zamudioi
sonorae
dioscoreoides
subpeltata
gradyi
guadalajarana
guiengola
mexiaea
humayensis
sciadophila
subreniformis
ocymoidea
15
ndhF
0.02

Figure 2.3. Majority rule consensus trees recovered from Bayesian analyses of the combined dataset (ITS + *ndhF*) with designated names of taxonomic units. Numbers above the branches indicate Maximum Likelihood bootstrap percentages (MLB), and numbers below the branches are Bayesian posterior probabilities (PP). Thick branches indicate MLB ≥ 70 and PP ≥ 0.95, and branches in dashed lines have Bayesian MLB < 50 and PP < 0.80. Branch length scale on lower right. Colors of clades correspond to colors used in figs. 2.2 and 2.4. Branch length scale on lower left of each tree. Numbers next to each section correspond to clades in Fig. 2.2 and section numbers in the taxonomic treatment. Subgroups within sect. *Alectoroctonum* were indicated with numbers after hyphen.




B
Joins Fig. 2.3A
angusta
acuta
subsect. Acutae
setiloba
polygonifolia
pediculifera
albomarginata
florida
capitellata
subsect. Chamaesyce
hyssopifolia
maculata
humifusa
setosa
celastroides
thymifolia
hirta
sect. Anisophyllum (13)
lacera
jaliscensis
subsect. Lacerae
eriantha
subsect. Erianthae
bifurcata
exstipulata
subsect. Exstipulatae
pumicicola
chersonesa
strigosa
radians
pulcherrima
cornastra
cuphosperma
dentata
pentadactyla
mayfieldii
subsect. Stormieae
hormorrhiza
pinetorum
cyathophora
zonosperma
heterophylla
colorata
sect. Poinsettia (14)
oerstediana
insulana
15-1
sp. nov YRA1428
macropodoides
ixtlana
sphaerorhiza
maysillesii
15-2
xalapensis
acerensis
graminea
graminea
ariensis
15-3
scandens
macropus
sp. nov. Steinmann6586
lagunensis
15-4
misella
sinaloensis
tresmariae
succedanea
cotinifolia
schlechtendalii
macvaughii
misera
hindsiana
gentryi
californica
15-5
equisetiformis
marginata
bicolor
15-6
cassythoides
alata
15-7
innocua
ipecacuanhae
corollata
polyphylla
aaron-rossii
15-8
leucocephala
caperata
15-9
antisyphilitica
rossiana
ceroderma
15-10
fulgens
delicatula
hexagona
15-11
bilobata
soobyi
15-12
segoviensis
sp. nov. tvd2006-1030
xanti
arteagae
cymosa
colletioides
15-13
gumaroi
francoana
zamudioi
sonorae
adiantoides
dioscoreoides
subpeltata
eglandulosa
whitei
gradyi
guadalajarana
calcicola
oaxacana
guiengola
guatemalensis
rzedowskii
mexiaea
humayensis
subreniformis
ocymoidea
sciadophila
poeppigii
15-14
sect. Alectoroctonum (15)
ITS + ndhF
0.03

Figure 2.4. Cladograms comparing major clades from this study and the "backbone" phylogeny (Horn et al., 2011). Numbers above the branches indicate Maximum Likelihood bootstrap percentages (MLB), and numbers below the branches are Bayesian posterior probabilities (PP). Thick branches indicate MLB ≥ 70 and PP ≥ 0.95, and branches in dashed lines have Bayesian MLB < 50 and PP < 0.80.

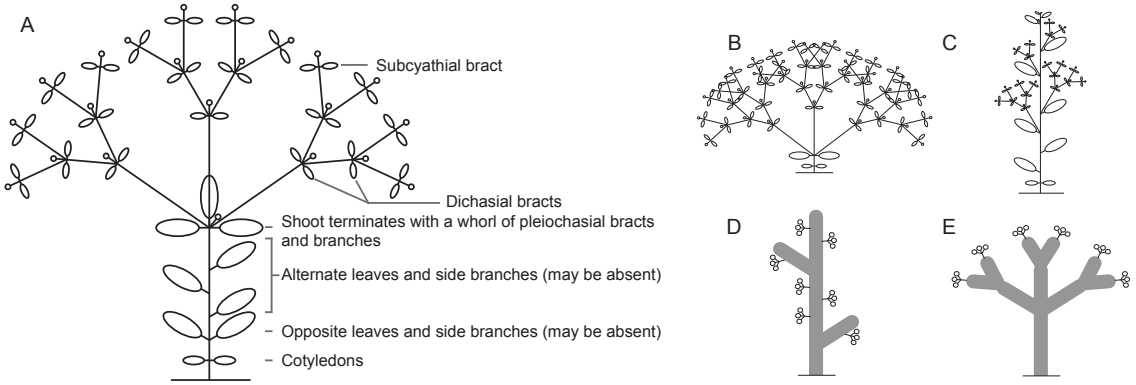


Figure 2.5. The basic growth forms in *Euphorbia* (A, Wheeler, 1941; Molero and Rovira, 1992) and its modifications (B–E). B, early termination of the main shoot; C, main shoot indeterminate and cymes axillary; D, similar to C but stems become succulent; and E, stem succulents with terminal cyathia or cyathial cymes.

LITERATURE CITED

ABOUHEIF, E. 2008. Parallelism as the pattern and process of mesoevolution. *Evolution & Development* 10: 3-5.

BINOJKUMAR, M. S., AND N. P. BALAKRISHNAN. 2010. The genus *Euphorbia* L. (Euphorbiaceae) in India - a taxonomic revision. Gajendra Singh Gahlot for Bishen Singh Mahendra Pal Singh, New Connaught Place, Dehra Dun, India.

BOISSIER, E. 1862. Euphorbiaceae. *In* A. P. d. Candolle [ed.], Prodromus systematis naturalis regni vegetabilis, vol. 15, part 2, 3-188. Victor Masson & Fils, Paris, France.

BROWN. 1913. Euphorbiaceae, Flora of tropical Africa Vol 6 Part 1.

BRUYNS, P. V., R. J. MAPAYA, AND T. HEDDERSON. 2006. A new subgeneric classification for *Euphorbia* (Euphorbiaceae) in southern Africa based on ITS and *psbA-trnH* sequence data. *Taxon* 55: 397-420.

CARTER, S. 1992. New herbaceous and woody species of *Euphorbia* (Euphorbiaceae) from Somalia. *Nord. J. Bot.* 12: 681-688.

CARTER, S., AND A. RADCLIFFE-SMITH. 1988. Euphorbiaceae (Part 2). *In* R. M. Polhill [ed.], Flora of Tropical East Africa. Published on behalf of the east African Governments by A.A. Balkema/Rotterdam/Brookfield.

CARTER, S., AND L. C. LEACH. 2001. *Euphorbia*. *In* G. V. Pope [ed.], Flora Zambesiaca, vol. 9, 339-433. Royal Botanic Gardens, Kew.

CREMERS, G. 1984. Les euphorbes coralliformes de Madagascar. *Bull. Jard. Bot. Belg.* 54: 23-64.

CROIZAT, L. 1940. New and critical Euphorbiaceae from eastern tropical Asia. *Journal of Arnold Arboretum* 21: 505-506.

DRESSLER, R. L. 1961. A synopsis of *Poinsettia* (Euphorbiaceae). *Annals of the Missouri Botanical Garden* 48: 329-341.

EBACH, M. C., D. M. WILLIAMS, AND J. J. MORRONE. 2006. Paraphyly is bad taxonomy. *Taxon* 55: 831-832.

GARCÍA, J. M. 2000. *Euphorbia gumaroi* Meyrán, una nueva especie del estado de Hidalgo, México. *Cactáceas y Suculentas Mexicanas* 45: 68-71.

GOVAERTS, R., D. G. FRODIN, AND A. RADCLIFFE-SMITH. 2000. World checklist and bibliography of Euphorbiaceae (and Pandaceae). Royal Botanic Gardens, Kew, Richmond, United Kingdom.

HASSALL, D. C. 1977. The genus *Euphorbia* in Australia. *Australian Journal of Botany* 25: 429-453.

HAYDEN, W. J. 1988. Ontogeny of the Cotyledonary Region of *Chamaesyce maculata* (Euphorbiaceae). *American Journal of Botany* 75: 1701-1713.

HOLMGREN, A. H., AND N. H. HOLMGREN. 1988. *Euphorbia aaron-rossii* (Euphorbiaceae), a new species from Marble and Grand Canyons of the Colorado River, Arizona. *Brittonia* 40: 357-362.

HORANDL, E. 2006. Paraphyletic versus monophyletic taxa - evolutionary versus cladistic classifications. *Taxon* 55: 564-570.

HUFT, M. J. 1979. A monograph of *Euphorbia* section *Tithymalopsis*. Ph.D. dissertation, University of Michigan, Ann Arbor, Michigan, USA.

HUFT, M. J. 1984. A review of *Euphorbia* (Euphorbiaceae) in Baja California. *Annals of the Missouri Botanical Garden* 71: 1021-1027.

KOLACZKOWSKI, B., AND J. W. THORNTON. 2009. Long-branch attraction bias and inconsistency in Bayesian phylogenetics. *Plos One* 4: e7891. doi:7810.1371/journal.pone.0007891.

KOONTZ, T. L., A. PETROFF, G. B. WEST, AND J. H. BROWN. 2009. Scaling relations for a functionally two-dimensional plant: *Chamaesyce setiloba* (Euphorbiaceae). *American Journal of Botany* 96: 877-884.

KOUTNIK, D. L. 1984. *Chamaesyce* (Euphorbiaceae) - a newly recognized genus in southern Africa. *South African Journal of Botany* 3: 262-264.

KOUTNIK, D. L. 1987. A taxonomic revision of the Hawaiian species of the genus *Chamaesyce* (Euphorbiaceae). *Allertonia* 4: 331-388.

MADDISON, D. R., AND W. P. MADDISON. 2005. MacClade 4.08: an analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MAYFIELD, M. H. 1997. A systematic treatment of *Euphorbia* subgenus *Poinsettia* (Euphorbiaceae). Ph.D. dissertation, the University of Texas at Ausin, Austin, Texas, USA.

MOLERO, J., AND A. M. ROVIRA. 1992. *Euphorbia* L. subsect. *Esula* (Boiss. in DC.) Pax in the Iberian Peninsula. Leaf surface, chromosome numbers and taxonomic treatment. *Collect. Bot. (Barcelona)* 21: 121-181.

MORAWETZ, J. J., B. WAGNER, R. RIINA, AND P. E. BERRY. 2010. *Euphorbia* seed atlas - part 2. *Euphorbia World* 6 (1): 25.

OLMSTEAD, R. G., AND J. A. SWEERE. 1994. Combining data in phylogenetic systematics - an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* 43: 467-481.

PAHLEVANI, A. H., AND H. AKHANI. 2011. Seed morphology of Iranian annual species of *Euphorbia* (Euphorbiaceae). *Botanical Journal of the Linnean Society* 167: 212-234.

PARK, K. R., AND R. K. JANSEN. 2007. A phylogeny of euphorbieae subtribe Euphorbiinae (Euphorbiaceae) based on molecular data. *Journal of Plant Biology* 50: 644-649.

PAX, F., AND K. HOFFMAN. 1921. Euphorbiaceae. Pp. 1-169 in Engler, Pflanzenw. Afrikas [Veg. Erde 9] 3, 2: 1-878.

PRENNER, G., AND P. J. RUDALL. 2007. Comparative ontogeny of the cyathium in *Euphorbia* (Euphorbiaceae) and its allies: exploring the organ flower inflorescence boundary. *American Journal of Botany* 94: 1612-1629.

PRENNER, G., N. I. CACHO, D. BAUM, AND P. J. RUDALL. 2011. Is *LEAFY* a useful marker gene for the flower-inflorescence boundary in the *Euphorbia* cyathium? *Journal of Experimental Botany*.

RAUH, W., AND R. D. MANGELSDORFF. 1999. *Euphorbia bemarahaensis* Rauh & Mangelsdorff, une nouvelle espèce du groupe *Euphorbia bosseri* Leandri et *Euphorbia platyclada* Rauh. *Succulentes* 21(4): 3-12.

SAGE, T. L., R. F. SAGE, P. J. VOGAN, B. RAHMAN, D. C. JOHNSON, J. C. OAKLEY, AND M. A. HECKEL. 2011. The occurrence of C_2 photosynthesis in *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae). *Journal of Experimental Botany*.

SCHLECHTENDAL, D. F. L. V. 1847. Plantae Leiboldianae. Dicotyleae. *Linnaea* 19: 234-320.

SHAW, J., E. B. LICKEY, E. E. SCHILLING, AND R. L. SMALL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94: 275-288.

SIMMONS, M. P. 2004. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* 31: 874-879.

SIMMONS, M. P., AND W. J. HAYDEN. 1997. Revision of the cerrado hemicryptophytic *Chamaesyce* of Boissier's "*Pleiadeniae*" (Euphorbiaceae). *Brittonia* 49: 155-180.

STEINMANN, V. W., AND J. M. PORTER. 2002. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* 89: 453-490.

THULIN, M., AND A. N. AL-GIFRI. 1995. *Euphorbia applanata* sp. nov. (Euphorbiaceae) from Yemen, with a note on *E. quaitensis*. *Nordic Journal of Botany* 15: 193-195.

WARD, D. B. 2001. New combinations in the Florida flora. *Novon* 11: 360-365.

WEBSTER, G. L. 1967. The genera of Euphorbiaceae in the southeastern United States. *Journal of the Arnold Arboretum* 48: 303-430.

WEBSTER, G. L., W. V. BROWN, AND B. N. SMITH. 1975. Systematics of photosynthetic carbon fixation pathways in *Euphorbia*. *Taxon* 24: 27-33.

WHEELER, L. C. 1941. *Euphorbia* subgenus *Chamaesyce* in Canada and the United States exclusive of Southern Florida. *Rhodora* 43: 97-154, 168-205, 223-286, pls. 654-668.

WHEELER, L. C. 1943. The genera of the living Euphorbieae. *American Midland Naturalist* 30: 456-503.

YANG, Y., AND P. E. BERRY. 2011. Phylogenetics of the Chamaesyce clade (*Euphorbia*, Euphorbiaceae): Reticulate evolution and long-distance dispersal in a prominent C_4 lineage. *American Journal of Botany* 98: 1486-1503.

ZIMMERMANN, N. F. A., C. M. RITZ, AND F. H. HELLWIG. 2010. Further support for the phylogenetic relationships within *Euphorbia* L. (Euphorbiaceae) from nrITS and *trnL–trnF* IGS sequence data. *Plant Systematics and Evolution* 286: 39-58.

CHAPTER III

PHYLOGENETICS OF SECTION *ANISOPHYLLUM* (THE CHAMAESYCE CLADE, *EUPHORBIA*, EUPHORBIACEAE): RETICULATE EVOLUTION AND LONG-DISTANCE DISPERSAL IN A PROMINENT C_4 LINEAGE

ABSTRACT

Premise of the study: The Chamaesyce clade of *Euphorbia* is the largest lineage of C_4 plants among the eudicots, with 350 species including both narrow endemics and cosmopolitan weeds. We sampled this group worldwide to address questions about subclade relationships, the origin of C_4 photosynthesis, the evolution of weeds, and the role of hybridization and long-distance dispersal in the diversification of the group.

Methods: Two nuclear (ITS and exon 9 of *EMB2765*) and three chloroplast markers (*matK*, *rpl16*, and *trnL-F*) were sequenced for 138 ingroup and six outgroup species. Exon 9 of *EMB2765* was cloned in accessions with >1% superimposed peaks.

Key results: The Chamaesyce clade is monophyletic and consists of three major subclades [1(2,3)]: (1) the Acuta clade, containing three North American species with C_3 photosynthesis and C_3-C_4 intermediates; (2) the Peplis clade, mostly North American and entirely C_4; and (3) the Hypericifolia clade, all C_4, with both New World and Old World groups. Incongruence between chloroplast and ITS phylogenies and divergent cloned copies of *EMB2765* exon 9 suggest extensive hybridization, especially in the Hawaiian Islands radiation.

Conclusions: The Chamaesyce clade originated in warm, arid areas of North America, where it evolved C_4 photosynthesis. From there, it diversified globally with extensive reticulate evolution and frequent long-distance dispersals. Although many species are weedy, there are numerous local adaptations to specific substrates and regional or island radiations, which have contributed to the great diversity of this group.

INTRODUCTION

Within the large genus *Euphorbia* L., with some 2000 species, the Chamaesyce clade (*Euphorbia* subgenus *Chamaesyce* section *Anisophyllum* Roeper) comprises a group of about 350 species that are remarkably distinct within the genus. This group is cosmopolitan in distribution, but with a majority of species native to the New World (210 vs. 140 native to the Old World), running counter to the prevailing pattern in most other large clades of *Euphorbia* that are more diverse in the Old World (Steinmann and Porter, 2002). The Chamaesyce clade is probably best known for its globally pervasive weedy species, such as *E. maculata* L. (spotted spurge), a mainly temperate species with an affinity for sidewalk cracks, and *E. hirta* L., a species widespread in warm temperate and tropical regions. The weediness displayed by these and other species is facilitated by precocious flowering, quick generation turnover (up to several generations per growing season), high seed set, and a specialized seed coat that becomes sticky when wet (Jordan and Hayden, 1992; Suzuki and Teranishi, 2005). On the other hand, many species in the Chamaesyce clade are quite restricted geographically, such as the eight species endemic to the Galapagos Islands (Burch, 1969) and the 29 taxa in 16 species limited to the Hawaiian Islands (Wagner et al., 1999). Although all members of the Chamaesyce clade share the pseudanthial cyathium that is a synapomorphy for *Euphorbia* (Prenner and Rudall, 2007), the clade differs markedly from the rest of the genus in having opposite, mostly asymmetrical leaves with interpetiolar stipules (Fig. 3.1). Most species are small, prostrate to ascending herbs, with a dichotomous branching pattern that is associated with the early abortion of the apical meristem (Fig. 3.1A; Degener and Croizat, 1938; Hayden, 1988). Another unique feature of the Chamaesyce clade within *Euphorbia* is the predominance of C_4 photosynthesis, which is both a physiological and anatomical system generally associated with plants adapted to warm, arid conditions (Fig. 3.1B, Sage et al., 2011a). All these factors lead to interesting questions concerning the geographical origin of the Chamaesyce clade, the evolution of C_4 photosynthesis in *Euphorbia*, adaptations for weediness and long-distance dispersal, and mechanisms that might explain the multiple radiations of species in different regions of the globe.

Taxonomic issues in the Chamaesyce clade have revolved mainly around the appropriate rank at which to recognize the group. Some botanists (for example, Wheeler, 1941; Burch, 1965; Hassall, 1977; Koutnik, 1987) recognized the group as a separate

genus, *Chamaesyce* Gray, because it is very easy to distinguish from other *Euphorbia* species. Others, from Boissier (1862) to Bruyns et al. (2006), treated the group as part of *Euphorbia*, usually as a section, and Boissier was correct in treating it as section *Anisophyllum* Roeper. To date, Boissier (1862) was the only botanist to propose subclades within the Chamaesyce clade, in which he recognized eight subsections. Six of these represented small, relatively well circumscribed groups of species. The other two subsections, however, were large and diverse, including both Old World and New World species; subsect. *Chamaesyce* included a group of 99 mostly prostrate species with solitary cyathia, and subsect. *Hypericifoliae* Boiss. comprised a group of 30 mostly erect species with clustered cyathia. All genus-wide molecular studies to date have unequivocally placed the Chamaesyce clade within *Euphorbia*, in the same subgeneric clade as the New World "Agaloma alliance", which includes *E. pulcherrima* Willd., the familiar Christmas poinsettia (Steinmann and Porter, 2002; Bruyns et al., 2006; Park and Jansen, 2007; Zimmermann et al., 2010). The problem with inferring deeper relationships within the Chamaesyce clade is that until now only 11 species have been molecularly sampled, so there has been insufficient coverage to assess any of the main questions raised above or to assess Bossier's subsectional classification.

Euphorbia is the only plant genus known to exhibit C_3, C_4, and CAM photosynthetic systems. Webster et al.'s (1975) carbon isotope ratio determinations in *Euphorbia* found that C_4 species were restricted to the Chamaesyce clade, although two species in this group endemic to the southwestern United States and northern Mexico, *E. acuta* Engelm. and *E. angusta* Engelm., had isotope ratios consistent with C_3 photosynthesis. This led Webster et al. to hypothesize that the Chamaesyce clade had originated in subtropical and warm temperate areas in North America from C_3 ancestors, with *E. acuta* and *E. angusta* representing a transitional stage. Sage et al. (2011b) subsequently used more refined techniques to confirm that *E. angusta* has a C_3 system, whereas the closely related *E. acuta* and *E. johnstonii* Mayfield are actually intermediate C_3-C_4 species. There are no clear reversals to C_3 photosynthesis in the Chamaesyce clade, although there is a radiation of 16 woody species in the Hawaiian Islands that includes several species restricted to wet montane forest understoreys or bogs and one species that forms trees up to 10 m tall (Fig. 3.1G, H; Koutnik, 1987; Lorence and Wagner, 1996). C_4

species that grow in such mesic habitats or as trees are highly unusual, and there is evidence that some of these species have experienced modifications of the specialized Kranz leaf anatomy (Herbst, 1971, 1972; Pearcy and Troughton, 1975; Sporck and Sack, 2010). By including more samples among these species in particular, we aim to better understand the dynamics of C_4 photosynthesis in the Chamaesyce clade.

In this study, we used comprehensive taxon sampling and sequencing of the nuclear ribosomal ITS region, the nuclear low-copy coding region exon 9 of *EMBRYO DEFECTIVE 2765*, and three chloroplast loci (*matK*, the *rpl16* intron, and the *trnL-F* spacer) to reconstruct the phylogenetic relationships within the Chamaesyce clade of *Euphorbia*. We first wanted to test the monophyly of the entire clade and then determine the precise placement of the C_3 and C_3- C_4 intermediate species in relation to the more numerous C_4 species. We then used the resulting phylogeny to determine the distribution of New World vs. Old World taxa in the clade, looking for evidence of long distance dispersal events and correlations with particular habitat types such as deep sand substrates or beach strand vegetation, as well as inferring the position of weedy taxa and their role in the diversification of the clade. Finally, after detecting evidence of reticulate evolution through contrasting nuclear and chloroplast phylogenies, we cloned the nuclear low-copy coding region exon 9 of *EMB2765* in a subset of species to detect the presence of multiple alleles and further evidence of hybridization.

MATERIALS AND METHODS

Taxon sampling—A total of 450 accessions from 138 species within the Chamaesyce clade were sequenced for this study. Out of these, 149 ingroup accessions were used in the analyses presented here, and duplicate accessions of a given taxon with identical or nearly identical sequences were excluded. In addition, six outgroup taxa were selected following previous molecular phylogenetic studies in *Euphorbia* (Steinmann and Porter, 2002; Bruyns et al., 2006; Park and Jansen, 2007; Zimmermann et al., 2010). Silica-dried material was obtained from collecting trips from 2004 to 2009 covering the major biogeographical regions where natural populations of Chamaesyce species occur: 1) North America: southern United States and northern Mexico; 2) the Caribbean: Dominican Republic, Puerto Rico, and Cuba; 2) South America: Argentina, Brazil,

Colombia, and Venezuela; 3) Africa: Morocco, Kenya, Tanzania, South Africa, and Madagascar; and 4) Eurasia: Portugal, Spain, Italy, Greece, Oman, and Russia. Additional silica-dried materials were obtained from collaborators from Thailand and northern Mexico. DNA of eight Hawaiian species was contributed by the Hawaiian Plant DNA Library (Morden et al., 1996; Randell and Morden, 1999). Leaf fragments were sampled from herbarium material to fill in sampling gaps, especially native species from Australia, Pacific and Atlantic islands, eastern Africa, and South America. Voucher information is presented in Appendix 3.1.

DNA extraction, PCR and sequencing—Total genomic DNA was extracted from silica-dried leaf fragments using DNeasy Plant Mini Kits (QIAGEN Inc., Valencia, California, USA) following the manufacturer's instructions, with modified protocols for herbarium material. Genomic DNA was diluted 10-50 times to reduce inhibition of PCR enzymes by secondary compounds.

More than 30 previously published primer pairs were tested for polymerase chain reaction (PCR) amplification specificity, numbers of phylogenetically informative sites, indel richness, and the presence of long poly-A/T regions that interrupt sequencing reactions. We also screened nuclear low-copy markers to verify that only one orthologous copy is amplified in the majority of ingroup taxa. Out of these, five regions were selected for this study: the nuclear ribosomal internal transcribed spacer (ITS) region; a nuclear low-copy coding region, exon 9 of *EMBRYO DEFECTIVE 2765* (*EMB2765*); the chloroplast (cpDNA) coding region *matK* with adjacent partial *trnK* intron, and non-coding regions *rpl16* intron and *trnL-F* spacer.

All PCR reactions from genomic DNA were carried out using *Ex Taq*™ (Takara Bio Inc., Otsu, Shiga, Japan). A negative control using nuclease-free water instead of template DNA was included in each PCR reaction to test for contamination. The PCR mixture contained 0.1 μL of 5 units/μL *Ex Taq* (increased to 0.15 μL with difficult samples), 1.5 μL 10×Ex Taq Buffer, 1.2 μL dNTP (2.5 mM), 0.5 μL of each primer (10 μM), 0.5 μL Betaine solution (5M, Sigma-Aldrich, Inc., St. Louis, Missouri, USA), 2 μL of diluted template DNA, and ddH_20 to bring the final volume to 15 μL.

The ITS region was amplified using primer pair ITS-I (Urbatsch et al., 2000) and ITS4 (White et al., 1990). When amplification failed, generally in herbarium samples,

internal primers ITS2 and ITS3 (White et al., 1990) were used to amplify the ITS region in two pieces with ITS-I – ITS2 and ITS3 – ITS4 respectively. The PCR profile consisted of an initial 2 min denaturing step at 95°C and 40 cycles of 45 s denaturation at 95°C, 45 s annealing at 48°C, and 45 s extension at 72°C, followed by a final extension of 4 min at 72°C. The primer pair trnK570f and matK1710r (Samuel et al., 2005) was used for amplifying the *matK* coding region and the adjacent partial *trnK* intron. When unsuccessful, two additional internal primers newly designed in this study were used to amplify the region in two pieces with trnK570f – matK1100r (5'-TTC TGG TTG AAA CCA CAC-3') and matK880f (5'-GCG TCT TTC TTG AAC GAA T-3') – matK1710r respectively. Similarly, the *rpl16* intron was amplified using primer pair rpl16-71f (Jordan et al., 1996) and rpl16-1516r (Kelchner and Clark, 1997), and internal primers were designed to amplify this region in two pieces in difficult materials, with rpl16-71f – rpl16-770r (5'- GAG AGG TAA CCC ATG ATC TC -3') and rpl16-431f (5'-AGA AGT GAT GGG AAC GAT GG-3') – rpl16-1516r respectively. The *trnL-F* spacer was amplified using the primer pair trnL-e and trnL-f (Taberlet et al., 1991). The PCR profile for all three cpDNA regions consisted of an initial 2 min denaturing step at 95°C followed by 40 cycles of 45 s denaturing at 95°C, 45 s annealing at 54°C, and 1.5-2 min per kb "slow and cold" extension at 65°C (Shaw et al., 2007), with a final extension of 8 min at 65°C. *EMB2765* was PCR-amplified using the primer pair EMB2765ex9F2 and EMB2765ex9R (Wurdack and Davis, 2009). The PCR profile consisted of an initial 2 min denaturing step at 95°C and 40 total cycles of 50 s denaturing at 95°C, a touchdown program of 1 min annealing at 60°C for 1 cycle, 59°C for 2 cycles, 58°C for 3 cycles, 57°C for 4 cycles, 55°C for 5 cycles, 52°C for 6 cycles and 50°C for 19 cycles, and a 1.5 min extension at 72°C for all 40 cycles to minimize PCR-induced recombination (Cronn et al., 2002), and then a final extension of 10 min at 72°C.

EMB2765 PCR products with greater than 1% superimposed peaks were purified with QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, California, USA) and cloned using TOPO TA Cloning Kit (Invitrogen, Carlsbad, California, USA) following the manufacturer's instruction. Transformed clones were incubated for 20 hrs at 37°C. Positive clones were picked and PCR-amplified directly. Each PCR reaction contained 0.05 μL Taq (5 units/μL, QIAGEN Inc., Valencia, California, USA), 1.5 μL 10x buffer,

0.5 μL $MgCl_2$ (2 mM), 1.2 μL dNTP mix (2.5 mM), 0.5 μL each of M13 primers (10 μM, supplied with the TOPO TA kit) and 10.95 μL of ddH_2O. Cycling conditions were: 94°C for 4 min for cell lysis; 35 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 1 min; followed by a final extension step of 72°C for 4 min.

All PCR products were examined by gel electrophoresis on 1% agarose gels. When positive, products were purified with ExoSap-IT® (USB Corporation, Cleveland, Ohio, USA). For weak PCR products, or products with primer dimers, the QIAquick PCR Purification Kit was used instead of ExoSap-IT. Cleaned PCR products were sequenced at the University of Michigan DNA Sequencing Core using the same PCR primers. For PCR products longer than 1 kb (*matK* and *rpl16*), internal PCR primers were also used for sequencing to ensure double coverage. For PCR-amplified positive clones, typically eight clones with the correct insertion size were sequenced once using the EMB2765ex9R primer only.

Phylogenetic analyses—Sequences were assembled and edited in Sequencher® v. 4.10.1 (Gene Codes, Ann Arbor, Michigan, USA). Sequence alignments were performed in MUSCLE v. 4 (Edgar, 2004) using the default parameters, and manually adjusted in MacClade v. 4.08 (Maddison and Maddison, 2005). The full-length data matrices are archived in TreeBASE (study number 11056), and sequences are deposited in GenBank (Appendix 3.1).

Segments of chloroplast gene regions (*matK*, *rpl16* and *trnL-F*) with poly-A/T length variation or variable short repeats of uncertain homology were excluded from the analyses. Two short chromosomal inversions were detected in the *rpl16* intron region (Fig. 3.2). Both regions were inverted and complemented for phylogenetic analysis without scoring them as binary data (Kim and Donoghue, 2008). A separate analysis was done excluding regions with the inversion.

Indels were not coded for ITS and *EMB2765*. For *matK*, *rpl16* and *trnL-F*, indels that could be unambiguously aligned were coded as binary characters following the simple gap coding criteria of Simmons and Ochoterena (2000), as implemented in the IndelCoder module of SeqState v. 1.4.1 (Müller, 2006).

Each of the three cpDNA gene regions was initially analyzed separately using Maximum Likelihood (ML) and Bayesian inference (BI). Congruence between the

individual chloroplast gene trees was visually inspected before concatenating the three regions into the first three character sets of the cpDNA matrix. Binary indels from all three cpDNA markers were concatenated and became the fourth character set of the cpDNA matrix.

EMB2765 sequences with less than 1% superimposed peaks were coded as ambiguous at those sites, but sequences with great than 1% superimposed peaks were excluded and replaced by sequences from clones. When multiple sequenced clones showed identical sequences, they were represented by a single sequence. ITS, *EMB2765,* and combined cpDNA matrices were each subjected to the analyses described below.

Models of sequence evolution that best fit each gene region were determined by the Akaike Information Criterion (AIC) implemented in MODELTEST v. 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004). Maximum likelihood (ML) analyses were performed in GARLI v. 1.0 (Zwickl, 2006) for ITS and *EMB2765* using the best-fit model. Grouping credibility was assessed with 1000 bootstrap replications. Since GARLI was unable to conduct partitioned analyses, the combined cpDNA dataset was analyzed using RAxML v. 7.0.3 (Stamatakis, 2006), partitioning each marker. Indels were excluded since neither GARLI nor RAxML is able to analyze binary data in their current versions. The nucleotide substitution model was set to GTR + γ as recommended by the RAxML manual. 1000 ML bootstrap replicates were performed, followed by a thorough search for the best tree.

Bayesian Inference was conducted in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Four independent runs of four chains each (three heated, one cold), starting from random trees, using the default temperature of 0.2, were run for 25 000 000 generations (10 000 000 for cpDNA). Trees were sampled every 5000 generations (1000 for cpDNA). Each analysis was conducted using the nucleotide substitution model GTR + I + γ as selected by AIC in MrModeltest v. 2.3 (Nylander, 2004). For the cpDNA data, the three concatenated gene regions, plus a binary indel dataset, were partitioned into four character sets allowing all parameters to be unlinked except branch length and topology. The binary indels were subject to "rates=gamma" since there is no invariable character in this dataset. A branch length prior "brlenspr=unconstrained:exponential(100.0)" was applied to both the cpDNA and

EMB2765 analyses to prevent unrealistically long branches (Brown et al., 2010; Marshall, 2010). All parameters were visually examined in Tracer v. 1.5 (Rambaut and Drummond, 2007) to verify stationary status. Trees from the first 2 500 000 generations were discarded as the burn-in period, and the remaining trees were used to compute the majority rule consensus.

Before combining ITS, *EMB2765*, *matK*, *rpl16* and *trnL-F* into a 5-locus dataset, suspected hybrid accessions were excluded if they had divergent copies of *EMB2765* in the 50% majority-rule consensus tree recovered from the Bayesian analysis. When *EMB2765* data was not available, accessions were also excluded if they had incongruent ITS and cpDNA placement with Bayesian posterior probability (PP) ≥ 0.95 or ML bootstrap ≥ 70%. The 114 remaining accessions were combined into the 5-locus dataset. No binary indel data was coded in this dataset. ML and Bayesian analyses were carried out following the same methods as for the cpDNA dataset, except the 5-locus dataset was partitioned into three character sets by ITS, *EMB2765* and cpDNA regions.

RESULTS

Monophyly of the Chamaesyce clade is highly supported by both ML and BI analyses of all datasets. Results also support a clade consisting of *E. angusta*, *E. acuta* and *E. johnstonii* as sister to the rest of the Chamaesyce clade (Figs. 3.3-3.5). These three species correspond to *Euphorbia* subsect. *Acutae* Boiss., as modified by Mayfield (1991), hereafter referred to as the "Acuta clade". Overall statistics of all gene regions sequenced for this study are summarized in Table 3.1, and results of the phylogenetic analyses are shown in Figs. 3.3-3.5.

Chloroplast matK, rpl16, trnL-F, and the combined cpDNA dataset—We were able to obtain sequences of all three cpDNA gene regions from 150 of the 155 total accessions. The remaining five had either one or two regions that did not amplify due to degraded template DNA. The cpDNA alignments were rich in highly variable polyA/T and microsatellite repeats, especially in *trnL-F*, in which 227 out of the 767 characters were excluded in three polyA/T sections and a 102 base pair (bp) microsatellite repeat region. After excluding polyA/T and microsatellite regions, the remaining alignments were well aligned yet indel-rich, especially in *rpl16*, in which 184 indels were coded

from the 1752 bp alignment. In the *matK* marker, the majority of indels came from the non-coding partial *trnK* intron region (Table 3.1).

Two short chromosomal inversions were detected in the *rpl16* intron region. The first, a 33 bp inversion starting from base pair 1280 in the initial alignment (Fig. 3.2A), was observed in all sequenced accessions of *E. hirta*, *E. jejuna* M.C.Johnst. & Warnock, *E. riebeckii* Pax, *E. schizolepis* F.Muell. ex Boiss., and *E. potentilloides* Boiss., and in one of the four sequenced accessions of *E. cinerascens* Engelm. Monophyly of these six species is strongly rejected by all other cpDNA and nrDNA markers, as well as the rest of *rpl16*. The second inversion in *rpl16* is 38 bp in length (Fig. 3.2B), and is found in *E. stictospora* Engelm., *E. velleriflora* (Klotzsch & Garcke) Boiss., and in one of the two sequenced accessions of *E. mendezii* Boiss., starting from base pair 1438 in the initial alignment. Monophyly of these three accessions is strongly supported by ITS and cpDNA, but not by *EMB2765*. Since only two base-pair substitutions occurred in the 33 bp inversion, and all three accessions in the 38 bp inversion had identical sequences, we reversed and complemented both inversions and included them in the alignment rather than coding them as binary data (Kim and Donoghue, 2008). In this manner, the inversion events were not scored for analysis, but the phylogenetic signal in corresponding aligned segments was retained. Analyses excluding both aligned segments after reverse direction in inverted taxa gave the same tree topology and highly similar branch support values (results not shown).

Analysis of the cpDNA sequences produced a well-resolved backbone within the Chamaesyce clade, with three highly supported subclades, which we call the Acuta clade, the Peplis clade, and the Hypericifolia clade (Fig. 3.3). The Peplis clade and the Hypericifolia clade are strongly supported as sister to each other, and together we call them “core Chamaesyce”. ML and BI analyses produced congruent tree topologies. However, a few clades that are supported by ML with bootstrap values ≥ 70% received Bayesian PP < 0.50. Similarly, a few clades with ≥ 0.95 Bayesian PP received ML bootstrap values of 60% or less. None of these discrepancies are located along the backbone, and they only affect interpretation of relationships among closely related species. These local discrepancies could be explained by the fact that BI incorporates the binary indel data, whereas ML implemented in RAxML does not.

Nuclear ribosomal ITS—The nuclear ribosomal ITS region was successfully PCR-amplified in all but two of the 155 accessions. Occasional superimposed peaks (< 1% in each sequence) were observed in a number of taxa. Higher rates of superimposed peaks (> 2% in each sequence) were found in all native Hawaiian taxa. When we cloned the ITS sequences of the Hawaiian taxa, we recovered more than eight different alleles, including a possible pseudogene copy as evidenced by an unusually variable 5.8s coding region (data not shown). Two other species, *E. leucantha* (Klotzsch & Garcke) Boiss. and *E. tamanduana* Boiss., had continuously superimposed ITS sequences, and their sequences were excluded from the ITS analyses.

The ITS region has relatively high proportion of variable (40.5%) sites compare to cpDNA loci (Table 3.1). BI and ML results are congruent with the same taxon grouping when ML bootstrap support is ≥ 70% and Bayesian PP is ≥ 0.95 (Fig. 3.3). Monophyly of the entire Chamaesyce clade, the Acuta clade, and core Chamaesyce are each well supported. Relationships among the major lineages within core Chamaesyce are less resolved compared to the cpDNA results, although in general fine-scale relationships are better resolved by ITS.

Well-supported clades (Bayesian PP ≥ 0.95 and ML bootstrap ≥ 70%) are generally congruent between ITS and cpDNA, but there are 16 species that show conflicting placement between ITS and cpDNA in well-supported clades (Fig. 3.3, species joined by lines between the trees). Also, the 17 taxa on the ITS phylogeny from *E. deltoidea* down to *E. jejuna* are grouped within the Peplis clade with moderate to weak support (Bayesian PP = 0.92, ML bootstrap < 50%), whereas cpDNA data strongly support placement of these taxa within the Hypericifolia clade (Bayesian PP = 1.00, ML bootstrap ≥ 85%). Given the low support levels of the branches leading to this group in the ITS phylogeny compared to the robust support values in the cpDNA tree, we included these taxa in the Hypericifolia clade in Fig. 3.3.

Nuclear low-copy coding region EMB2765—PCR amplification and direct sequencing of PCR products were successfully carried out in 124 out of the 154 accessions for *EMB2765*. Among them, 94 had less than 1% superimposed peaks, and the remaining 30 accessions with higher levels of superimposed peaks were cloned. Monophyly of the Chamaesyce clade, the Acuta clade, and core Chamaesyce are each

highly supported, but relationships among major lineages within core Chamaesyce are poorly resolved (Fig. 3.4). Branches that are well-supported are generally congruent among *EMB2765*, ITS, and cpDNA (Figs. 3.3 and 3.4). When placement of species in the ITS and cpDNA phylogenies conflict with each other (Fig. 3.3), they often correspond to divergent placements of *EMB2765* copies, as seen in *E. eichleri* Müll.Arg., *E. carissoides* F.M.Bailey, and *E. porteriana* (Small) Oudejans (Fig. 3.4). *EMB2765* also reveals a number of cases in which multiple divergent alleles were recovered even when there is no significant conflicting placement between ITS and cpDNA, such as in *E. maculata* (Fig. 3.4, in pink) and all native Hawaiian species in our sampling (Fig. 3.4, in red). The majority of cloned accessions in the Hypericifolia clade have alleles that are resolved in different positions within that clade, and these separations are at least moderately supported by Bayesian PP ≥ 0.80 or ML bootstrap ≥ 50%. In contrast, the majority of accessions cloned in the Acuta clade and the Peplis clade have alleles that are clustered together with their conspecific copies (Fig. 3.4, in green), except in the case of *E. hooveri* Wheeler, which has two divergent alleles, one of them nested within accessions of *E. albomarginata* Torr. & A.Gray and the other nested within *E. serpens* Kunth (Fig. 3.4, in orange). In addition, there are four species that have divergent copies resolving in both the Peplis clade and the Hypericifolia clade, namely *E. blodgettii* Engelm. ex Hitchc., *E. garberi* Engelm. ex Chapm*, E. porteriana*, and *E. klotzschii* Oudejans (Fig. 3.4, in brown).

Five-locus dataset—Data from all five loci were combined after removal of 35 accessions that were identified as possible hybrids (Appendix 3.2). The 114 taxa remaining in the 5-locus dataset produced a well-supported phylogeny, with most branches having Bayesian PP ≥ 0.95 and ML bootstrap ≥ 70% (Fig. 3.5).

DISCUSSION

Three major subclades within the Chamaesyce clade—In agreement with previous molecular studies (Steinmann and Porter, 2002; Bruyns et al., 2006; Park and Jansen, 2007; Zimmermann et al., 2010), all our datasets (ITS, cpDNA, *EMB2765* and 5-locus) strongly support the monophyly of the Chamaesyce clade and its nested position within *Euphorbia.* Three major lineages within the Chamaesyce clade are strongly

supported by both the cpDNA and the 5-locus datasets, namely the Acuta clade, the Peplis clade, and the Hypericifolia clade (Figs. 3.3, 3.5 and 3.6). Morphologically, species in the Acuta clade can be distinguished from the core Chamaesyce by their reduced stipules, lack of the typical C_4 Kranz anatomy (Webster et al., 1975), and decussate rather than distichous leaves. In the core Chamaesyce clade, however, there is no single character that can readily distinguish a species in the Peplis clade from those in the Hypericifolia clade. Nonetheless, it is notable that the majority of species in the Peplis clade are glabrous, perennial herbs with entire leaf margins. Species in the Hypericifolia clade are considerably more diverse, varying from annual and perennial herbs to woody perennials, often with toothed leaf margins and usually with some kind of pubescence.

There is very little correspondence between Boissier's (1862) large subsections *Hypericifoliae* and *Chamaesyce* and either the Peplis or Hypericifolia clades identified from our molecular data. Therefore, Boissier's previous classification appears to be of little value in designating major monophyletic groups. His other small subsections are nested within the Hypericifolia clade (two were not represented in our sampling), except for subsect. *Acutae*, which corresponds to our Acuta clade.

Evolution of C_4 photosynthesis— The C_4 photosynthetic system evolved at least 62 times in the angiosperms, with 36 independent occurrences in the eudicots (Sage et al., 2011a). Within Euphorbiaceae, the only known C_4 species are members of the Chamaesyce clade. This is supported by genus-wide surveys of Kranz anatomy, CO_2 compensation points and $^{13}C/^{12}C$ isotope ratios (Webster et al., 1975; Batanouny et al., 1991). With 350 species, the Chamaesyce clade is the most species-rich C_4 eudicot lineage, containing around one fifth of all C_4 eudicot species. The question of where the C_3 to C_4 transition occurred, however, has been unclear because of the uncertainty of photosynthetic states and lack of knowledge about the phylogenetic relationships of the species.

Due to their general morphological resemblance and their largely overlapping distribution, Boissier (1862) grouped *E. acuta*, *E. angusta*, and *E. lata* Engelm. into subsect. *Acutae.* This classification was followed by Webster et al. (1975), who included all three species in the transitional group between C_3 outgroups and the remaining C_4 species. Mayfield (1991) subsequently modified this circumscription by removing the C_4

E. lata from subsect. *Acutae* and adding to the group a newly described species, *E. johnstonii*, a northern Mexican endemic that also possesses reduced stipules and lacks Kranz anatomy. Mayfield's taxonomy is confirmed by the molecular data presented here, with all four datasets grouping *E. acuta, E. angusta*, and *E. johnstonii* in the Acuta clade (= subsect. *Acutae*), whereas *E. lata* was recovered nested within the Peplis clade (Figs. 3.3 and 3.5).

Webster et al.'s scenario of a transitional C_3 Acuta clade was complicated by the findings of Sage et al. (2011a), who provided detailed data indicating that both *E. acuta* and *E. johnstonii* are in fact C_3-C_4 intermediates. In *E. acuta*, which was examined in more detail, there is low activity of key C_4 enzymes, and therefore it has a C_3-like carbon isotope ratio in its leaves. However, Kranz-like anatomy and localization of glycine decarboxylase in the bundle sheath cells of this species indicate that it is able to scavenge CO_2 produced by photorespiration in enlarged bundle sheath cells. This trait is considered to be an early and key step in the evolution from C_3 to C_4 photosynthesis. On the other hand, *E. angusta* was confirmed to be a true C_3 species, and the species now excluded from subsect. *Acutae, E. lata,* showed typical C_4 features in anatomy, gas exchange, and activities of key photosynthetic enzymes. Thus, the results of Sage et al. (2011a) confirm the recircumsription of subsect. *Acutae* by Mayfield (1991), and there is no full transition from C_3 to C_4 in the Acuta clade.

According to our current knowledge on phylogenetic relationships and photosynthetic states, there are three possible evolutionary scenarios for the evolution of C_4 photosynthesis in the Chamesyce clade: 1) it could have evolved at least twice from C_3 ancestors, once within the Acuta clade, and another time on the stem leading to core Chamaesyce; 2) alternatively, C_4 photosynthesis could have evolved once in the common ancestor of the entire Chamaesyce clade and then have gone through various degrees of reversal to C_3 in the Acuta clade; or 3) an ancestral C_3-C_4 intermediate type in the common ancestor of the Chamaesyce clade could have given rise to all the C_3, C_4, and intermediate types in the extant species of the clade (Christin et al., 2010). Given the complexities of the intermediate photosynthetic types in *E. acuta* and *E. johnstonii*, and the small size and sister relationship of subsect. *Acutae* to the rest of the clade, we cannot

yet be certain which of these scenarios might explain the evolution of C_3, C_4, and intermediate systems within the Chamaesyce clade.

North American origin of the Chamaesyce clade—According to evidence from morphology, geographic centers of diversity, chromosome counts, and photosynthetic types, Webster et al. (1975) hypothesized that the Chamaesyce clade originated in subtropical and warm temperate areas of North America. This scenario is strongly supported by our molecular phylogenetic analysis (Figs. 3.3-3.5). The outgroup lineage sister to the Chamaesyce clade is mainly North American and corresponds to the former *Euphorbia* subgenus *Agaloma* (Raf.) House (Steinmann and Porter, 2002). Within the Chamaesyce clade, the entire Acuta clade and all but two species that are deeply nested in the Peplis clade, *E. peplis* L. and *E. serpens*, are endemic to the southern United States and Mexico (Fig. 3.5). Species in the Hypericifolia clade, in contrast, have many different distribution patterns, including both cosmopolitan weeds as well as narrow endemics in both the New World and the Old World. Even so, a small clade consisting of *E. astyla* Engelm. ex Boiss. and *E. jejuna*, two Chihuahuan Desert endemics, is sister to the rest of the Hypericifolia clade, and all of the Old World groups are deeply nested in predominantly New World groups. Consequently, our data are consistent with a North American origin for the Chamaesyce clade as well as for each of the three major subclades. This makes it very likely that C_4 photosynthesis originated in this area as well.

Long-distance dispersal events from the New World to the Old World—There is a group of species in the Hypericifolia clade that occurs either on oceanic islands or in coastal areas of Old World continents, represented in our sampling by seven species beginning with *E. atoto* G.Forst. (Fig. 3.3, in blue; Fig. 3.5, shaded box). While cpDNA and *EMB2765* do not fully resolve this group, both ITS and the 5-locus datasets support the monophyly of the seven species. Both datasets recover *E. mesembryanthemifolia* Jacq., a shrub native to the Caribbean, as the group's closest New World relative, followed by *E. hypericifolia* L., a weedy species native to the Neotropics. Both of these closely related New World species are characterized by relatively large leaves and a more or less woody, ascending habit. Therefore, this oceanic Old World group most likely originated once from an ascending and shrubby ancestor in the Neotropics. Subsequent dispersals have occurred throughout the Pacific coastlines, with widespread species such

as *E. atoto* and *E. chamissonis* (Klotzsch & Garcke) Boiss., as well as others that colonized coastal Australia such as *E. coghlanii* F.M.Bailey and *E. psammogeton* P.S. Green, southeast Asia (e.g., *E. reniformis* Blume) and the Indian Ocean (e.g., *E. mertonii* Fosberg and *E. indica* Lam.). None of the native Hawaiian species is recovered in this oceanic group, however, despite Hawaii's intermediate geographic position between the New World and the members of the *E. atoto* group. Therefore, a close relationship between Hawaiian Chamaesyce and *E. atoto,* as proposed by Degener and Croizat (1938) and Koutnik (1982), is not supported by our molecular data.

Many species in the Chamaesyce clade possess a seed coat that becomes mucilaginous and sticky when wet (Fig. 3.1C; Jordan and Hayden, 1992), and this type of seed coat is otherwise rare in *Euphorbia*. Mucilaginous seed coats have been shown to facilitate seed germination in other plant groups, particularly in weedy species or in desert habitats (Gutterman and Shem-Tov, 1997; Ebrahimzadeh et al., 2000; Penfield et al., 2001). The small, sticky seeds of the Chamaesyce clade can easily adhere to birds and thus enhance the likelihood of long-distance dispersal (Jordan and Hayden, 1992; Steinmann and Porter, 2002). A sticky seed coat is notably absent in the C_3 *E. angusta,* which could be interpreted as retaining the ancestral state of the clade, although it is present in the closely related *E. acuta* and *E. johnstonii*. A mucilaginous seed coat is also missing in inland Hawaiian species, but in this case it has been attributed to a secondary loss in insular habitats (Jordan and Hayden, 1992). Certain species such as *E. mesembryanthemifolia* and *E. atoto* also lack a mucilaginous seed coat. Instead, their seeds are able to float in seawater, which could explain their distribution on islands and in coastal areas as discussed above (Carlquist, 1966; Jordan and Hayden, 1992).

In contrast to the oceanic dispersal pattern exemplified by the shrubby *E. atoto* and its allies, there is another Old World group in the Hypericifolia clade that shows evidence of long-distance dispersal between inland continental habitats. This clade (Fig. 3.3, in tan; Fig. 3.5, shaded) consists of small, prostrate herbs, with a number of African and Eurasian species such as *E. humifusa* Willd. and the seven species from *E. arabica* Hochst. & Steud. ex Anderson to *E. zambesiana* Benth. It also includes inland Australian species such as *E. australis* Boiss., *E. dallachyana* Baill., *E. schultzii* Benth., and *E.* sp. nov. Australia. Some species in this inland group show incongruent relationships between

ITS and cpDNA, and some of them also have divergent *EMB2765* copies. These include inland African species such as *E. tettensis* Klotzsch, *E. neopolycnemoides* Pax & K.Hoffm., *E. mossambicensis* (Klotzsch & Garcke) Boiss., *E. lissosperma* S.Carter, and *E. eylesii* Rendle, as well as inland Australian species like *E. carissoides* and *E. schizolepis* (Fig. 3.3, in tan and with lines connecting incongruent placements; Fig. 3.4). However, accessions of many of the Old World species we were able to sample in this group came from herbarium specimens and were difficult to amplify or clone, so we may have failed to detect additional copies of *EMB2765*. Better sampling with fresh leaf material among the inland Old World species is needed to better understand the relationships among species in this region.

Another Old World group in the Hypericifolia clade consists of only two species sampled here (Fig. 3.3, in purple): *E. forsskalii* J.Gay is native to Africa, the Mediterranean region, and the Arabian Peninsula, whereas *E. makinoi* Hayata is native to eastern and southeastern Asia. Both ITS and cpDNA support the two as sister to each other, and together they are sister to *E. dioeca* Kunth, a widespread New World species. Like the previous group, this group would benefit from freshly collected material to verify the relationship suggested here, as well as to check for multiple copies of *EMB2765.*

Contrary to the different kinds of long-distance dispersals evoked within the Hypericifolia clade, the Peplis clade is entirely New World except for *E. peplis*, which has a Macaronesian, Mediterranean, and European distribution. This species is nested in a clade of six other species, and all seven of them are specialized on either inland deep sand deposits or sandy beach habitats (Figs. 3.3-3.5). *Euphorbia peplis* is thus the only species in the Peplis clade that appears to represent a dispersal event from the New World to the Old World.

Finally, more recent human-assisted dispersal has probably contributed to the cosmopolitan distribution of weeds such as *E. maculata*, *E. hirta*, *E. prostrata* Aiton, and *E. thymifolia* L., although we cannot rule out the possibility of pre-human dispersal events.

Widespread reticulate evolution—Divergent copies of *EMB2765* and incongruence between chloroplast and nuclear datasets allow us to hypothesize 35 taxa of

possible hybrid origin among the species of the Chamaesyce clade sampled here (Appendix 3.2). To untangle their relationships, we made three assumptions: 1) the chloroplast genome is contributed by the maternal parent, and thus a species of hybrid origin would group with the species most closely related to the maternal parent in the cpDNA tree; 2) due to concerted evolution, ITS can be homogenized either towards the paternal or maternal sequence (Alvarez and Wendel, 2003). Therefore, ITS and cpDNA could be incongruent if our ITS sequence had retained the paternal copy in a taxon of hybrid origin; 3) if we found two copies of *EMB2765*, we expected divergent copies to cluster with both the maternal and paternal parents, due to the biparental nature of nuclear low-copy genes; 4) when a third copy of *EMB2765* was found that resolved in a different phylogenetic position from the other two copies, it could be indicative of further hybridization events in the history of that taxon.

We should note that due to the broad scale of this study, in which we sequenced between two and four accessions for most taxa in our unreduced 450-accession dataset, the hybrid relationships proposed here (taxa joined by lines in Fig. 3.3 and taxa with divergent copies of *EMB2765* in Fig. 3.4; summarized in Appendix 3.2) are meant to be taken as working hypotheses for more detailed, population-level sampling involving both cytological and molecular studies. Because of the high number of taxa of possible hybrid origin that emerged from this study, we cannot examine each one in detail here. Instead, three of the most notable species or species complexes of hypothesized hybrid origin are presented below as examples.

Allopolyploid origin of the woody Hawaiian Chamaesyce—With 16 recognized species, the Hawaiian Chamaesyce clade represents one of the most notable radiations of woody taxa in the Hawaiian Archipelago (Fig. 3.1, G and H; Ziegler, 2002). Monophyly of the Hawaiian Chamaesyce clade was reported by Motley and Raz (2004) based on ITS sequence data, with extensive taxon sampling among Pacific Island species, but relatively little sampling from North America. Their study suggested that the closest relatives of the Hawaiian Chamaesyce clade were from the New World instead of other Pacific Islands. Our expanded sampling also supports the monophyly of the Hawaiian Chamaesyce clade (Fig. 3.3, in red) and recovers four small annual species commonly found in disturbed habitats in the southern United States, northern Mexico, and the Caribbean as the closest

relatives of the group. These include *E. stictospora, E. velleriflora*, *E. mendezii,* and *E. leucantha* (Fig. 3.3, ITS), which are all morphologically quite similar to each other. A fifth species, *E. cinerascens* (Fig. 3.1F), is a perennial species endemic to the Chihuahuan Desert, and it was identified by cpDNA as an additional member of the sister clade to Hawaiian Chamaesyce (Fig. 3.3, cpDNA). Cloning of *EMB2765* PCR products detected three copies in taxa of the Hawaiian Chamaesyce clade (Fig. 3.4, in red). Each species surveyed had all three copies, except for *E. multiformis* Gaudich. ex Hook. & Arn., which had only two copies. One of these copies supports the Hawaiian species as being closely related to *E. stictospora*, *E. velleriflora*, *E. mendezii*, *E. leucantha*. There are also two copies of *E. cinerascens* that are placed close to this clade, but with low support (Fig. 3.4, blue), which is consistent with the cpDNA pattern observed in Fig. 3.3. A second *EMB2765* copy in the Hawaiian species gives weak support for them being sister to the third, divergent copy of *EMB2765* in *E. cinerascens*. The third copy of *EMB2765* in the Hawaiian species, however, does not reveal a highly supported sister group for this clade. Given the high chromosome numbers in counts of the four Hawaiian species surveyed thus far compared to other closely related Chamaesyce species (Fig. 3.4; 2n = 38, Carr, 1985), allopolyploidy appears to have evolved early within the native Hawaiian species of the Chamaesyce clade. Also, since *E. cinerascens* has multiple copies of *EMB2765,* and its placement in the ITS phylogeny is different from the relationships inferred by the cpDNA tree, this indicates that it may also have originated by interspecific hybridization.

According to our earlier assumptions, both *E. cinerascens* and the Hawaiian Chamaesyce clade appear to share the same or a closely related maternal genome, related to the clade of *E. stictospora*, *E. velleriflora*, *E. mendezii*, *E. leucantha*. A different shared paternal parent for both *E. cinerascens* and the Hawaiian Chamaesyce clade is suggested by the second copy of *EMB2765*, albeit with weak support ("ancestral taxon 1" in Fig. 3.7A). This initial hybrid may have served as the maternal parent in a subsequent hybridization event ("ancestral taxon 2" in Fig. 3.7A), as evidenced by the third divergent copy of *EMB2765* in the Hawaiian Chamaesyce. It would presumably have been this secondary hybrid that eventually reached the Hawaiian Islands through long-distance dispersal and subsequently radiated into the 16 species present there now.

Our results are consistent with the finding that a number of other Hawaiian angiosperm radiations are of North American origin involving hybrids and/or polyploids as well (Carr, 1998; Baldwin and Wagner, 2010), including the Hawaiian silversword alliance (Barrier et al., 1999), the Hawaiian mints (Lindqvist and Albert, 2002), *Viola* (Ballard and Sytsma, 2000), and *Cuscuta* (Stefanovic and Costea, 2008). These radiations appear to be associated with dispersal by birds and with hybridization prior to long-distance dispersal (Baldwin and Wagner, 2010). Allopolyploid taxa can exhibit great adaptive plasticity through increased heterozygosity, better masking of recessive deleterious alleles, and lower susceptibility to inbreeding depression (Comai, 2005), and this may facilitate colonization of new niches, such as in the Hawaiian Islands.

Apart from the Hawaiian radiation, there are also eight species in the Chamaesyce clade that are endemic to the Galapagos Islands (Burch, 1969). Although we were not able to sample any taxa from this area, it would be an excellent group to study to determine if polyploidy and hybridization were involved in their radiation as well.

Euphorbia serpens species complex— Euphorbia serpens, its sister species *E. albomarginata*, and five other species that appear to involve *E. serpens* as one of their parents, together form a complex which we infer to have highly reticulate relationships (Fig. 3.7B). Three of the species in this complex are very similar morphologically and are monophyletic in both the ITS and cpDNA phylogenies. These include *E. blodgettii*, which is widespread from the southeastern United States to Central America in somewhat disturbed habitats, and *E. garberi* and *E. porteriana* (Fig. 3.1J), which are both narrow endemics restricted to limestone outcrops in southern Florida. The cpDNA data places these three species together and sister to a small clade consisting of *E. dioeca* (Mexico to South America) and the Old World *E. forsskalii* and *E. makinoi* in the Hypericifolia clade, whereas ITS data places them nested among accessions of *E. serpens* in the Peplis clade (Fig. 3.3). In the *EMB2765* phylogeny, each of these species has a copy of *EMB2765* nested among multiple *E. serpens* accessions (Fig. 3.4, in brown, with only 2 of 20 accessions of *E. serpens* shown), in agreement with the ITS placement. Both *E. garberi* and *E. porteriana* also have a second copy of *EMB2765* that is closely related to *E. dioeca*, in the Hypericifolia clade. This topology is consistent with the relationships shown in the cpDNA tree (Fig. 3.3), except that an *EMB2765* copy of *E. blodgettii* is

presumably missing. A third *EMB2765* copy of *E. garberi* and a second copy of *E. blodgettii* are both clustered with other tropical New World species of the Hypericifolia clade that are also specialized on limestone substrates, such as *E. deltoidea* Engelm. ex Chapm. and *E. turpinii* Boiss. We hypothesize that a hybridization event occurred between *E. dioeca* or a closely related extant or ancestral species, as the maternal donor, and *E. serpens* as the paternal donor. This initial hybrid plant subsequently hybridized with *E. deltoidea* or a closely related species (Fig. 3.7B), followed by differentiation of *E. garberi*, *E. blodgettii*, and probably *E. porteriana*. Both *E. garberi* and *E. porteriana* are sympatric with *E. deltoidea*, and all three species are restricted to pine rocklands on limestone outcrops in southern Florida; *E. blodgettii* also occurs in southern Florida, but extends into the southeastern United States and Central America.

Another suggested hybridization event between the Peplis clade and the Hypericifolia clade also involves *E. serpens*. *Euphorbia klotzschii* from southern South America is nested among accessions of *E. serpens* in the Peplis clade with cpDNA data, whereas ITS sequence data place it sister to *E. serpyllifolia* Pers. in the Hypericifolia clade (Fig. 3.3). *EMB2765* recovered two copies of *E. klotzschii* that correspond to the different ITS and cpDNA placements (Fig. 3.4). This implies that *E. serpens* could have been the maternal donor, and *E. serpyllifolia*, or a closely related species, was the paternal donor that led to *E. klotzschii*.

A third proposed hybrid species origin involves *E. serpens* and *E. albomarginata,* both of which are small, prostrate, glabrous herbs with white, membranous stipules and entire leaf margins. *Euphorbia hooveri*, a species that is morphologically quite distinct from *E. serpens* and *E. albomarginata* (see Fig. 3.1, I and K), was recovered in both the ITS and cpDNA phylogenies nested among *E. albomarginata* accessions in the Peplis clade (Fig. 3.3). However, *E. hooveri* has two *EMB2765* copies, one nested among *E. albomarginata* accessions and the other nested with *E. serpens* accessions (Fig. 3.4, in orange). This suggests that *E. hooveri* may be of hybrid origin from ancestors allied to *E. albomarginata* and *E. serpens*. *Euphorbia hooveri* is a rare summer annual restricted to mud flats in ephemeral vernal pools in the Central Valley of California, whereas both putative parental species occur on a variety of soil types nearby in more upland habitats (Hickman, 1993).

One of the caveats of inferring parentage and reticulate relationships within a species complex is that population level sampling is required to account for complications such as lineage sorting, chromosome races, introgression, and other confounding factors. In our unreduced 450-accession dataset, we analyzed ITS and cpDNA data in 20 accessions of *E. serpens* throughout its full range of distribution, as well as three accessions of *E. blodgettii*, two accessions of *E. porteriana*, and four accessions of *E. albomarginata*. When the multiple accessions are analyzed together, all five putative hybrid species discussed above continue to have either ITS or cpDNA sequences deeply nested in *E. serpens* or *E. albomarginata* with strong support (data not shown), showing the same pattern as seen from the reduced dataset shown in Fig. 3.3. Although this does not provide conclusive evidence for the hybrid origin of these taxa, it does show a consistent pattern within the more densely sampled species.

Euphorbia maculata—Euphorbia maculata is a small, prostrate summer annual, able to go through multiple overlapping generations within a single growing season (Suzuki and Teranishi, 2005), and it is one of the most common weeds across temperate North America and is naturalized worldwide. While both ITS and cpDNA analyses place it sister to the North American species *E. meganaesos* Featherman and *E. glyptosperma* Engelm. in the Hypericifolia clade (Fig. 3.3), two distinct *EMB2765* alleles were recovered (Fig. 3.4). The first allele corresponds to the ITS and cpDNA placement, grouped together with species that have chromosome numbers of 2n = 22, while the other allele is closely related to *E. dioeca*, a species that may also be involved in the *E. serpens* species complex as well as other hybrid relationships. With a relatively high chromosome count (2n = 40, Xue et al., 2007; Fig. 3.4), *E. maculata* is likely of allopolyploid origin from species closely related to *E. dioeca* and *E. glyptosperma* (Fig. 3.7C).

CONCLUSION

Through a complex suite of character switches, including physiology and anatomy (C_4 photosynthesis), seed morphology (sticky surface and small size), and life-history (reduced vegetative growth and prolonged reproductive stages), the Chamaesyce clade of *Euphorbia* has successfully adapted to warm and dry areas in subtropical North America, diversified locally into three major clades, and subsequently achieved

worldwide distribution through multiple long-distance dispersal events. During this process, genetic mixing through reticulate evolution and changes in ploidy level have produced new species with novel adaptations. This study provides a phylogenetic framework for further study into the physiology, biogeography, character evolution, and conservation status of the most diverse C_4 lineage among the eudicots. It also demonstrates the ongoing evolutionary potential of weedy plant lineages through dispersal followed by local adaptation, producing diverse derivative endemic lineages such as the Hawaiian radiation.

Table 3.1. Summary of chloroplast and nuclear gene regions used in this study. The cpDNA matrix comes from concatenated *matK*, *rpl16*, *trnL-F,* and the binary indel dataset. The 5-locus dataset comes from concatenated exon 9 of *EMB2765*, ITS, *matK*, *rpl16* and *trnL-F*.

	Chloroplast gene regions					Nuclear gene regions		5-locus dataset
	matK	*rpl16*	*trnL-F*	combined cp indels	combined cpDNA	ITS	*EMB2765* exon 9	
Number of accessions	153	154	150	154	154	153	124	114
Range of raw length* (bp)	839-1944	446-1366	258-481	334	802-3572	346-1068	621-767	–
Excluded characters	32	117	227	0	376	–	–	–
Aligned length (after exclusion)	2128	1752	540	334	4754	1213	767	6286
Variable sites (proportion)	691 (32.5%)	613 (35.0 %)	203 (37.6 %)	334 (100%)	1844 (38.8%)	491 (40.5%)	321 (41.9%)	2090 (33.2%)
No. of indels coded	88	184	62	–	334	–	–	–
Nucleotide substitution model selected by AIC	TVM+γ	GTR+I+γ	TVM+γ	–	TVM+I+γ	GTR+I+γ	TrN+I+γ	–

* Lower ends of raw lengths are from partial sequences that the full-length sequences failed to amplify or sequence.

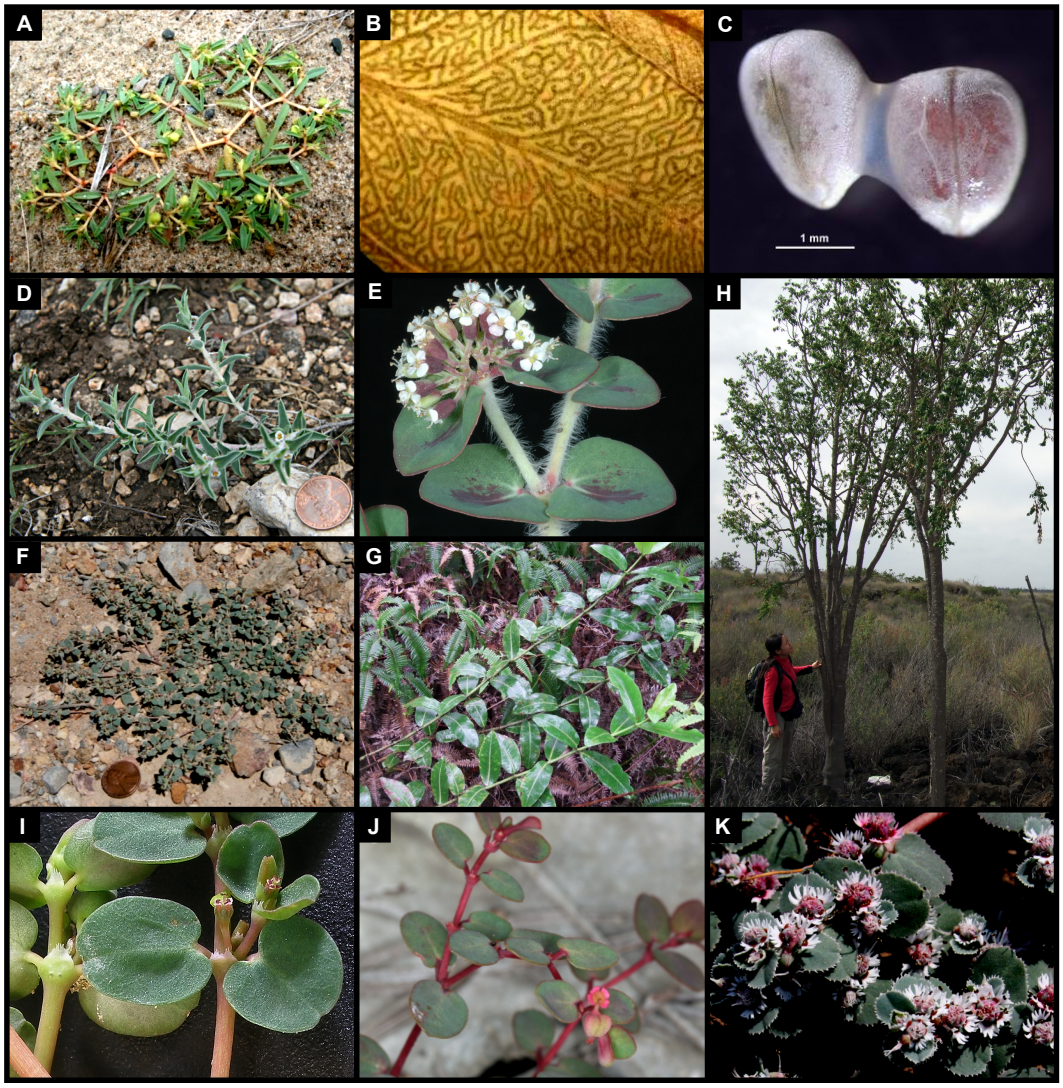


Figure 3.1. Diversity of morphology and habitats in the Chamaesyce clade of *Euphorbia*. A. *Euphorbia polygonifolia*, showing the typical prostrate, dichotomously branching growth form of the Chamaesyce clade (*Berry 7916*, MICH). B. Dark-green veins associated with Kranz anatomy that are often visible on C_4 Chamaesyce leaves (*E. deppeana* Boiss., *Lau 2817*, BISH). C. Two seeds of *E. polygonifolia* (*Berry 8023*, MICH), showing mucilaginous seed coats after a drop of water was added. D. *E. acuta*, a C_3 species in the Acuta clade (*Yang 23*, MICH). E. *E. umbellulata,* showing dichotomous branching and well-developed cyathial gland appendages (*Yang 91*, MICH). F. *E. cinerascens*, a North America species closely related to the woody Hawaiian Chamaesyces (*Yang 6*, MICH). G. *E. remyi var. remyi*, a C_4 wet forest understory shrub endemic to the island of Kauai. H. *E. olowaluana*, a C_4 tree and pioneer species on recently formed lava fields, Hawaii. I-K. Members of the *E. serpens* species complex. I. *E. serpens,* a prostrate herb widespread in the New World and introduced to the Old World (*Aedo 18005*, MA). J. *E. porteriana*, an ascending herb restricted to limestone outcrops in southern Florida (*Yang 131*, MICH). K. *E. hooveri*, an annual species endemic to vernal pools in the Central Valley of California. E from V.W. Steinmann and I from C. Aedo.

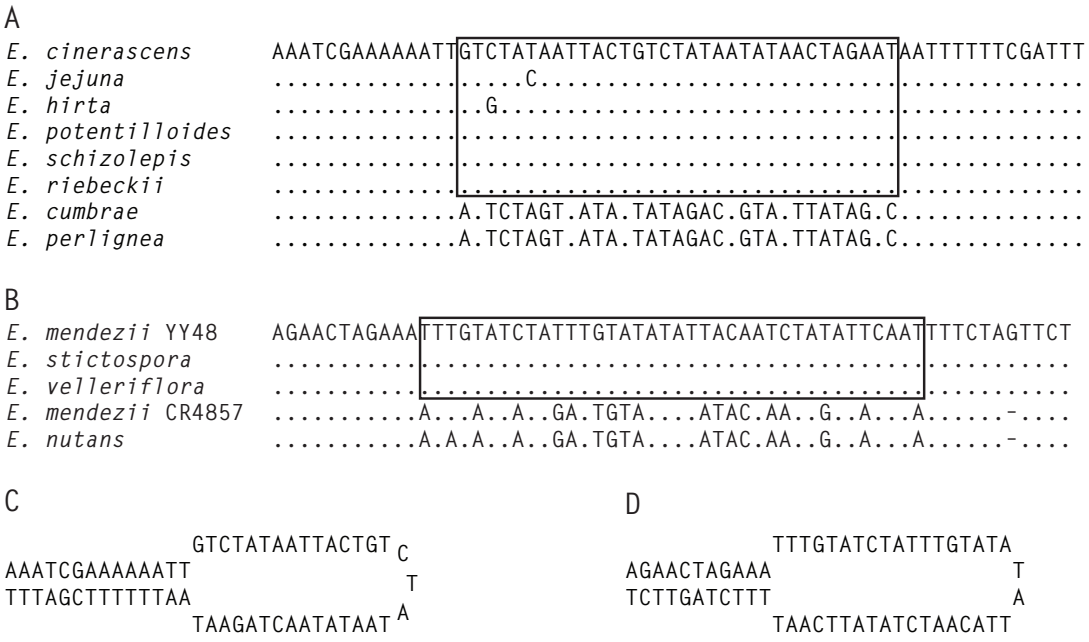


Figure 3.2. Short inversions and flanking inverted repeats found in the *rpl16* intron region. A. The box identifies the 33 bp inversion shared by *E. cinerascens*, *E. jejuna*, *E. hirta*, *E. potentilloides*, *E. schizolepis* and *E. riebeckii*, flanked by 14 bp inverted repeats on both sides. B. The box identifies the 38 bp inversion shared by *E. stictospora*, *E. velleriflora,* and one of the two sequenced accessions of *E. mendezii*, flanked on both sides by 11 bp inverted repeats. C. Secondary stem-loop structure of the DNA region shown in A inferred for *E. cinerascens*. D. Secondary stem-loop structure of the DNA region shown in B inferred for *E. stictospora*. Dots represent bases that are identical to the first row in the alignment, and dashes indicate gaps created by a single base pair insertion.

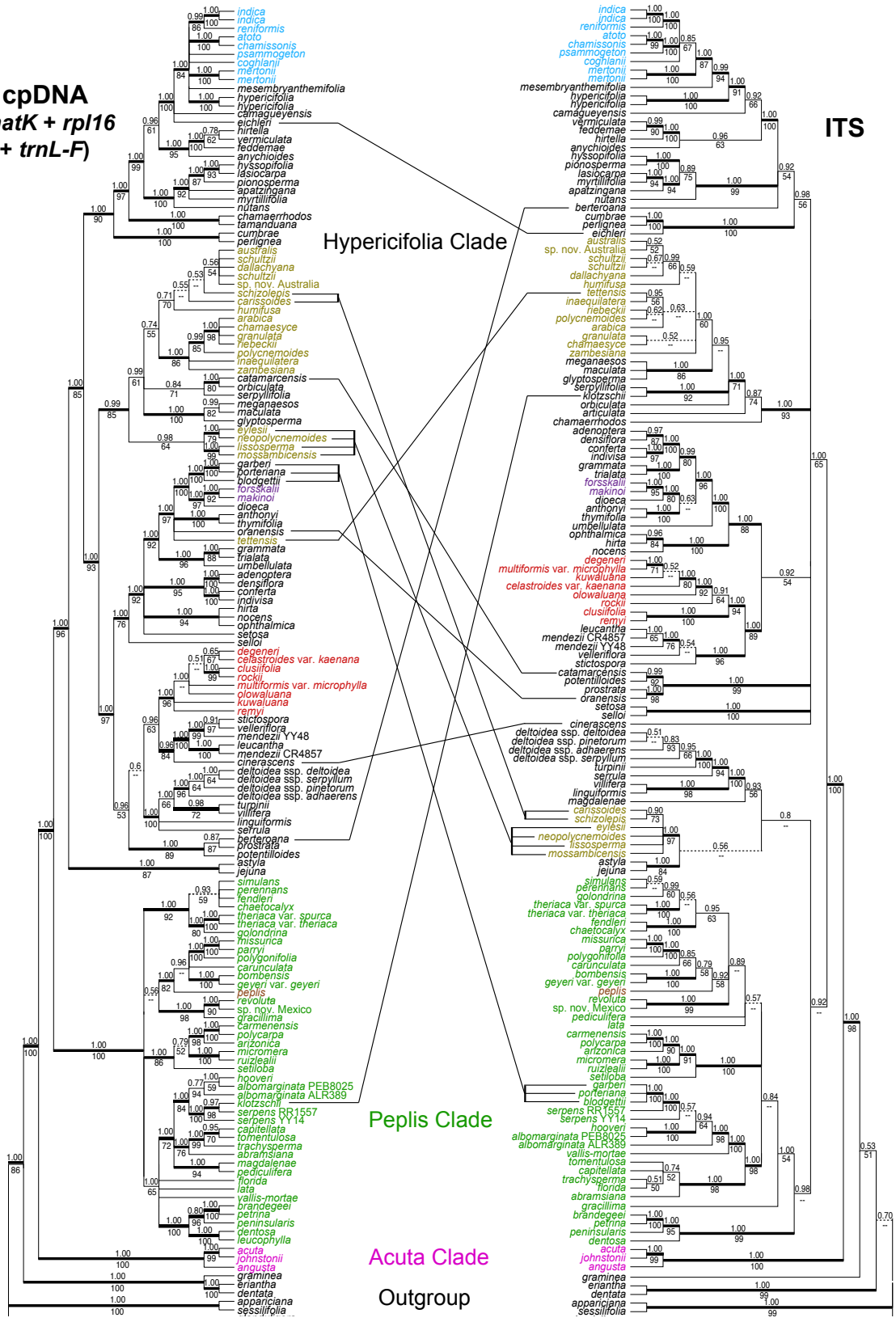

cpDNA
(matK + rpl16
+ trnL-F)
ITS
Hypericifolia Clade
Peplis Clade
Acuta Clade
Outgroup
indica
indica
reniformis
atoto
chamissonis
psammogeton
coghlanii
mertonii
mertonii
mesembryanthemifolia
hypericifolia
hypericifolia
camagueyensis
eichleri
hirtella
vermiculata
feddemae
anychioides
hyssopifolia
lasiocarpa
pionosperma
apatzingana
myrtillifolia
nutans
chamaerrhodos
tamanduana
cumbrae
perlignea
australis
schultzii
dallachyana
schultzii
sp. nov. Australia
schizolepis
carissoides
humifusa
arabica
chamaesyce
granulata
riebeckii
polycnemoides
inaequilatera
zambesiana
catamarcensis
orbiculata
serpyllifolia
meganaesos
maculata
glyptosperma
eylesii
neopolycnemoides
lissosperma
mossambicensis
garberi
porteriana
blodgettii
forsskalii
makinoi
dioeca
anthonyi
thymifolia
oranensis
tettensis
grammata
trialata
umbellulata
adenoptera
densiflora
conferta
indivisa
hirta
nocens
ophthalmica
setosa
selloi
degeneri
celastroides var. kaenana
clusiifolia
rockii
multiformis var. microphylla
olowaluana
kuwaluana
remyi
stictospora
velleriflora
mendezii YY48
leucantha
mendezii CR4857
cinerascens
deltoidea ssp. deltoidea
deltoidea ssp. serpyllum
deltoidea ssp. pinetorum
deltoidea ssp. adhaerens
turpinii
villifera
linguiformis
serrula
berteroana
prostrata
potentilloides
astyla
jejuna
simulans
perennans
fendleri
chaetocalyx
theriaca var. spurca
theriaca var. theriaca
golondrina
missurica
parryi
polygonifolia
carunculata
bombensis
geyeri var. geyeri
peplis
revoluta
sp. nov. Mexico
gracillima
carmenensis
polycarpa
arizonica
micromera
ruizlealii
setiloba
hooveri
albomarginata PEB8025
albomarginata ALR389
klotzschii
serpens RR1557
serpens YY14
capitellata
tomentulosa
trachysperma
abramsiana
magdalenae
pediculifera
florida
lata
vallis-mortae
brandegeei
petrina
peninsularis
dentosa
leucophylla
acuta
johnstonii
angusta
graminea
eriantha
dentata
appariciana
sessilifolia

Figure 3.3. Majority rule consensus trees recovered from Bayesian analyses of the chloroplast DNA data (*matK* + *rpl16* + *trnL-F* + indels, cpDNA) and the nuclear ITS data. Numbers above the branches are Bayesian posterior probabilities (PP) and numbers below the branches are Maximum Likelihood bootstrap percentages (MLB). Thick branches indicate PP ≥ 0.95 and MLB ≥ 70%, and branches in dashed lines have Bayesian PP < 0.80 and MLB < 50%. Presumed hybrid accessions with different placement between the ITS and cpDNA phylogeny that are well-supported by MLB and PP are connected by lines. Taxa belonging to the Acuta clade near the bottom are colored pink; taxa in the Peplis clade are colored green, except *E. peplis*, the only taxa in the Peplis clade that is native to the Old World and therefore colored darker brown. Taxa in the Hypericifolia clade are colored black if they are native to the New World, whereas the four groups native to the Old World are colored as follows: the oceanic group in blue, the continental group in tan, *E. forsskalii* and *E. makinoi* in purple, and the Hawaiian group in red.

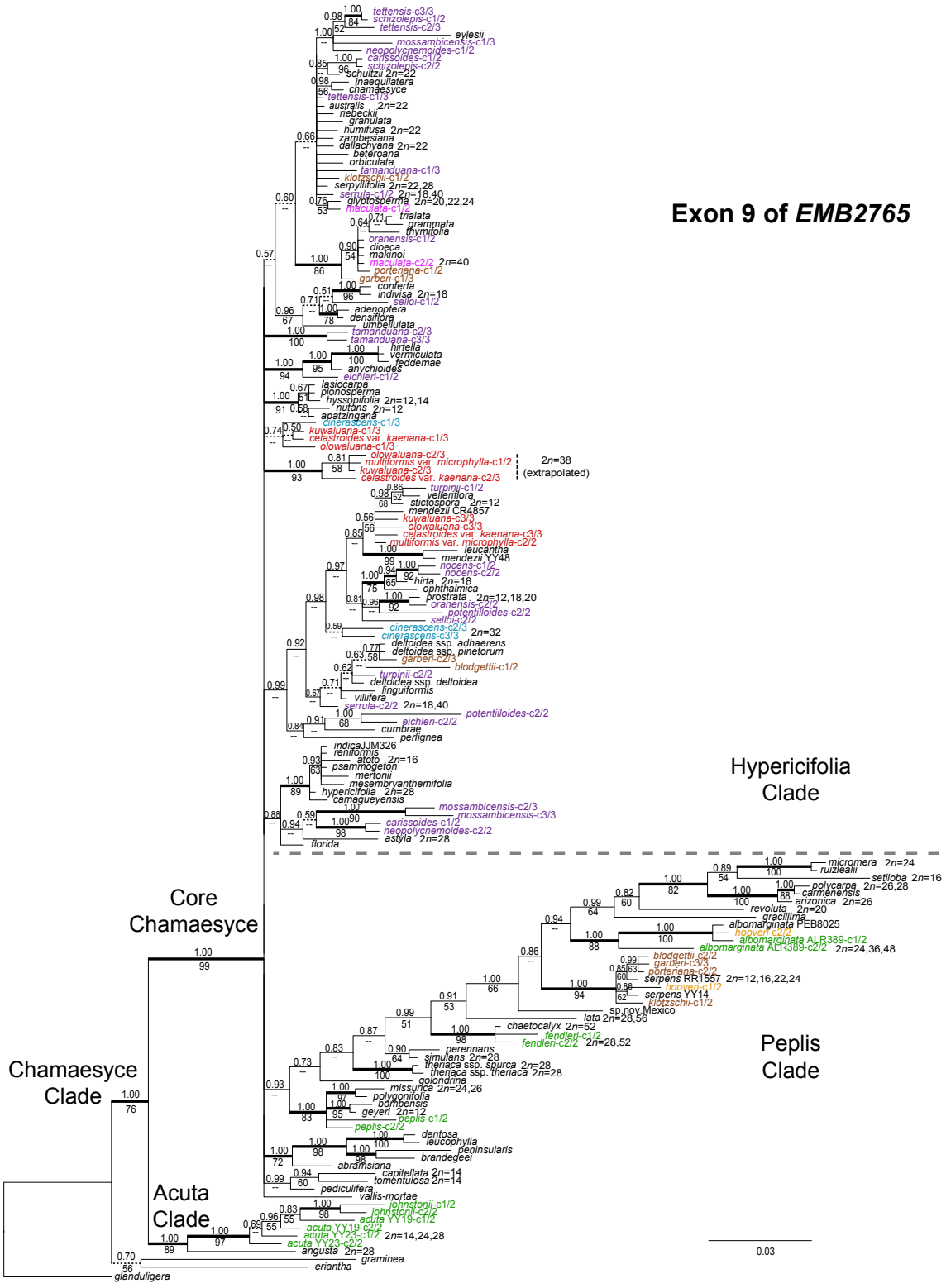

Exon 9 of *EMB2765*
Hypericifolia Clade
Core Chamaesyce
Chamaesyce Clade
Acuta Clade
Peplis Clade
0.03

Figure 3.4. Majority rule consensus tree recovered from Bayesian analyses of the nuclear coding region exon 9 of *EMB2765*. Numbers above the branches are Bayesian posterior probabilities (PP) and numbers below the branches are Maximum Likelihood bootstrap percentages (MLB). Thick branches indicate PP ≥ 0.95 and MLB ≥ 0.70%, and branches in dashed lines have Bayesian PP < 0.80 and MLB < 50%. Branch length scale on lower right. Previously published chromosome numbers are listed next to their respective taxa (Perry, 1943; Hans, 1973; Urbatsch et al., 1975; Hassall, 1976; Carr, 1985; Xue et al., 2007; Powell, *in prep.*). Cloned accessions in the Acuta and Peplis clades with their conspecific copies clustered together are colored green; divergent cloned copies of *E. hooveri* are colored orange. Divergent cloned copies of the *E. serpens* complex that span both the Peplis clade and the Hypericifolia clade are colored brown. Within the Hypericifolia clade, accessions of *E. maculata* are in pink, Hawaiian endemics in red, and the closely related *E. cinerascens* in blue. Remaining accessions with divergent cloned copies are colored purple. The separation of the Hypericifolia clade from the Peplis clade in this tree is inferred from the more robust cpDNA and 5-locus phylogenies and is indicated by a dashed line.

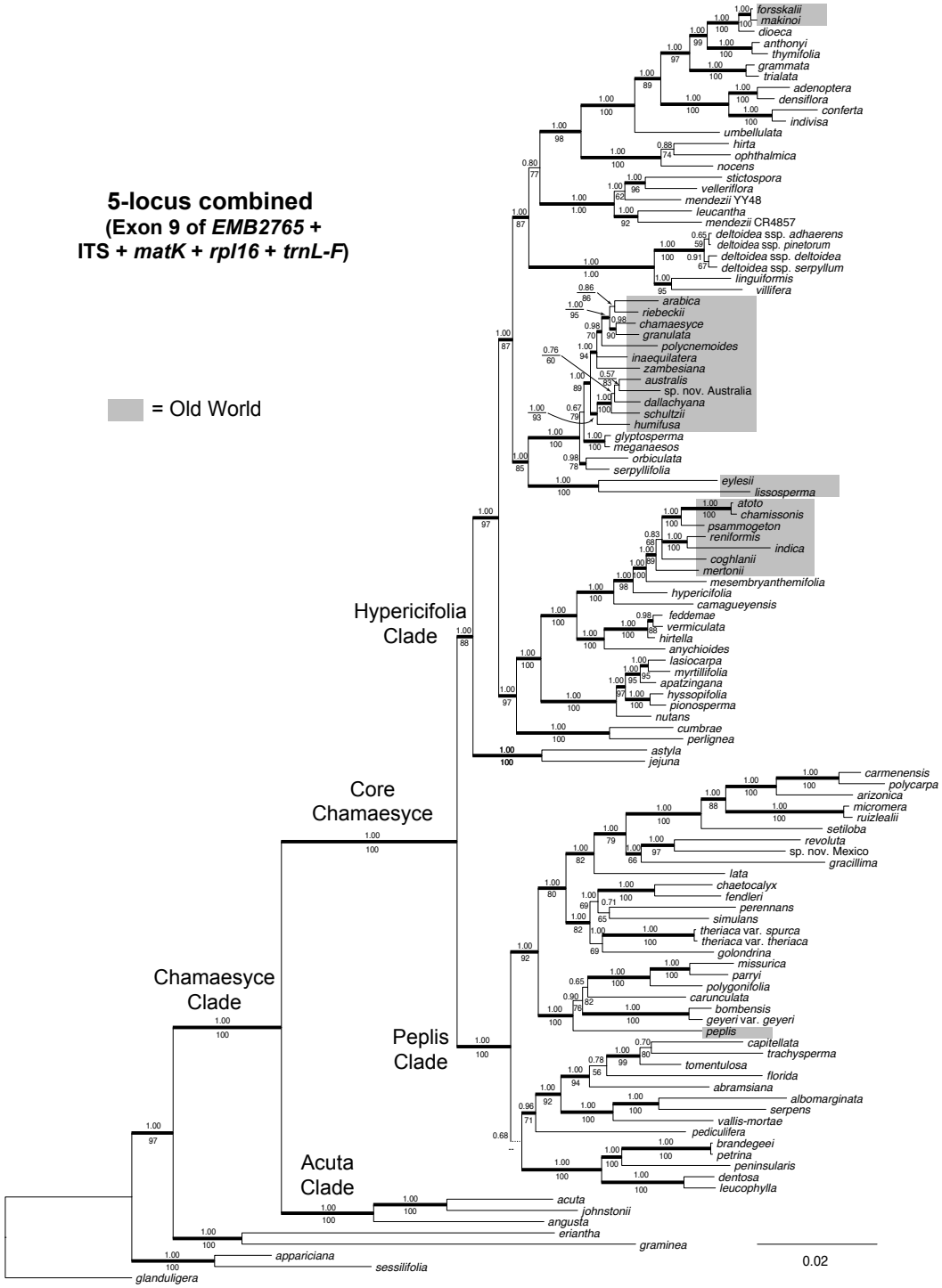


Figure 3.5. Majority rule consensus tree recovered from Bayesian analyses of the 5-locus dataset (exon 9 of *EMB2765* + ITS + *matK* + *rpl16* + *trnL-F*), with putative hybrid taxa removed (see Appendix 3.2). Numbers above the branches indicate Bayesian posterior probabilities (PP) and numbers below the branches are Maximum Likelihood bootstrap percentages (MLB). Thick branches indicate Bayesian posterior probabilities (PP) ≥ 0.95 and Maximum Likelihood bootstrap percentages (MLB) ≥ 0.70%, and branches in dashed lines have Bayesian PP < 0.80 and MLB < 50%. Branch length scale on lower right.

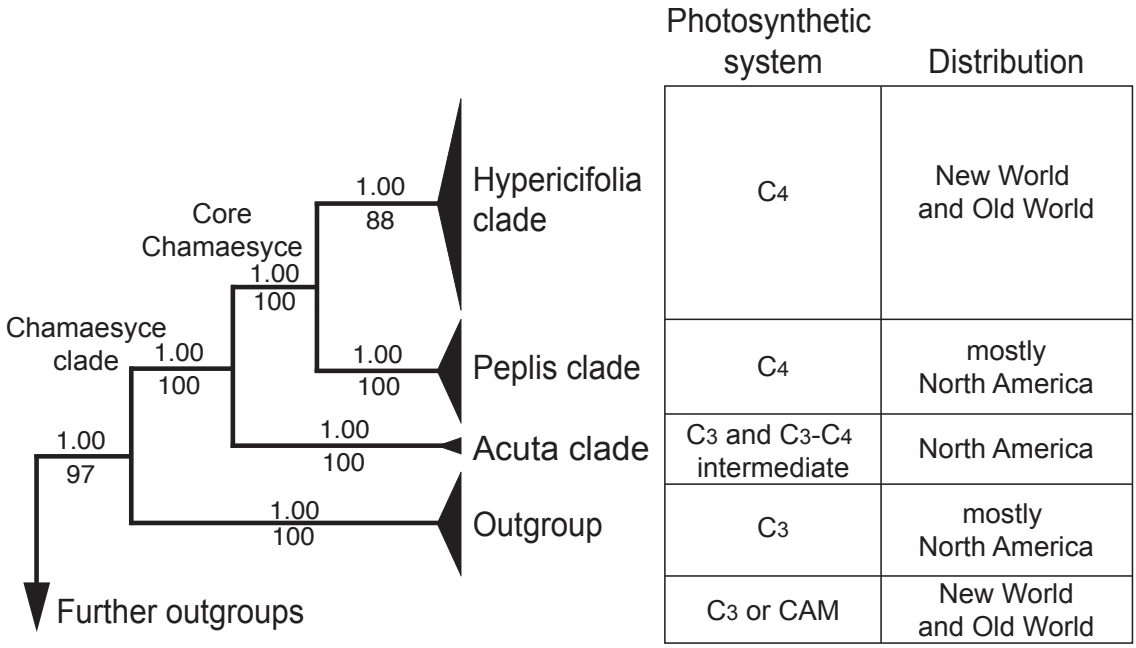


Figure 3.6. Summary of the three major subclades within the Chamaesyce clade recovered from results of the 5-locus dataset (exon 9 of *EMB2765* + ITS + *matK* + *rpl16* + *trnL-F*), with corresponding photosynthetic systems and geographical distributions indicated. Numbers above the branches indicate Bayesian posterior probabilities and numbers below the branches are Maximum Likelihood bootstrap percentages. Photosynthetic systems are from Webster et al. (1975) and Sage et al. (2011a); closely related outgroups follow Steinmann and Porter (2002).

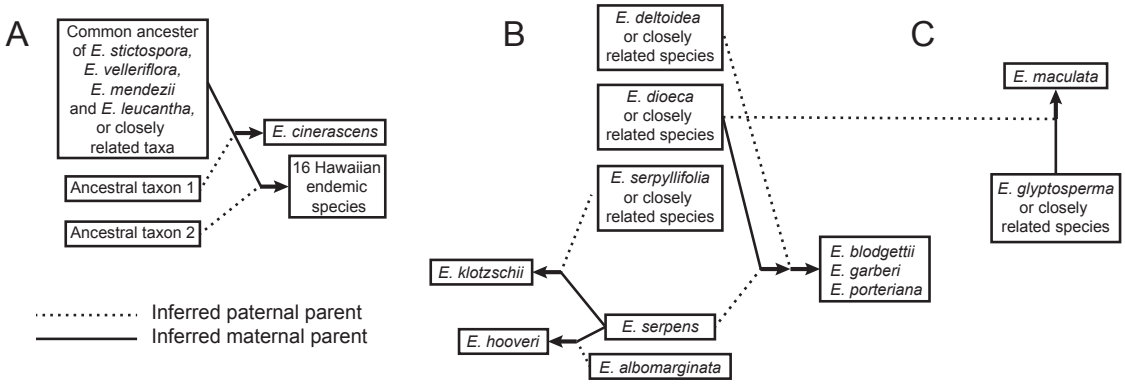


Figure 3.7. Hypothetical hybrid relationships inferred from the ITS, cpDNA, and 5-locus datasets. Arrows go from putative parents towards derived hybrid taxa. The inferred paternal parent is indicated by dotted lines, and maternal parent by solid lines. A. Endemic Hawaiian Chamaesyce and proposed New World progenitors. B. *E. serpens* species complex. C. *E. maculata.*

Appendix 3.1. Voucher and GenBank accession numbers for plant materials used in this study. Abbreviations: BISH = Bishop Museum; BRI = Queensland Herbarium, Australia; CORD = Universidad Nacional de Córdoba Herbarium, Argentina; DAV = University of California, Davis Herbarium; HAW = University of Hawai'i Herbarium; IEB = Instituto de Ecología, Pátzcuaro, Mexico; LSU = Louisiana State University Herbarium; MICH = University of Michigan Herbarium; MO = Missouri Botanical Garden Herbarium; PRE = South African National Biodiversity Institute Herbarium, South Africa; SD = San Diego Natural History Museum; SP = Instituto de Botânica, Brazil; SRSC = Sul Ross State University Herbarium.

Taxon, collection locality, collection number (herbarium), GenBank accession: ITS, exon 9 of *EMB2765*, *rpl16* intron, *trnL-F* spacer, *matK*.

Ingroup—***Euphorbia abramsiana*** L.C.Wheeler, Mexico: Sonora, *T. Van Devender2006-644* (MICH), HQ645217, HQ650889, HQ645369, HQ645523, HQ645673; ***Euphorbia acuta*** Engelm., USA: Texas, *Y. Yang 19* (MICH), –, [HQ650891 (clone 1), HQ650890 (clone 2)], –, –, –; ***Euphorbia acuta*** Engelm., USA: Texas, *Y. Yang 23* (MICH), HQ645218, [HQ650893 (clone 1), HQ650892 (clone 2)], HQ645370, HQ645524, HQ645674; ***Euphorbia adenoptera*** Bertol., Dominican Republic, *B. van Ee 636* (MICH), HQ645219, HQ650894, HQ645371, HQ645525, HQ645675; ***Euphorbia albomarginata*** Torr. & A.Gray, USA: California, *P.E. Berry 8025* (MICH), HQ645220, HQ650895, HQ645372, HQ645526, HQ645676; ***Euphorbia albomarginata*** Torr. & A.Gray, Mexico: Sonora, *A.L. Reina-G. 2006-389* (MICH), HQ645221, [HQ650897 (clone 1), HQ650896 (clone 2)], HQ645373, HQ645527, HQ645677; ***Euphorbia angusta*** Engelm., USA: Texas, *Y. Yang 41* (MICH), HQ645222, HQ650898, HQ645374, HQ645528, HQ645678; ***Euphorbia anthonyi*** Brandegee, Mexico, *R. Moran 5917* (SD), HQ645223, –, HQ645375, HQ645529, HQ645679; ***Euphorbia anychioides*** Boiss., Mexico, *Y. Yang 107* (MICH), HQ645224, HQ650899, HQ645376, HQ645530, HQ645680; ***Euphorbia apatzingana*** McVaugh, Mexico, *Y. Yang 89* (MICH), HQ645225, HQ650900, HQ645377, HQ645531, HQ645681; ***Euphorbia arabica*** Hochst. & Steud. ex Anderson, Ethiopia, *M. Gilbert 168* (MO), HQ645227, –, HQ645379, HQ645533, HQ645683; ***Euphorbia arizonica*** Engelm., USA: Texas, *Y. Yang 31* (MICH), HQ645228, HQ650901, HQ645380, HQ645534, HQ645684; ***Euphorbia articulata*** Burm., N/A, AF537446 (downloaded from GenBank), –, –, –, –; ***Euphorbia astyla*** Engelm. ex Boiss., USA: Texas, *B.H. Warnock 20328* (SRSC), HQ645229, HQ650902, HQ645381, HQ645535, HQ645685; ***Euphorbia atoto*** G.Forst., New Hebrides, *G.L. Webster 19361* (DAV), HQ645230, HQ650903, HQ645382, HQ645536, HQ645686; ***Euphorbia australis*** Boiss., Australia, *D. Halford Q9233a* (BRI), HQ645231, HQ650904, HQ645383, HQ645537, HQ645687; ***Euphorbia berteroana*** Balb. ex Spreng., Argentina, *B. van Ee 647* (MICH), HQ645232, –, HQ645384, HQ645538, HQ645688; ***Euphorbia blodgettii*** Engelm. ex Hitchc., USA: Florida, *Y. Yang* 138 (MICH), HQ645233, [HQ650905 (clone 1), HQ650906 (clone 2)], HQ645385, HQ645539, HQ645689; ***Euphorbia bombensis*** Jacq., USA: Florida, *Y. Yang 177* (MICH), HQ645234, HQ650907, HQ645386, HQ645540, HQ645690; ***Euphorbia brandegeei*** Millsp., Mexico, *B. van Ee 706* (MICH), HQ645235, HQ650908, HQ645387, HQ645541, HQ645691; ***Euphorbia camagueyensis*** (Millsp.) Urb., Cuba, *J. Gutierrez. HAJB 81994* (MICH),

HQ645236, HQ650909, HQ645388, HQ645542, HQ645692; ***Euphorbia capitellata*** Engelm., Mexico: Sonora, *A.L. Reina-G. 2006-916* (MICH), HQ645237, HQ650910, HQ645389, HQ645543, HQ645693; ***Euphorbia carissoides*** F.M. Bailey, Australia, *K.R. McDonald 5073* (BRI), HQ645239, [HQ650911 (clone 1), HQ650912 (clone 2)], HQ645391, HQ645545, –; ***Euphorbia carmenensis*** N.E.Rose, Mexico: Baja California Sur, *V.W. Steinmann 6450* (MICH), HQ645240, HQ650913, HQ645392, HQ645546, HQ645695; ***Euphorbia carunculata*** Waterf., USA: Texas, *B.H. Warnock 20916* (SRSC), HQ645241, –, HQ645393, HQ645547, HQ645696; ***Euphorbia catamarcensis*** (Croizat) Subils, Argentina, *F.N. Biurrun 4748* (CORD), HQ645242, –, HQ645394, HQ645548, HQ645697; ***Euphorbia celastroides*** **var.** ***kaenana*** Sherff, USA: Hawaii, MMR C-2-60, HQ645243, [HQ650916 (clone 1), HQ650915 (clone 2), HQ650914 (clone 3)], HQ645395, HQ645549, HQ645698; ***Euphorbia chaetocalyx*** (Boiss.) Tidestr., USA: Texas, *Y. Yang 30* (MICH), HQ645244, HQ650917, HQ645396, HQ645550, HQ645699; ***Euphorbia chamaerrhodos*** Boiss., Brazil, da Silva 2945 (SP), HQ645245, –, HQ645397, HQ645551, HQ645700; ***Euphorbia chamaesyce*** L., Greece, Riina, R. 1558 (MICH), HQ645246, HQ650918, HQ645398, HQ645552, HQ645701; ***Euphorbia chamissonis*** (Klotzsch & Garcke) Boiss., Malaysia, *J. Beaman 9736* (DAV), HQ645247, –, HQ645399, HQ645553, HQ645702; ***Euphorbia cinerascens*** Engelm., USA: Texas, *Y. Yang 6* (MICH), HQ645248, [HQ650919 (clone 1), HQ650921 (clone 2), HQ650920 (clone 3)], HQ645400, HQ645554, HQ645703; ***Euphorbia clusiifolia*** Hook. & Arn., USA: Hawaii, *T.J. Motley 1576* (BISH), HQ645249, –, HQ645401, HQ645555, HQ645704; ***Euphorbia coghlanii*** F.M.Bailey, Australia, *D. Halford Q8601* (BRI), HQ645250, –, HQ645402, HQ645556, HQ645705; ***Euphorbia conferta*** (Small) B.E.Sm., USA: Florida, *Y. Yang 162* (MICH), HQ645251, HQ650922, HQ645403, HQ645557, HQ645706; ***Euphorbia cumbrae*** Boiss., Mexico, *Y. Yang 49* (MICH), HQ645252, HQ650923, HQ645404, HQ645558, HQ645707; ***Euphorbia dallachyana*** Baill., Australia, *D. Halford Q8109* (BRI), HQ645261, HQ650930, HQ645413, HQ645567, HQ645716; ***Euphorbia degeneri*** Sherff, USA: Hawaii, *C.W. Morden 1274* (HAW), HQ645253, –, HQ645405, HQ645559, HQ645708; ***Euphorbia deltoidea*** **subsp.** ***adhaerens*** (Small) Oudejans, USA: Florida, *Y. Yang 147* (MICH), HQ645254, HQ650924, HQ645406, HQ645560, HQ645709; ***Euphorbia deltoidea*** **subsp.** ***deltoidea*** Engelm. ex Chapm., USA: Florida, *Y. Yang 159* (MICH), HQ645255, HQ650925, HQ645407, HQ645561, HQ645710; ***Euphorbia deltoidea*** **subsp.** ***pinetorum*** (Small) Oudejans, USA: Florida, *Y. Yang 145* (MICH), HQ645256, HQ650926, HQ645408, HQ645562, HQ645711; ***Euphorbia deltoidea*** **subsp.** ***serpyllum*** (Small) Oudejans, USA: Florida, *Y. Yang 132* (MICH), HQ645257, –, HQ645409, HQ645563, HQ645712; ***Euphorbia densiflora*** (Klotzsch and Garcke) Klotzsch, Mexico: Sonora, *A.L. Reina-G. 2006-149* (MICH), HQ645258, HQ650927, HQ645410, HQ645564, HQ645713; ***Euphorbia dentosa*** I.M.Johnst., Mexico: Baja California Sur, *Y. Yang 204* (MICH), HQ645259, HQ650928, HQ645411, HQ645565, HQ645714; ***Euphorbia dioeca*** Kunth, Mexico, *Y. Yang 102* (MICH), HQ645260, HQ650929, HQ645412, HQ645566, HQ645715; ***Euphorbia eichleri*** Müll.Arg., Argentina, *B. van Ee 671* (MICH), HQ645264, [HQ650933 (clone 1), HQ650934 (clone 2)], HQ645416, HQ645570, HQ645719; ***Euphorbia eylesii*** Rendle, Namibia, *Giess 10005* (PRE), HQ645265, HQ650935, HQ645417, HQ645571, HQ645720; ***Euphorbia feddemae*** McVaugh, Mexico, *Y. Yang 112* (MICH), HQ645266, HQ650936, HQ645418, HQ645572,

HQ645721; ***Euphorbia fendleri*** Torr. & A.Gray, USA: Texas, *Y. Yang 7* (MICH), HQ645267, [HQ650938 (clone 1), HQ650937 (clone 2)], HQ645419, HQ645573, HQ645722; ***Euphorbia florida*** Engelm., Mexico: Sonora, *A.L. Reina-G. 2006-476* (MICH), HQ645268, HQ650939, HQ645420, HQ645574, HQ645723; ***Euphorbia forsskalii*** J.Gay, French Guinea, *J.G. Adam 25916* (MO), HQ645269, –, HQ645421, HQ645575, HQ645724; ***Euphorbia garberi*** Engelm. ex Chapm., USA: Florida, *Y. Yang 164* (MICH), HQ645270, [HQ650940 (clone 1), HQ650941 (clone 2), HQ650942 (clone 3)], HQ645422, HQ645576, HQ645725; ***Euphorbia geyeri* var. *geyeri*** Engelm. & A.Gray, USA: Texas, *B.H. Warnock 20915* (SRSC), HQ645271, HQ650943, HQ645423, HQ645577, HQ645726; ***Euphorbia glyptosperma*** Engelm., USA: Texas, *Y. Yang 35* (MICH), HQ645273, HQ650945, HQ645425, HQ645579, HQ645728; ***Euphorbia golondrina*** L.C. Wheeler, USA: Texas, *Y. Yang 27* (MICH), HQ645274, HQ650946, HQ645426, HQ645580, HQ645729; ***Euphorbia gracillima*** S.Watson, Mexico: Sonora, *A.L.Reina-G. 2006-579* (MICH), HQ645275, HQ650947, HQ645427, HQ645581, HQ645730; ***Euphorbia grammata*** (McVaugh) Oudejans, Mexico, *Y. Ramirez-Amezcua 697* (MICH), HQ645276, HQ650948, HQ645428, HQ645582, HQ645731; ***Euphorbia granulata*** Forssk., Morocco, *R. Riina 1800* (MICH), HQ645277, HQ650949, HQ645429, HQ645583, HQ645732; ***Euphorbia hirta*** L., Mexico: Sonora, *A.L. Reina-G. 2006-470* (MICH), HQ645278, HQ650950, HQ645430, HQ645584, HQ645733; ***Euphorbia hirtella*** Boiss., Argentina, *B. van Ee 621* (MICH), HQ645279, HQ650951, HQ645431, HQ645585, HQ645734; ***Euphorbia hooveri*** Wheeler, USA: California, *P.E. Berry 7761* (MICH), HQ645280, [HQ650952 (clone 1), HQ650953 (clone 2)], HQ645432, HQ645586, HQ645735; ***Euphorbia humifusa*** Willd., Russia, *W. Jin 16* (MICH), HQ645281, HQ650954, HQ645433, HQ645587, HQ645736; ***Euphorbia hypericifolia*** L., USA: Florida, *Y. Yang 128* (MICH), HQ645282, HQ650955, HQ645434, HQ645588, HQ645737; ***Euphorbia hypericifolia*** L., Puerto Rico, *W. Jin 36* (MICH), HQ645353, –, HQ645506, HQ645656, HQ645809; ***Euphorbia hyssopifolia*** L., Mexico: Sonora, *T.R. Van Devender 2006-463* (MICH), HQ645283, HQ650956, HQ645435, HQ645589, HQ645738; ***Euphorbia inaequilatera*** Sond., Tanzania, *J.J. Morawetz 452* (MICH), HQ645284, HQ650957, HQ645436, HQ645590, HQ645739; ***Euphorbia indica*** Lam., Madagascar, *B. van Ee 1025* (MICH), HQ645350, –, HQ645503, HQ645653, HQ645806; ***Euphorbia indica*** Lam., Oman, *J.J. Morawetz 326* (MICH), HQ645352, HQ651029, HQ645505, HQ645655, HQ645808; ***Euphorbia indivisa*** (Engelm.) Tidestr., Mexico: Sonora, *T.R. Van Devender 2006-723* (MICH), HQ645285, HQ650958, HQ645437, HQ645591, HQ645740; ***Euphorbia jejuna*** M.C.Johnst. & Warnock, USA: Texas, *B.L. Turner 24-416* (SRSC), HQ645286, –, HQ645438, –, HQ645741; ***Euphorbia johnstonii*** Mayfield, Mexico, *R.F. Sage s.n.* (MICH), HQ645287, [HQ650959 (clone 1), HQ650960 (clone 2)], HQ645439, HQ645592, HQ645742; ***Euphorbia klotzschii*** Oudejans, Argentina, *B. van Ee 619* (MICH), HQ645314, [HQ650996 (clone 1), HQ650995 (clone 2)], HQ645467, HQ645620, HQ645770; ***Euphorbia kuwaluana*** O.Deg. & Sherff, USA: Hawaii, *C.W. Morden 2222* (HAW), HQ645288, [HQ650962 (clone 1), HQ650961 (clone 2), HQ650963 (clone 3)], HQ645440, HQ645593, HQ645743; ***Euphorbia lasiocarpa*** Klotzsch, Jamaica, *B. van Ee 764* (MICH), HQ645289, HQ650964, HQ645441, HQ645594, HQ645744; ***Euphorbia lata*** Engelm., USA: Texas, *Y. Yang 13* (MICH), HQ645290, HQ650965, HQ645442, HQ645595, HQ645745; ***Euphorbia leucantha*** (Klotzsch & Garcke) Boiss., Mexico, *Y. Yang 98*

(MICH), HQ645291, HQ650966, HQ645443, HQ645596, HQ645746; ***Euphorbia leucophylla*** Benth., Mexico: Baja California Sur, *V.W. Steinmann 6437* (MICH), –, HQ650967, HQ645444, HQ645597, HQ645747; ***Euphorbia linguiformis*** McVaugh, Mexico, *Y. Yang 97* (MICH), HQ645292, HQ650968, HQ645445, HQ645598, HQ645748; ***Euphorbia lissosperma*** S.Carter, Kenya, *R.B. Faden 74/778* (MO), HQ645293, –, HQ645446, HQ645599, HQ645749; ***Euphorbia maculata*** L., USA: Michigan, *P.E. Berry 7762* (MICH), HQ645294, [HQ650970 (clone 1), HQ650969 (clone 2)], HQ645447, HQ645600, HQ645750; ***Euphorbia magdalenae*** Benth., Mexico, Dominguez L., M. 1476 (IEB), HQ645295, –, HQ645448, HQ645601, HQ645751; ***Euphorbia makinoi*** Hayata, Taiwan, *C. Lin 690* (MO), HQ645296, HQ650971, HQ645449, HQ645602, HQ645752; ***Euphorbia meganaesos*** Featherman, USA: Louisiana, *R. Neyland 1092* (LSU), HQ645297, –, HQ645450, HQ645603, HQ645753; ***Euphorbia mendezii*** Boiss., Mexico, *Y. Yang 48* (MICH), HQ645298, HQ650972, HQ645451, HQ645604, HQ645754; ***Euphorbia mendezii*** Boiss., Mexico, *P. Carrillo-Reyes 4857* (IEB), HQ645299, HQ650973, HQ645452, HQ645605, HQ645755; ***Euphorbia mertonii*** Fosberg, Seychelles, *D. Potter 920501-04* (DAV), HQ645300, HQ650974, HQ645453, HQ645606, HQ645756; ***Euphorbia mertonii*** Fosberg, Madagascar, *B. van Ee 1086* (MICH), HQ645351, –, HQ645504, HQ645654, HQ645807; ***Euphorbia mesembryanthemifolia*** Jacq., USA: Florida, *Y. Yang 136* (MICH), HQ645301, HQ650975, HQ645454, HQ645607, HQ645757; ***Euphorbia micromera*** Boiss., USA: Texas, *Y. Yang 36* (MICH), HQ645302, HQ650976, HQ645455, HQ645608, HQ645758; ***Euphorbia missurica*** Raf., USA: Texas, *Y. Yang 29* (MICH), HQ645303, HQ650977, HQ645456, HQ645609, HQ645759; ***Euphorbia mossambicensis*** (Klotzsch & Garcke) Boiss., South Africa, *R. Becker 1338* (MICH), HQ645304, [HQ650980 (clone 1), HQ650978 (clone 2), HQ650979 (clone 3)], HQ645457, HQ645610, HQ645760; ***Euphorbia multiformis* var. *microphylla*** Boiss., USA: Hawaii, *M.J. Spork s.n.*, HQ645305, [HQ650981 (clone 1), HQ650982 (clone 2)], HQ645458, HQ645611, HQ645761; ***Euphorbia myrtillifolia*** L., Jamaica, *B. van Ee 754* (MICH), HQ645306, –, HQ645459, HQ645612, HQ645762; ***Euphorbia neopolycnemoides*** Pax & K.Hoffm., South Africa, *R. Becker 1339* (MICH), HQ645307, [HQ650984 (clone 1), HQ650983 (clone 2)], HQ645460, HQ645613, HQ645763; ***Euphorbia nocens*** (L.C.Wheeler) V.W.Steinm., Mexico, *Y. Yang 43* (MICH), HQ645308, [HQ650986 (clone 1), HQ650985 (clone 2)], HQ645461, HQ645614, HQ645764; ***Euphorbia nutans*** Lag., USA: Michigan, *P.E. Berry 7763* (MICH), HQ645309, HQ650987, HQ645462, HQ645615, HQ645765; ***Euphorbia olowaluana*** Sherff, USA: Hawaii, *M.J. Spork s.n.*, HQ645310, [HQ650988 (clone 1), HQ650990 (clone 2), HQ650989 (clone 3)], HQ645463, HQ645616, HQ645766; ***Euphorbia ophthalmica*** Pers., Mexico, *Y. Yang 101* (MICH), HQ645311, HQ650991, HQ645464, HQ645617, HQ645767; ***Euphorbia oranensis*** (Croizat) Subils, Argentina, *B. van Ee 685* (MICH), HQ645312, [HQ650993 (clone 1), HQ650992 (clone 2)], HQ645465, HQ645618, HQ645768; ***Euphorbia orbiculata*** Kunth, Colombia, *R. Riina 1589* (MICH), HQ645313, HQ650994, HQ645466, HQ645619, HQ645769; ***Euphorbia parryi*** Engelm., USA: Texas, *B.H. Warnock 18715* (SRSC), HQ645315, –, HQ645468, HQ645621, HQ645771; ***Euphorbia pediculifera*** Engelm., Mexico: Sonora, *T.R. Van Devender 2006-938* (MICH), HQ645317, HQ650997, HQ645470, HQ645623, HQ645773; ***Euphorbia peninsularis*** I.M.Johnst., Mexico: Baja California Sur, *Y. Yang 201* (MICH), HQ645318,

HQ650998, HQ645471, HQ645624, HQ645774; ***Euphorbia peplis*** L., Greece, *R. Riina 1566* (MICH), HQ645319, [HQ650999 (clone 1), HQ651000 (clone 2)], HQ645472, HQ645625, HQ645775; ***Euphorbia perennans*** (Shinners) Warnock & M.C.Johnst., USA: Texas, *Y. Yang 3* (MICH), HQ645320, HQ651001, HQ645473, HQ645626, HQ645776; ***Euphorbia perlignea*** McVaugh, Mexico, *V.W. Steinmann 3045* (MICH), HQ645321, HQ651002, HQ645474, HQ645627, HQ645777; ***Euphorbia petrina*** S.Watson, Mexico: Sonora, *A.L. Reina-G. 2006-1403* (MICH), HQ645322, –, HQ645475, HQ645628, HQ645778; ***Euphorbia pionosperma*** V.W.Steinm. & Felger, Mexico, *V.W. Steinmann 1006* (IEB), HQ645323, HQ651003, HQ645476, HQ645629, HQ645779; ***Euphorbia polycarpa*** Benth., Mexico: Sonora, *T.R. Van Devender 2006-551* (MICH), HQ645325, HQ651004, HQ645478, HQ645630, HQ645781; ***Euphorbia polycnemoides*** Hochst. ex Boiss., Malawi, *J. Pawek 12716* (MO), HQ645324, –, HQ645477, –, HQ645780; ***Euphorbia polygonifolia*** L., Canada, *P.E. Berry 7765* (MICH), HQ645326, HQ651005, HQ645479, HQ645631, HQ645782; ***Euphorbia porteriana*** (Small) Oudejans, USA: Florida, *Y. Yang 131* (MICH), HQ645327, [HQ651006 (clone 1), HQ651007 (clone 2)], HQ645480, HQ645632, HQ645783; ***Euphorbia potentilloides*** Boiss., Argentina, *G. Ocampo 1557* (IEB), HQ645328, [HQ651008 (clone 1), HQ651009 (clone 2)], HQ645481, HQ645633, HQ645784; ***Euphorbia prostrata*** Aiton, Mexico: Sonora, *A.L. Reina-G. 2006-473* (MICH), HQ645329, HQ651010, HQ645482, HQ645634, HQ645785; ***Euphorbia psammogeton*** P.S.Green, Australia, *D. Halford Q8340a* (BRI), HQ645330, HQ651011, HQ645483, HQ645635, HQ645786; ***Euphorbia remyi*** A.Gray ex Boiss, USA: Hawaii, *C.W. Morden 1365* (HAW), HQ645331, –, HQ645484, HQ645636, HQ645787; ***Euphorbia reniformis*** Blume, Thailand, *H-J. Esser 08-03* (MICH), HQ645332, HQ651012, HQ645485, HQ645637, HQ645788; ***Euphorbia revoluta*** Engelm., Mexico: Sonora, *A.L. Reina-G. 2006-661* (MICH), HQ645333, HQ651013, HQ645486, HQ645638, HQ645789; ***Euphorbia riebeckii*** Pax, Oman, *J.J. Morawetz 361a* (MICH), HQ645334, HQ651014, HQ645487, HQ645639, HQ645790; ***Euphorbia rockii*** C.N.Forbes, USA: Hawaii, *T.J. Motley 1699* (BISH), HQ645335, –, HQ645488, HQ645640, HQ645791; ***Euphorbia ruizlealii*** Subils, Argentina, *B. van Ee 675* (MICH), HQ645336, HQ651015, HQ645489, HQ645641, HQ645792; ***Euphorbia schizolepis*** F.Muell. ex Boiss., Australia, *B. Wannan 2640* (BRI), HQ645337, [HQ651017 (clone 1), HQ651016 (clone 2)], HQ645490, HQ645642, HQ645793; ***Euphorbia schultzii*** Benth., Australia, *D. Halford Q9220a* (BRI), HQ645238, –, HQ645390, HQ645544, HQ645694; ***Euphorbia schultzii*** Benth., Australia, *I.D. Cowie 5234* (BRI), HQ645338, HQ651018, HQ645491, HQ645643, HQ645794; ***Euphorbia selloi*** (Klotzsch & Garcke) Boiss., Argentina, *G. Ocampo 1558* (IEB), HQ645339, [HQ651020 (clone 1), HQ651019 (clone 2)], HQ645492, HQ645644, HQ645795; ***Euphorbia serpens*** Kunth, Greece, *R. Riina 1557* (MICH), HQ645340, HQ651021, HQ645493, HQ645645, HQ645796; ***Euphorbia serpens*** Kunth, USA: Texas, *Y. Yang 14* (MICH), HQ645341, HQ651022, HQ645494, HQ645646, HQ645797; ***Euphorbia serpyllifolia*** Pers., Mexico, *Y. Yang 46* (MICH), HQ645342, HQ651023, HQ645495, HQ645647, HQ645798; ***Euphorbia serrula*** Engelm., Mexico: Sonora, *T.R. Van Devender 2006-406* (MICH), HQ645343, [HQ651025 (clone 1), HQ651024 (clone 2)], HQ645496, HQ645648, HQ645799; ***Euphorbia setiloba*** Engelm. ex Torr., Mexico: Sonora, *A.L. Reina-G. 2006-478* (MICH), HQ645345, HQ651026, HQ645498, HQ645650, HQ645801; ***Euphorbia setosa*** (Boiss.) Müll.Arg., Brazil, *I. Cordeiro 3025*

(MICH), HQ645346, –, HQ645499, HQ645651, HQ645802; ***Euphorbia simulans*** (L.C.Wheeler) Warnock & M.C.Johnst., USA: Texas, *Y. Yang 2* (MICH), HQ645347, HQ651027, HQ645500, HQ645652, HQ645803; ***Euphorbia* sp. nov.** Australia, Australia, *R. Booth 3536* (BRI), HQ645348, –, HQ645501, –, HQ645804; ***Euphorbia* sp. nov.** Mexico, Mexico, *V.W. Steinmann 1007* (IEB), HQ645349, HQ651028, HQ645502, –, HQ645805; ***Euphorbia stictospora*** Engelm., USA: Texas, *Y. Yang 24* (MICH), HQ645355, HQ651031, HQ645508, HQ645658, HQ645811; ***Euphorbia tamanduana*** Boiss., Brazil, *M. Caruzo 136* (MICH), –, [HQ651032 (clone 1), HQ61033 (clone 2), HQ651034 (clone 3)], HQ645509, HQ645659, HQ645812; ***Euphorbia tettensis*** Klotzsch, South Africa, *N. Zambatis 2024* (PRE), HQ645356, [HQ651035 (clone 1), HQ61036 (clone 2), HQ651037 (clone 3)], HQ645510, HQ645660, HQ645813; ***Euphorbia theriaca*** L.C. Wheeler, USA: Texas, *A.M. Powell 6349* (SRSC), HQ645357, HQ651038, HQ645511, HQ645661, HQ645814; ***Euphorbia theriaca* var. *spurca*** M.C.Johnst., USA: Texas, *Y. Yang 37* (MICH), HQ645354, HQ651030, HQ645507, HQ645657, HQ645810; ***Euphorbia thymifolia*** L., Mexico: Sonora, *T.R. Van Devender 2006-628* (MICH), HQ645358, HQ651039, HQ645512, HQ645662, HQ645815; ***Euphorbia tomentulosa*** S.Watson, Mexico: Baja California Sur, *Y. Yang 196* (MICH), HQ645359, HQ651040, HQ645513, HQ645663, HQ645816; ***Euphorbia trachysperma*** Engelm., Mexico: Sonora, *T.R. Van Devender 2007-688* (MICH), HQ645360, –, HQ645514, HQ645664, HQ645817; ***Euphorbia trialata*** (Huft) V.W.Steinm., Mexico, *Y. Yang 88* (MICH), HQ645361, HQ651041, HQ645515, HQ645665, HQ645818; ***Euphorbia turpinii*** Boiss., Dominican Republic, *B. van Ee 643* (MICH), HQ645362, [HQ651042 (clone 1), HQ651043 (clone 2)], HQ645516, HQ645666, HQ645819; ***Euphorbia umbellulata*** Engelm. ex Boiss., Mexico, *Y. Yang 99* (MICH), HQ645363, HQ651044, HQ645517, HQ645667, HQ645820; ***Euphorbia vallis-mortae*** (Millsp.) J.T.Howell, USA: California, *P.E. Berry 8027* (MICH), HQ645364, HQ651045, HQ645518, HQ645668, HQ645821; ***Euphorbia velleriflora*** (Klotzsch & Garcke) Boiss., Mexico: Sonora, *T.R. Van Devender 2006-513* (MICH), HQ645365, HQ651046, HQ645519, HQ645669, HQ645822; ***Euphorbia vermiculata*** Raf., Canada, *M.J. Oldham 20515* (MICH), HQ645366, HQ651047, HQ645520, HQ645670, HQ645823; ***Euphorbia villifera*** Scheele, USA: Texas, *Y. Yang 26* (MICH), HQ645367, HQ651048, HQ645521, HQ645671, HQ645824; ***Euphorbia zambesiana*** Benth., Tanzania, *J.C. Lovett 4703* (MO), HQ645368, HQ651049, HQ645522, HQ645672, HQ645825.

Outgroup—***Euphorbia appariciana*** Rizzini, Brazil, *M. Caruzo 138* (MICH), HQ645226, –, HQ645378, HQ645532, HQ645682; ***Euphorbia dentata*** Michx., USA: Texas, *Y. Yang 40* (MICH), HQ645316, –, HQ645469, HQ645622, HQ645772; ***Euphorbia eriantha*** Benth., USA: Texas, *Y. Yang 1* (MICH), HQ645262, HQ650931, HQ645414, HQ645568, HQ645717; ***Euphorbia glanduligera*** Pax, Angola, *P.V. Bruyns 10692* (MICH), HQ645272, HQ650944, HQ645424, HQ645578, HQ645727; ***Euphorbia graminea*** Jacq., Mexico, *V.W. Steinmann 5818* (MICH), HQ645263, HQ650932, HQ645415, HQ645569, HQ645718; ***Euphorbia sessilifolia*** Klotzsch ex Boiss., Brazil, *M. Caruzo 133* (MICH), HQ645344, –, HQ645497, HQ645649, HQ645800.

Appendix 3.2. Presumably hybrid taxa that were excluded from the 5-locus dataset, inferred from divergent copies of *EMB2765* exon 9 or divergent placement between ITS and cpDNA phylogenies.

Euphorbia berteroana, E. blodgettii, E. carissoides, E. catamarcensis, E. celastroides var. kaenana, E. chamaerrhodos, E. cinerascens, E. clusiifolia, E. degeneri, E. dentata, E. eichleri, E. garberi, E. hooveri, E. klotzschii, E. kuwaluana, E. maculata, E. magdalenae, E. mossambicensis, E. multiformis var. microphylla, E. neopolycnemoides, E. olowaluana, E. oranensis, E. porteriana, E. potentilloides, E. prostrata, E. remyi, E. rockii, E. schizolepis, E. schultzii, E. selloi, E. serrula, E. setosa, E. tamanduana, E. tettensis, E. turpinii.

LITERATURE CITED

ALVAREZ, I., AND J. F. WENDEL. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417-434.

BALDWIN, B. G., AND W. L. WAGNER. 2010. Hawaiian angiosperm radiations of North American origin. *Annals of Botany* 105: 849-879.

BALLARD, H. E., AND K. J. SYTSMA. 2000. Evolution and biogeography of the woody Hawaiian violets (*Viola*, Violaceae): Arctic origins, herbaceous ancestry and bird dispersal. *Evolution* 54: 1521-1532.

BARRIER, M., B. G. BALDWIN, R. H. ROBICHAUX, AND M. D. PURUGGANAN. 1999. Interspecific hybrid ancestry of a plant adaptive radiation: Allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotic gene duplications. *Molecular Biology and Evolution* 16: 1105-1113.

BATANOUNY, K., W. STICHLER, AND H. ZIEGLER. 1991. Photosynthetic pathways and ecological distribution of *Euphorbia* species in Egypt. *Oecologia* 87: 565-569.

BOISSIER, E. 1862. *Euphorbia* sect. *Anisophyllum*. *In* A. P. d. Candolle [ed.], Prodromus systematis naturalis regni vegetabilis, vol. 15, part 2, 11-52. Victor Masson & Fils, Paris, France.

BROWN, J. M., S. M. HEDTKE, A. R. LEMMON, AND E. M. LEMMON. 2010. When trees grow too long: Investigating the causes of highly inaccurate Bayesian branch-length estimates. *Systematic Biology* 59: 145-161.

BRUYNS, P. V., R. J. MAPAYA, AND T. HEDDERSON. 2006. A new subgeneric classification for *Euphorbia* (Euphorbiaceae) in southern Africa based on ITS and *psbA-trnH* sequence data. *Taxon* 55: 397-420.

BURCH, D. 1965. A taxonomic revision of the genus *Chamaesyce* (Euphorbiaceae) in the Caribbean. Ph.D. dessertation, University of Florida, Gainesville, Florida, USA.

BURCH, D. 1969. Notes on the Galapagos Euphorbieae (Euphorbiaceae). *Annals of the Missouri Botanical Garden* 56: 173-178.

CARLQUIST, S. 1966. The biota of long-distance dispersal. III. Loss of dispersibility in the Hawaiian flora. *Brittonia* 18: 310-335.

CARR, G. D. 1985. Additional chromosome numbers of Hawaiian flowering plants. Pacific Science 39: 302-306.

CARR, G. D. 1998. Chromosome evolution and speciation in Hawaiian flowering plants. *In*: T. F. Stuessy, M. Ono [eds.], Evolution and speciation of island plants, pp. 5–47. Cambridge University Press, Cambridge, UK.

CHRISTIN, P.-A., R. P. FRECKLETON, AND C. P. OSBORNE. 2010. Can phylogenetics identify C_4 origins and reversals? *Trends in Ecology & Evolution* 25: 403-409.

COMAI, L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* 6: 836-846.

CRONN, R., M. CEDRONI, T. HASELKORN, C. GROVER, AND J. F. WENDEL. 2002. PCR-mediated recombination in amplification products derived from polyploid cotton. *Theoretical and Applied Genetics* 104: 482-489.

DEGENER, O., AND L. CROIZAT. 1938. *Chamaesyce*. *In* O. Degener [ed.], Flora Hawaiiensis. Published privately.

EBRAHIMZADEH, H., V. NIKNAM, AND A. A. MAASSOUMI. 2000. Mucilage content and its sugar composition in *Astragalus* species from Iran. *Pakistan Journal of Botany* 32: 131-140.

EDGAR, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797.

GUTTERMAN, Y., AND S. SHEM-TOV. 1997. Mucilaginous seed coat structure of *Carrichtera annua* and *Anastatica hierochuntica* from the Negev Desert highlands of Israel, and its adhesion to the soil crust. *Journal of Arid Environments* 35: 695-705.

HANS, A. S. 1973. Chromosomal conspectus of the Euphorbiaceae. *Taxon* 22: 591-636.

HASSALL, D. C. 1976. Numerical and cytotaxonomic evidence for generic delimitation in Australian Euphorbiaceae. *Australian Journal of Botany* 24: 633-640.

HASSALL, D. C. 1977. Systematic studies in Australian Euphorbieae (Euphorbiaceae). Ph.D. dissertation, University of Queensland, St. Lucia, Queensland, Australia.

HAYDEN, W. J. 1988. Ontogeny of the cotyledonary region of *Chamaesyce maculata* (Euphorbiaceae). *American Journal of Botany* 75: 1701-1713.

HERBST, D. 1971. Disjunct foliar veins in Hawaiian Euphorbias. *Science* 171: 1247-1248.

HERBST, D. 1972. Ontogeny of foliar venation in *Euphorbia forbesii*. *American Journal of Botany* 59: 843-850.

HICKMAN, J. C. 1993. The Jepson Manual: higher plants of California. University of California Press, Berkeley, California, USA.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

JORDAN, M. S., AND W. J. HAYDEN. 1992. A survey of mucilaginous testa in *Chamaesyce*. *Collectanea Botanica (Barcelona)* 21: 79-89.

JORDAN, W. C., M. W. COURTNEY, AND J. E. NEIGEL. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). *American Journal of Botany* 83: 430-439.

KELCHNER, S. A., AND L. G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution* 8: 385-397.

KIM, S. T., AND M. J. DONOGHUE. 2008. Molecular phylogeny of *Persicaria* (Persicarieae, Polygonaceae). *Systematic Botany* 33: 77-86.

KOUTNIK, D. L. 1982. A taxonomic revision of the Hawaiian species of the genus *Chamaesyce* (Euphorbiaceae). Ph.D. dissertation, Univ. California, Davis.

KOUTNIK, D. L. 1987. A taxonomic revision of the Hawaiian species of the genus *Chamaesyce* (Euphorbiaceae). *Allertonia* 4: 331-388.

LINDQVIST, C., AND V. A. ALBERT. 2002. Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany* 89: 1709-1724.

LORENCE, D. H., AND W. L. WAGNER. 1996. A new species of *Chamaesyce* section *Sclerophyllae* (Euphorbiaceae) from Kaua'i. *Novon* 6: 68-71.

MADDISON, D. R., AND W. P. MADDISON. 2005. MacClade 4.08: an analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MARSHALL, D. C. 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: Lost in the land of long trees. *Systematic Biology* 59: 108-117.

MAYFIELD, M. H. 1991. *Euphorbia johnstonii* (Euphorbiaceae), a new species from Tamaulipas, Mexico, with notes on *Euphorbia* subsection *Acutae*. *SIDA Contributions to Botany* 14: 573-579.

MORDEN, C. W., V. C. CARAWAY, AND T. J. MOTLEY. 1996. Development of a DNA library for native Hawaiian plants. *Pacific Science* 50: 324-335.

MOTLEY, T. J., AND L. RAZ. 2004. Origins, evolution, and systematics of Hawaiian and Pacific *Chamaesyce* (Euphorbiaceae). Botany 2004: Annual Meeting of the Botanical Society of America in Salt Lake City, Utah, USA [online abstract, http://www.2004.botanyconference.org/engine/search/index.php?func=detail&aid=360].

MÜLLER, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular Phylogenetics and Evolution* 38: 667-676.

NYLANDER, J. A. A. 2004. MrModeltest, version 2.3 for Mac. Program distributed by the author, website: http://www.abc.se/~nylander/ [accessed 04 January 2010].

PARK, K. R., AND R. K. JANSEN. 2007. A phylogeny of Euphorbieae subtribe Euphorbiinae (Euphorbiaceae) based on molecular data. *Journal of Plant Biology* 50: 644-649.

PEARCY, R. W., AND J. TROUGHTON. 1975. C_4 photosynthesis in tree form *Euphorbia* species from Hawaiian rainforest sites. *Plant Physiology* 55: 1054-1056.

PENFIELD, S., R. C. MEISSNER, D. A. SHOUE, N. C. CARPITA, AND M. W. BEVAN. 2001. *MYB61* is required for mucilage deposition and extrusion in the *Arabidopsis* seed coat. *Plant Cell* 13: 2777-2791.

PERRY, B. A. 1943. Chromosome number and phylogenetic relationships in the Euphorbiaceae. *American Journal of Botany* 30: 527-543.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

POSADA, D., AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793-808.

PRENNER, G., AND P. J. RUDALL. 2007. Comparative ontogeny of the cyathium in *Euphorbia* (Euphorbiaceae) and its allies: exploring the organ flower inflorescence boundary. *Am. J. Bot.* 94: 1612-1629.

RAMBAUT, A., AND A. DRUMMOND. 2007. Tracer v1.4. Computer program and documentation distributed by the author, website: http://beast.bio.ed.ac.uk/Tracer [accessed 14 Jul 2010].

RANDELL, R. A., AND C. W. MORDEN. 1999. Hawaiian Plant DNA library II: endemic, indigenous, and introduced species. *Pacific Science* 53: 401-417.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

SAGE, R. F., P.-A. CHRISTIN AND E. J. EDWARDS. 2011a. The C_4 plant lineages of planet Earth. *Journal of Experimental Botany*. Advance access published online March 16, 2011, pp. 1-15, doi:10.1093/jxb/err048.

SAGE, T. L., R. F. SAGE, P. J. VOGAN, B. RAHMAN, D. C. JOHNSON, J. C. OAKLEY, AND M. A. HECKEL. 2011b. The occurrence of C_2 photosynthesis in *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae). *Journal of Experimental Botany*. Advance access published online March 31, 2011, pp. 1-15, doi:10.1093/jxb/err059.

SAMUEL, R., H. KATHRIARACHCHI, P. HOFFMANN, M. H. J. BARFUSS, K. J. WURDACK, C. C. DAVIS, AND M. W. CHASE. 2005. Molecular phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* 92: 132-141.

SHAW, J., E. B. LICKEY, E. E. SCHILLING, AND R. L. SMALL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94: 275-288.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

SPORCK, M. J., AND L. SACK. 2010. Adaptive radiation of leaf venation and the meaning of disjunct veins in the C_4 Hawaiian *Euphorbia*. Botany 2010: Annual Meeting of the Botanical Society of America in Providence, Rhode Island, USA [online abstract, website http://2010.botanyconference.org/engine/search/index.php?func=detail&aid=475].

STAMATAKIS, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

STEFANOVIC, S., AND M. COSTEA. 2008. Reticulate evolution in the parasitic genus *Cuscuta* (Convolvulaceae): over and over again. *Botany* 86: 791-808.

STEINMANN, V. W., AND J. M. PORTER. 2002. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* 89: 453-490.

SUZUKI, N., AND S. TERANISHI. 2005. Phenology and life cycle of the annual, *Chamaesyce maculata* (L.) Small (Euphorbiaceae), with multiple overlapping generations in Japan. *Ecological Research* 20: 425-432.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.

URBATSCH, L. E., B. G. BALDWIN, AND M. J. DONOGHUE. 2000. Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chlorplast DNA restriction site data. *Systematic Botany* 25: 539-565.

URBATSCH, L. E., J. D. BACON, R. L. HARTMAN, M. C. JOHNSTON, T. J. WATSON, JR., AND G. L. WEBSTER. 1975. Chromosome numbers for North American Euphorbiaceae. *American Journal of Botany* 62: 494-500.

WAGNER, W. L., D. R. HERBST, AND S. H. SOHMER. 1999. Manual of the flowering plants of Hawai`i, revised edition with supplement by W. L. Wagner and D. R. Herbst, pp. 1855–1918. Bishop Museum Special Publication, vol. 97. University of Hawai'i Press, Honolulu, Hawai'i, USA.

WEBSTER, G. L., W. V. BROWN, AND B. N. SMITH. 1975. Systematics of photosynthetic carbon fixation pathways in *Euphorbia*. *Taxon* 24: 27-33.

WHEELER, L. C. 1941. *Euphorbia* subgenus *Chamaesyce* in Canada and the United States exclusive of Southern Florida. *Rhodora* 43: 97-154, 168-205, 223-286, pls. 654-668.

WHITE, T. J., T. BRUYNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H.

Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications, 315-324. Academic Press, San Diego, California, USA.

WURDACK, K. J., AND C. C. DAVIS. 2009. Malpighiales phylogenetics: Gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. *American Journal of Botany* 96: 1551-1570.

XUE, H. G., S. D. ZHOU, X. J. HE, AND Y. YU. 2007. Karyotype in fifteen populations belonging to thirteen species of *Euphorbia* (Euphorbiaceae) in China. *Acta Phytotaxonomica Sinica* 45: 619-626.

ZIEGLER, A. C. 2002. Hawaiian natural history, ecology, and evolution. University of Hawai'i Press, Honolulu, Hawai'i, USA.

ZIMMERMANN, N., C. RITZ, AND F. HELLWIG. 2010. Further support for the phylogenetic relationships within *Euphorbia* L. (Euphorbiaceae) from nrITS and *trnL–trnF* IGS sequence data. *Plant Systematics and Evolution* 286: 39-58.

ZWICKL, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin, Austin, Texas, USA.

CHAPTER IV

RADIATION OF WOODY HAWAIIAN CHAMAESYCE (EUPHORBIA, EUPHORBIACEAE): OLDER TO YOUNGER ISLAND DISPERSALS, AND FURTHER EVIDENCE FOR HYBRID ORIGIN AND RECENT INTERSPECIFIC HYBRIDIZATIONS

ABSTRACT

Although the majority of species in *Euphorbia* sect. *Anisophyllum* (Euphorbiaceae) are herbaceous and more or less weedy, a clade of 16 species that are all woody perennials diversified on the Hawaiian Islands. They are found in a broad range of habitats from coastal vegetation to high elevation bogs, and range in habit from subshrubs to trees. Some of these species are the only C_4 plants that are adapted to wet forest understory environments. To investigate the phylogenetic relationships and evolutionary dynamics within the Hawaiian clade, we sampled 104 Hawaiian individuals including 15 of the 16 species on six major Hawaiian islands. Concatenated chloroplast sequences from more than 8 kb of non-coding regions support older to younger island dispersals along the Hawaiian island chain. Nuclear ITS, *LEAFY* and *G3pdhC* markers support a hybrid origin of the Hawaiian Chamaesyce clade, as well as recent interspecific hybridization among the Hawaiian species.

INTRODUCTION

The Hawaiian Islands are one of the most remote island archipelagos in the world. Built by the successive emergence of volcanic islands (see Fig. 4.7 and relative ages of the islands in Table 4.1), the island chain provides evolutionary biologists with a natural system of time-calibrated experiments of colonization and adaptive radiation (Ziegler, 2002). Among Hawaii's native angiosperm flora, one of their most notable features is the repeated evolution of woody taxa from small, herbaceous mainland ancestors. This phenomenon has been studied in a number of angiosperm lineages such as the silversword alliance (Asteraceae, Baldwin et al., 1991), violets (Violaceae, Ballard and

Sytsma, 2000), *Plantago* (Plantaginaceae, Dunbar-Co et al., 2008), *Silene* (Caryophyllaceae, Eggens et al., 2007), *Echium* (Boraginaceae, Bohle et al., 1996), *Schiedea* (Caryophyllaceae,Willyard et al., 2011), and of note here, *Euphorbia* (subgenus *Chamaesyce* Raf.) sect. *Anisophyllum* Roeper (Euphorbiaceae; Koutnik, 1987). Hereafter we use the conventional name "Hawaiian Chamaesyce" for species in sect. *Anisophyllum* that are native to the Hawaiian Islands, to distinguish them from other *Euphorbia* species that also occur there.

There are 29 infraspecific taxa recognized within the 16 species of Hawaiian Chamaesyce (Table 4.1). They are found in all major island habitats, from coastal vegetation to dry forests, wet forests and bogs, ranging in habit from subshrubs and shrubs to trees up to 10 m tall. Among them, six species and four additional varieties are listed as endangered, and many others are listed as species of concern by the United States Fish & Wildlife Service (http://www.fws.gov/endangered/). Many taxa are endemic to a single island (Koutnik, 1987; Lorence and Wagner, 1996; Wagner et al., 1999). For example, *E. deppeana* is now only known from two populations on Oahu, with around 50 individuals (Cliff Morden, pers. comm.; Koutnik, 1987); *E. eleanoriae*, long hidden among volcanic cliffs of Kauai's Na Pali coast, was only described in 1996 (Lorence and Wagner, 1996).

Section *Anisophyllum* comprises about 350 species in *Euphorbia* subgenus *Chamaesyce* (Yang and Berry, 2011). Members of the section are generally small prostrate herbs, often more or less weedy. All but three basal members exhibit C_4 photosynthesis, which is a specialized adaptation that has a competitive advantage in low CO_2 situations and in warm, often dry environments (Sage, 2004). In contrast to most other C_4 plants, however, Hawaiian Chamaesyce are all woody, and they include some of the only known C_4 trees (Pearcy and Troughton, 1975). Species such as *E. remyi* are shrubs specialized in wet forest understory (Fig. 4.1B). These species are atypical C_4 plants, and they represent an important system to examine questions related to the evolution of C_4 photosynthesis.

Monophyly of the Hawaiian Chamaesyce was supported by the ITS dataset of Motley and Raz (2004), with extensive taxon sampling among Pacific Island species, but relatively little sampling from North America. Their study suggested that the close

relatives of the Hawaiian Chamaesyce were from the New World instead of other Pacific Islands. This result was subsequently confirmed by more comprehensive taxon sampling across sect. *Anisophyllum* (Yang and Berry, 2011), including eight Hawaiian Chamaesyce species, using nuclear ribosomal ITS, three chloroplast (cpDNA) markers, and one nuclear low-copy locus exon 9 of *EMB2765* (Fig. 4.2B). In the ITS data set, the sister group of Hawaiian Chamaesyce was a clade of four small herbaceous species found in the southern United States, northern Mexico, and the Caribbean including *E. stictospora*, *E. velleriflora*, *E. mendezii* and *E. leucantha*. The chloroplast data set shows an additional species nested in the sister clade of Hawaiian Chamaesyce, namely *E. cinerascens*, from the southern United States and northeastern Mexico. A third data set using the nuclear *EMB2765* recovered one copy in the majority of continental species, but three copies in Hawaiian Chamaesyce; one copy supports the Hawaiian species as closely related to *E. stictospora*, *E. velleriflora* and *E. mendezii*, generally consistent with the ITS data. A second copy weakly supports Hawaiian species grouped with *E. cinerascens*, partly consistent with cpDNA data; while the third copy does not reveal a highly supported sister group for Hawaiian Chamaesyce, probably due to the limited resolution of *EMB2765*. Given the high chromosome numbers in counts of all four Hawaiian Chamaesyce species surveyed thus far (2n = 38, Carr, 1985) compared to closely related non-Hawaiian Chamaesyce species (Fig. 4, Yang and Berry, 2011), allopolyploidy has likely contributed to the origin of Hawaiian Chamaesyce. Also, taking the patterns in chloroplast markers and ITS into account, Yang and Berry (2011) hypothesized that the origin of Hawaiian Chamaesyce went through two rounds of interspecific hybridizations, as shown in Fig. 4.2A.

In this study, we greatly increased both taxon and molecular sampling within Hawaiian Chamaesyce. Using ITS, eight chloroplast non-coding regions, and two additional nuclear low-copy loci, we aim to further investigate the hypothesized hybrid origin of Hawaiian Chamaesyce, as well as to address questions about the diversification of the group within the Hawaiian radiation. Specifically, did Hawaiian Chamaesyce arrive first to older islands and then successively disperse to younger islands along the island chain? Also, did high elevation taxa originate from high elevation taxa of a different island or from low elevation taxa of the same island?

MATERIALS AND METHODS

Taxon sampling—DNA accessions representing 26 of the total 29 Hawaiian Chamaesyce taxa were obtained from the Hawaiian Plant DNA Library (Morden et al., 1996; Randell and Morden, 1999). Together with additional samples from greenhouses and field collections during a field trip to the Hawaiian Islands in February 2009, accessions from 27 of 29 Hawaiian Chamaesyce taxa were obtained in a total of more than 300 DNA accessions. Depending on the range and variations for each taxon, between one and twelve accessions per taxon were included for this study. For species like *E. deppeana*, that have only two wild populations with around 50 individuals in total, only one accession was included; on the opposite side of the spectrum, *E. celastroides* var. *amplectens* and *E. degeneri* are both found on all major Hawaiian Islands, and twelve and ten accessions were included respectively, representing multiple populations from different islands. For each of the remaining taxa, we included accessions from multiple populations covering the range of distribution as much as possible. A total of 104 DNA accessions were selected for this study, covering all six major Hawaiian Islands: Kauai, Oahu, Maui, Molokai, Lanai and Hawaii. In order to distinguish different accessions of the same taxon, we include DNA numbers following taxon names for all the ingroup Hawaiian Chamaesyce in the following text. In addition, eleven closely related outgroup species were selected based on phylogenetic reconstruction across sect. *Anisophyllum* (Yang and Berry, 2011).

PCR, cloning and sequencing—In addition to genomic DNA obtained from the Hawaiian Plant DNA Library, genomic DNA was extracted from newly collected silica-dried leaf fragments using DNeasy Plant Mini Kits (QIAGEN, Valencia, California, USA) following the manufacturer's instructions. All genomic DNA samples were diluted 20 times before subjecting to PCR to reduce inhibition of PCR enzymes by secondary compounds.

PCR amplification, sequencing, and alignment of the ITS followed the same protocol as Yang and Berry (2011). Sequences with continuous superimposed peaks due to multiple alleles of length variation were excluded. Two of these excluded sequences, *E. celastroides* var. *kaenana* 5840 and *E. kuwaleana* 5700 were cloned following the

same protocol as Yang and Berry (2011) to evaluate ITS allelic variation within each genome. Sequenced clones were added back to the ITS matrix for subsequent phylogenetic analyses.

The *trnH-psbA* spacer [primers: trn H (GUG) and psb A (Hamilton, 1999)] was sequenced in all ingroup accessions. Based on a preliminary maximum parsimony analysis of *trnH-psbA* sequences, 68 out of the initial 104 ingroup accessions were selected for sequencing additional cpDNA markers. Fifteen of the most variable chloroplast non-coding regions from Shaw et al. (2005; 2007) were tested in a subset of accessions for primer specificity, percentage of variable sites and poly-A/T length variations. Among them, four regions were chosen for this study (Shaw et al., 2005; Shaw et al., 2007): the *rpl14-rpl36* spacer (primers: rpL14 and rpL36), *psbB-psbH* spacer (primers: psbB and psbH), *atpI-atpH* spacer (primers: atpI and atpH), and *psbD-trnT* spacer (PCR primers: psbD and $trnT^{(GGU)}$-R; additional sequencing primers: psbD-trnT F881 5' TTG ATC TTG CGT TCT GGA ATC 3', and psbD-trnT-R1138 5' CCT AAC CTA TTG CAT GAT GAC 3'). Three additional chloroplast non-coding regions that had been previously used for phylogenetic studies in Euphorbiaceae were also sequenced: *rpl16* intron, *trnL* intron [primers: trnL-c and trnL-d (Taberlet et al., 1991)] and *trnL-F* spacer. PCR amplification, sequencing, and alignment followed the same cpDNA protocol as Yang and Berry (2011). In total, eight chloroplast non-coding regions were sequenced in 79 DNA accessions (68 ingroups and 11 outgroups).

More than ten primer pairs were tested for amplifying nuclear low-copy regions in Hawaiian Chamaesyce. Among them, the second intron of *LEAFY* (*LFY*) was amplified by primer pair LFY F2 (5' CGT GGS AAA AAG AAY GGY YTD GAT TA 3') and LFY R1 (5' CAT TTT DGG YTT GTT KAT GTA 3'; Ivalu Cacho, pers. comm.). Positive PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, California, USA). Intron of *glyceraldehyde 3-phosphate dehydrogenase subunit C* (*G3pdhC*) was PCR amplified using primer pair GPDX7F and GPDX9R (Strand et al., 1997). Positive bands were excised and purified using QIAquick Gel Extraction Kit (QIAGEN, Valencia, California, USA). Purified PCR products of both *G3PDHC* and *LEAFY* were cloned. Cloning, PCR amplification of clones, sequencing of amplified clones, sequence assembly and alignment followed the same protocol as Yang

and Berry (2011), except that at least 24 clones from each PCR product were sequenced. Copy-specific primers were designed for both *LFY* and *G3pdhC*.

Copies of *LFY* were PCR amplified using copy-specific primer pair LFY1 F629 (5' TTC AGA CAC CTT TTG GGT T 3') and LFY1 R1415 (5' CTC GAC TTG ATT AGC ATA TTC TTG G 3'), and primer pair LFY2 F177 (5' GGG TCC ACA GTA TAC CTA CCT AC 3') and LFY2 R1415 (5' CCA ACA TGA TTA GCA TAT TCC TGC 3'). Each PCR reaction contained 0.2 μL PfuUltra II Fusion HS DNA Polymerase (Agilent Technologies, Inc., Santa Clara, California, USA), 1.5 μL 10x buffer, 1.5 μL dNTP mix (2.5 mmol/L), 0.5 μL of each primer (10 μmol/L), 3 μL diluted template DNA, and 7.8 μL ddH_2O for a final volume of 15 μL. Cycling conditions were: 94 °C for 4 min; 35 cycles of 95 °C for 20 s, 51°C for 20 s, 72 °C for 1 min; and a final extension step of 72 °C for 3 min. Positive PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, California, USA).

G3pdhC was PCR amplified using copy-specific primer pair GDX7 1F59 (5' TTC ACG CCA TCA CTG GTT AGT C 3') and GDX7 1R900 (5' TTA GGT TTC AGC AAG AGA ATC 3'), and primer pair GDX7 2F144 (5' CTC CTT TGA ACT TGT GAT ACT G 3') and GDX7 2R850 (5'- CAG YAA CAG AAA TGC TAA TGC CC - 3'), using the same PCR protocols as for *LFY*. PCR products were examined on a 1.5% agarose gel, and positive bands were excised from and purified using the QIAquick Gel Extraction Kit (QIAGEN, Valencia, California, USA). Cloning, PCR amplification of clones, sequencing of amplified clones, sequence assembly and alignment followed the same protocol as Yang and Berry (2011), except the cloning kit used was Zero Blunt TOPO PCR cloning kit for sequencing (Invitrogen, Carlsbad, California, USA), and between 8 to 32 clones were sequenced for each PCR product.

Phylogenetic analyses—Sequences were assembled and edited in the program Sequencher v. 4.10.1 (Gene Codes, Ann Arbor, Michigan, USA), aligned in the program MUSCLE v. 4 (Edgar, 2004) using the default parameters, and manually adjusted in the program MacClade v. 4.08 (Maddison and Maddison, 2005).

For the ITS data set, no indels were coded and no characters were excluded. For cpDNA, segments with poly A/T length variation were excluded. Two short chromosomal inversions in *rpl16* and one in *trnH-pshA* were detected by visual

examination of the alignment. All three inversions were inverted and complemented for phylogenetic analysis without scoring them as binary data (Kim and Donoghue, 2008; Yang and Berry, 2011). Indels that could be unambiguously aligned were coded as binary characters following the simple gap coding criteria of Simmons and Ochoterena (2000), as implemented in the IndelCoder module of the program SeqState v. 1.4.1 (Müller, 2006). Each of the eight cpDNA regions was initially analyzed separately using maximum parsimony. Congruencies were visually inspected before concatenating them into the first character set of the cpDNA matrix; binary indels from all eight cpDNA regions were concatenated and became the second character set of the cpDNA matrix.

For nuclear low-copy genes *LFY* and *G3pdhC*, all copies for each gene were initially pooled together into a *LFY* master matrix and a *G3pdhC* master matrix. For *G3pdhC*, copy 1, copy 2-4, and copy 5-6 were separated from the master matrix and these three matrixes were each analyzed individually, since these three data sets do not align well in certain areas; whereas all copies of *LFY* were analyzed together in one matrix. In each separated matrix, segments with poly A/T length variation were excluded. Indels that could be unambiguously aligned were coded as binary characters following the simple gap coding criteria of Simmons and Ochoterena (2000), as implemented in the IndelCoder module of the program SeqState v. 1.4.1 (Müller, 2006). Binary indels were concatenated to the nucleotide character set and became the second character set for each matrix.

ITS, cpDNA, and *LFY* were each subjected to the maximum parsimony (MP) and Bayesian inference (BI) as described next. Maximum likelihood is unable to take binary indel characters into account and therefore was not used in this study. *G3pdhC* copy 1, copy 2-4 and copy 5-6 were analyzed using MP only, since there were very few variable sites within each copy and model-based methods were unnecessary.

Maximum parsimony was implemented in the program PAUP* (Swofford, 2002). Heuristic searches were performed with 1 000 random addition replicates holding 1 trees per step and keeping best trees only, MaxTrees=10 000, with TBR branching swapping algorithm and saving 1 tree per replicate. Clade support was assessed by 500 bootstrap replicates as implemented in PAUP* with the following search settings: keep best tree only, stepwise addition, swap best tree only, MaxTrees=1 000, 1 000 random replications

of sequence addition, holding 1 tree at each step, keep best trees only, TBR branch swapping, and multitrees on.

Bayesian inference was conducted in the program MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Two independent runs of four chains each (three heated, one cold), starting from random trees, using the default temperature of 0.2, were run for 5 million generations. Trees were sampled every 100 generations. Each analysis was conducted using the nucleotide substitution model GTR+I+γ selected by AIC in MrModeltest v. 2.3 (Nylander, 2004). A branch length prior "brlenspr=unconstrained:exponential(100.0)" was applied to prevent unrealistically long branches (Brown et al., 2010; Marshall, 2010). Both of the cpDNA and the *LFY* data sets were partitioned into two character sets, allowing all parameters to be unlinked except branch length and topology. The binary indels were subject to "rates=gamma" since only variable characters were coded in this data set. All parameters were visually examined in the program Tracer v. 1.5 (Rambaut and Drummond, 2007) to verify stationary status. Trees from the first 1 million generations were discarded as the burn-in period, and the remaining trees were used to compute the majority rule consensus.

RESULTS

Overall statistics of all gene regions sequenced for this study are summarized in Table 4.2, and results of the phylogenetic analyses are shown in Figs. 4.3-4.6.

ITS data set—Maximum parsimony and BI recovered the same topology when BI posterior probability (PP) was > 0.95 and MP bootstrap percentage (BS) > 60% among ingroup taxa (Fig. 4.3). Monophyly of Hawaiian Chamaesyce is highly supported (BS = 100; PP = 1.00). All ingroup ITS sequences show 10 or more superimposed peaks, which is highly elevated compared to outgroups. In addition, 18 of the ingroup accessions have continuously superimposed peaks from allele length variation and were excluded from the alignment.

Many taxa are polyphyletic in our ITS phylogeny, even those ones that are morphologically and ecologically distinctive, such as *E. celastroides* var. *lorifolia, E. degeneri,* and *E. halemanui. Euphorbia celastroides* var. *lorifolia* 5295 and 5299 were collected from the same population, yet ITS sequences from these two accessions are

well separated on the phylogeny, with accession 5295 grouped with other *E. celastroides* accessions and 5299 sister to *E. olowaluana*. Cloning results of *E. kuwaleana* 5700 and *E. celastroides* var. *kaenana* 5840 revealed many different alleles. One allele, 5840c4 has a very long branch compared to other ingroup taxa with highly elevated base pair substitution at the ITS2 region.

Although ITS sequences are highly superimposed and messy, there are nonetheless a number of well-supported clades (Fig. 4.3). Each clade occupies similar habitat types on a single island or the island group of Maui, Molokai and Lanai. High elevation, high precipitation and relatively large-leaved species of Oahu (*E. rockii*, *E. clusiifolia* and *E. herbstii*) and Kauai (*E. remyi* and *E. halemanui*) each form a monophyletic clade. All accessions of *E. olowaluana* (Hawaii) also form a clade with little sequence variation.

cpDNA data set—Two short chromosomal inversions were detected in the *rpl16* intron region. The first one is a 33-bp inversion starting from base pair 860 in the initial alignment. It was present in two outgroup accessions (*E. hirta* and *E. cinerascens*) and in six ingroup accessions (*E. remyi* var. *remyi* 5305 and Y356, *E. celastroides* var. *hanapepensis* 4169, *multiformis* var. *multiformis* 4766, *celastroides* var. *tomentella* 5597, and *celastroides* var. *stokesii* 5315). Monophyly of these eight accessions is strongly rejected by all other cpDNA markers (Fig. 4.4). The second inversion in *rpl16* is 38 bp long and is found in *E. stictospora*, *E. velleriflora*, and *E. mendezii*, starting from base pair 949 in the initial alignment. Monophyly of these three accessions is strongly supported by all cpDNA regions. A third cpDNA inversion was detected in the *trnH-psbA* spacer. It is 23-bp long, starting from base pair 791 in the initial alignment, and is present in two outgroup accessions (*E. setosa* and *E. linguiformis*) and all three *E. celastroides* var. *amplectens* accessions collected on Lanai (only Y396 is shown in Fig. 4.4; the rest are not shown). Monophyly of these five accessions is strongly rejected by all other cpDNA markers. Since there is no base pair substitution in any of these three inversion regions, we reversed and complemented all inversions and included them in the alignment without coding the inversion events as binary data (Yang and Berry, 2011).

After flipping back chromosomal inversions and excluding regions of poly A/T length variations, the resulting cpDNA alignments were well aligned. Percentages of

variable characters range from 3.7% in *trnL* to 9.4% in *trnH-psbA*. Branch lengths within Hawaiian Chamaesyce are much shorter compared to the outgroups.

Compared to ITS, the cpDNA data set has a lower percentage of both variable and parsimony informative sites (Tab. 4.2), but more total variable and informative characters. A "younger island clade" is well supported (Fig. 4.4). Within it there are four subclades that are either entirely on Oahu, or comprised of younger island taxa nested in a grade of Oahu accessions. The rest of the cpDNA tree consists of a number of older island clades that are each endemic to either Kauai or Oahu, and together they form the "older island polytomy".

LFY data set—Four copies were recovered from the *LFY* dataset. Copy-specific primer pair LFY2 F177 and LFY2 R1415 amplified copies 1 and 2, and primer pair LFY1 F629 and LFY1 R1415 amplified copies 3 and 4 (Fig. 4.5). Maximum parsimony agrees with BI in most cases when BS > 55 and PP > 0.95. The major disagreement is that monophyly of copy 1 is strongly supported by BI (PP = 1.00) while the BS support level is less than 50. This is probably due to the fact that sequences of copy 1 share an insertion of around 200 bp in the middle, and this insertion is relatively variable compared to the rest of the *LFY* matrix. Only one copy was recovered from the outgroups, and all the outgroups form a grade with both copies 3 and 4 nested in it. No apparent close relatives for copies 1 and 2 were detected.

Many ingroup accessions lost one or two copies out of the four copies (Fig. 4.5). Only seven accessions have been PCR amplified by both copy-specific primer pairs. Out of these seven accessions, both *E. olowaluana* 5619 and *E. multiformis* var. *microphylla* 5624 have all four copies; both *E. kuwaleana* 5700 and *E. degeneri 2219* have copies 1, 3 and 4; while *E. rockii* 2223 has copies 1, 2 and 4; *E. clusiifolia* 1353 has only copy 1 and one copy of either copy 3 or 4 (unclear due to low resolution0; and *celastroides var. kaenana* 5840 has copy 1, 2, and one of copies 3 and 4. Presence versus absence of copies varies even within a single species. Among the different accessions of *E. olowaluana*, accession 5619 has all four copies, while 5131, Y350 and Y352 are all missing copy 2 (copies 3 and 4 not examined), and 5116 has both copies 1 and 2 present (copies 3 and 4 not examined). The same happens in many other taxa, such as in *E. halemanui*, where accession Y357 has both copies 1 and 2, and 4780 has copy 2 missing.

Among accessions of *E. celastroides* var. *kaenana*, accession 2934 is missing copy 2, while both 5840 and 2776 have copies 1 and 2. Among all accessions examined, each accession has at least one of copies 1 and 2, and at least one of copies 3 and 4.

G3pdhC data set—Six copies of *G3pdhC* were detected within Hawaiian Chamaesyce. Copy-specific primer pair GDX7 1F59 and GDX7 1R900 amplified copy 1; primer pair GDX7 2F144 and GDX7 2R850 preferentially amplified copies 2 and 3 but also occasionally picked up other copies; while copies 4, 5 and 6 were detected from initial PCR and cloning using the non-copy-specific primer pair GPDX7F and GPDX9R (Strand et al., 1997). A master alignment including all sequences recovered in *G3pdhC* was not well aligned, mainly because copy 1, copies 2-4 and copies 5-6 do not align well with each other in places. Therefore the master alignment was divided into three matrixes that aligned well within each matrix: copy 1, copies 2-4, and copies 5-6. Each of the three matrixes was analyzed separately using MP only, since there are only a few base pair substitutions within each copy. As for the outgroups, copy 1 and copy 6 were detected in *E. stictospora*, and copy 5 was detected in *E. velleriflora*. No outgroup is associated with copies 2-4, and therefore the phylogeny was rooted with copy 4, which has a long branch length leading from copies 2 and 3 (Fig. 4.6).

Relationships within each copy of *G3pdhC* are poorly resolved. However, certain taxon groupings were shared among copies 1, 2 and 3, the three copies with relatively high number of sequences available. Only one tree was recovered from MP analysis of copies 5-6, which only contains five accessions. Therefore a phylogram was presented with no bootstrap necessary (Fig. 4.6).

DISCUSSION

Patterns of seed dispersal among island and habitat types—The chloroplast genome of angiosperms is generally maternally inherited (Sears, 1980). Assuming that this is the case in Hawaiian Chamaesyce, we should be able to infer dynamics of seed dispersal from the cpDNA phylogeny.

Seed dispersal is predominantly from older to younger islands—In our cpDNA phylogeny (Fig. 4.4), there is a well-supported clade of mostly younger island accessions (Hawaii, Molokai, Lanai, Maui, also Oahu). Within it there are four subclades that are

each either entirely in Oahu, or else consists of younger island accessions nested in a grade of Oahu accessions. Among these younger islands, Molokai, Lanai and Maui are geographically proximate, and they have been connected by land at least once during the past 1-2 million years (Ziegler, 2002). Such geographic proximity and past land connections is reflected by our cpDNA phylogeny: each of the four subclades within the younger island clade either have taxa from all three islands or else have no accessions from any of these three islands. The rest of the Hawaiian Chamaesyce form a polytomy consisting of a number of small and each well-supported clades from either Kauai or Oahu. Overall accessions from the same island or island group appear to cluster with each other, and therefore crossing the water barrier between islands seems to be the limiting factor in range expansion in Hawaiian Chamaesyce.

Although younger island accessions tend to nest in grade of older island accessions, seed dispersal does not appear to follow a strict stepping stone pattern. In the Hawaii-Oahu clade (Fig. 4.4), *E. olowaluana*, which is endemic to Hawaii, is nested in a grade of Oahu accessions. It appears that seed dispersal occurred from Oahu directly to Hawaii, skipping islands in the middle, although this could also be an artifact of incomplete sampling or extinction. Seed dispersal can also go in reverse from younger to older islands. This would presumably happen more often in widespread species that are more readily moving among islands. Only one such case is readily identifiable in our cpDNA phylogeny: *E. degeneri* 338 from Kauai is nested in the Maui-Lanai-Molokai-Oahu clade 2 that is otherwise from younger islands. Although additional dispersal events from younger to older islands could be identified with increased taxon and molecular sampling, the predominant trend appears to be from older to younger islands, with accessions in Molokai, Lanai, Maui and Hawaii coming from Oahu (Fig. 4.7). Relationships among taxa on Oahu and Kauai, however, are unclear, due to the low resolution of deep notes in our cpDNA phylogeny. Taxa of both islands may be derived from older, or even currently submerged islands further up the Hawaiian island chain (Givnish et al., 2009; Heads, 2011).

Seed dispersal among different habitat types—High elevation (mesic forest, wet forest and bog) taxa of Oahu and Kauai are well separated in the cpDNA phylogeny. Therefore high elevation taxa likely evolved independently on Oahu and Kauai, the two

oldest islands.

Among the four subclades of the younger islands clade (Fig. 4.4), the Hawaii-Oahu clade is mainly mid-elevation (dry forest), whereas both of the Maui-Lanai-Molokai-Oahu clades are predominantly low elevation (scrub and coastal strand). This indicates dispersal from Oahu to Hawaii, and from Oahu to Maui-Lanai-Molokai are via low- to mid- elevation taxa. Colonization's of the older islands, Kauai and Oahu, are less clear, with accessions sister to each other occupying similar or very different vegetation types. This is likely the consequence of the longer history of island occupation on Kauai, repeated colonization from even older islands, or extinction.

Both Kauai and Oahu have the highest number of taxa (Table 4.1). Although geographically larger in size, as well as higher in elevation, the younger islands of Maui and Hawaii have fewer taxa. Both Maui and Hawaii also lack taxa that live in bogs and mesic to wet forests, despite the presence of these habitat types. All the mesic to wet forests and bog taxa on Kauai and Oahu are single-island endemics, whereas lower elevation taxa more often occur on more than one island, especially *E. celastroides*, *E. degeneri* and *E. multiformis*. Therefore it appears that seed dispersals occur predominantly among lower elevation taxa. Higher elevation taxa evolved from taxa of lower elevation on the same island, instead of from taxa of similar habitat types from adjacent islands.

Similar patterns of older to younger island colonization, and coastal to woodland colonization, are evident in Hawaiian *Plantago*, with Kauai being most species-rich (Dunbar-Co et al., 2008). Species number of Hawaiian lobelias, however, peaks on Maui (Givnish et al., 2009). All six major lineages in Hawaiian lobeliads have gone through parallel divergence in morphology as well as parallel island colonization across major Hawaiian Islands. It is likely that due to an earlier arrival and/or more rapid divergence, the Hawaiian lobeliads are more "saturated" in terms of colonizing empty niches compared to Hawaiian *Plantago* and Hawaiian Chamaesyce.

The ability of dispersal over the water in section *Anisophyllum* appears to correlate with presence of a mucilaginous seed coat. Majority of species in sect. *Anisophyllum* have a mucilaginous seed coat that becomes sticky when wet, a trait that is rare in other *Euphorbia* species (Jordan and Hayden, 1992). Mucilaginous seed coats

retain water and can facilitate germination in dry conditions (Gutterman and Shem-Tov, 1997; Penfield et al., 2001). A previous survey of the mucilaginous seed coat in sect. *Anisophyllum* suggested its presence in most continental species but an absence in many island species. All six Hawaiian Chamaesyce species that have been surveyed have lost their seed coat, except *E. celastroides*, one of the two species occurring on all major Hawaiian Islands (Jordan and Hayden, 1992). The correlation between the seed coat and long-distance dispersal, combined with the small seed size in sect. *Anisophyllum* (1-2 mm long) suggests a possible mechanism for arriving on Hawaii and dispersal among Hawaiian Islands by adhesion to migrating birds (Jordan and Hayden, 1992). On the other hand, Hawaiian Chamaesyce has non-floating seeds except for *E. degeneri*, a coastal strand species found on all major Hawaiian islands (Lauren Raz, personal communication; Carlquist, 1966). This implies an additional dispersal mechanism among Hawaiian Islands.

Recent interspecific hybridization—Both *E. multiformis* var. *microphylla* 5622 and 5624 collected at the Pohakuloa Training Area (PTA) of Hawaii share the same cpDNA haplotype with *E. olowaluana* Y350, 403 and 5619, which are also collected from PTA (Fig. 4.4). In the ITS phylogeny, however, *E. multiformis* var. *microphylla* accessions are well separated from a monophyletic *E. olowaluana* (Fig. 4.3). Due to the close proximity of *E. multiformis* var. *microphylla* 5622 and 5624 with *E. olowaluana* Y350, 403 and 5619, interspecific hybridization most likely occurred between the two species. Such hybridization events are likely to be recent due to the shared cpDNA haplotype across eight non-coding regions in a total of more than 8 kb. Morphologically intermediate wild populations of Hawaiian Chamaesyce have been discovered in a number of taxa (Koutnik, 1987), and cross-pollination experiments in greenhouses suggest that many species are interfertile (Cliff Morden, pers. comm.).

Homogenization of ITS sequences—Given the elevated level of superimposed peaks in all ingroup ITS sequences, divergent alleles of ITS likely coexist within each ingroup accession. Some of these alleles may be preferentially amplified, while others will either become minor superimposed peaks, or are not detectable in sequencing results. In addition, concerted evolution may homogenize divergent ITS alleles towards one allele or another, resulting in divergent ITS sequences among closely related accessions. Given

all the complications of ITS sequences, it is notable that a number of clades are still well supported (Fig. 4.3), and each well-supported clade is characterized by occupying one or similar habitats, and is geographically restricted to either one single island or the island group of Maui, Lanai and Molokai. Taxa that are high elevation, single-island endemics tend to group with high elevation taxa of the same islands.

Another notable well-supported clade consists of all accessions of *E. olowaluana*, an endemic of the island of Hawaii. Since Hawaii is less than 0.5 million years old, *E. olowaluana* is probably also of recent origin with a small founder population with very little sequence polymorphism in ITS; or alternatively, have gone through recent and rapid homogenizations of ITS.

Further evidence for hybrid origin of Hawaiian Chamaesyce—Yang and Berry (2011) hypothesized the hybrid origin of Hawaiian Chamaesyce based on divergent alleles from the nuclear low-copy region exon 9 of *EMB2765*, and the relatively high chromosome numbers in all four Hawaiian species that have been examined. In this study, we substantially expanded both the taxon and molecular sampling within Hawaiian Chamaesyce. ITS shows highly elevated level of superimposed peaks compared to outgroups, suggesting that multiple divergent alleles exist in each ingroup accession. The ITS phylogeny disagrees with the cpDNA phylogeny extensively, but they largely agree with each other for the outgroups. This further supports the hybrid origin of Hawaiian Chamaesyce.

Two additional nuclear low-copy genes, *LFY* and *G3pdhC* both show increased number of copies compared to outgroups. Two out of the four copies of *LFY*, and three out of the six copies of *G3pdhC* grouped together with the outgroups we identified from ITS and cpDNA. The remaining copies do not point to any definite outgroups, probably because only the outgroups identified by ITS and cpDNA were included in this study. This is similar to the pattern recovered from *EMB2765*, in which two copies clustered with the same outgroups identified by ITS and cpDNA, while a third copy nested in a polytomy within sect. *Anisophyllum* with no clear affinity with any mainland species (Yang and Berry, 2011). Such pattern of having divergent gene copies each having different outgroup affinities further support hybrid origin of Hawaiian Chamaesyce.

Similar allopolyploid origin of Hawaiian radiation also occurred in the

silversword alliance (Barrier et al., 1999), and probably also in the Hawaiian mints (Lindqvist and Albert, 2002). Allopolyploids can exhibit adaptive plasticity through increased heterozygosity, better masking of recessive deleterious alleles, and lower susceptibility to inbreeding depression (Comai, 2005). All these advantages likely facilitate colonization of new habitats, such as in the Hawaiian Islands.

Loss of duplicated copies in LFY—All the ingroup accessions examined have at least one copy of *LFY* copies 1 and 2, and at least one of copies 3 and 4. This is not likely an artifact of preferential amplification of one copy over another. For the copy-specific primer pair LFY2 F177 and LFY2 R1415, either only one clear band is present in the initial PCR product, and one copy was detected in the sequencing products from the 24 clones sequenced; or else two bands of equal intensity were present in the initial PCR product that were around 200 bp apart, and the clones were roughly 50% of one copy or the other. A similar situation occurred with the other copy-specific primer pair LFY1 F629 and LFY1 R1415. Sequences of clones always either have only one copy, or have two copies of equal representation.

The second intron of *LFY* is by far the most frequently used low-copy nuclear gene, especially in resolving reticulate evolutionary patterns. *LFY* is a master regulator for the whole floral network in flower meristems; it is found in all land plants and is generally single-copy in angiosperms (Moyroud et al., 2010). It is functionally conserved, and even in taxa with lineage-specific duplications, they do not seem to have diverged in function (Maizel et al., 2005). Until now multiple copies of *LFY* have only been recorded in recently formed polyploids (Wada et al., 2002; Kim et al., 2008; Volz and Renner, 2009; Kim et al., 2010; Wei et al., 2010), with no evidence of persisting through more than a few speciation events. Such a tendency for keeping only one copy can potentially create pseudocongruence between *LFY* and nuclear ribosomal regions (Grimm and Denk, 2010). However, until now no empirical evidence of active *LFY* gene loss has been reported. Here we document for the first time recent and recurrent gene loss in *LFY*.

In addition to the woody Hawaiian Chamaesyce, insular woody taxa have evolved multiple times within sect. *Anisophyllum*, including a number of Pacific and Caribbean taxa, as well as another smaller island radiation of eight woody species in the Galapagos Islands (Burch, 1969; Yang and Berry, 2011). An interesting discovery in *Arabidopsis*

thaliana shows that MADS box proteins *soc1-3 ful-2* double mutants develop into highly branched shrubs, suggesting that transformation from small annual herbs into woody plants can be achieved by altering a small number of genes (Melzer et al., 2008). This supports the evolutionary potential of a characteristically weedy plant group such as sect. *Anisophyllum*, and provides a potential molecular mechanism for explaining repeated evolution of woody island taxa across various angiosperm groups in general.

Conclusions—With comprehensive taxon sampling including 27 of 29 Hawaiian Chamaesyce taxa, our cpDNA data set suggests that seed dispersals in Hawaiian Chamaesyce were generally from older to younger islands among low- to mid- elevation taxa; and high elevation taxa evolved independently on Kauai and Oahu. All nuclear data sets (ITS, *LFY* and *G3pdhC*) show divergent alleles and increased numbers of copies compared to outgroups, further supporting the hybrid origin of Hawaiian Chamaesyce. In the *LFY* data set, we for the first time documented recent, recurrent and non-random gene losses. In addition, shared cpDNA haplotypes between co-occurring species support recent interspecific hybridization.

Future directions—Angiosperm radiations on the Hawaiian Islands are notoriously difficult to resolve due to recent and rapid speciation and ongoing gene flow (Howarth and Baum, 2005; Bacon et al., 2011). Similarly, our study suggests a complex reticulate relationship in a prominent radiation on the Hawaiian Islands.

To further resolve such recent and rapid divergence, many unlinked loci per individual, and many individuals per taxa are required to resolve the short branch length, and distinguish lineage sorting from introgression. Due to the hybrid origin and high copy numbers in each gene, copy-specific primers are often unable to distinguish copies, and cloning is necessary for separating these copies. Since many loci are required for each individual, and many individuals have to be investigated for each taxon, using current cloning techniques becomes very time-consuming and expensive. Therefore targeted next-generation sequencing techniques are the logical choice to separate alleles without cloning in future studies (Griffin et al., 2011).

Table 4.1. Distribution of the 29 Hawaiian Chamaesyce taxa on five major Hawaiian Islands. Habitat types are sorted top to bottom from higher elevation and wetter habitats to lower elevation and dryer ones, whereas ages of islands are sorted left to right from older to younger (Koutnik, 1987; Lorence and Wagner, 1996; Wagner and Sohmer, 1999; Ziegler, 2002; Morden and Gregoritza, 2005). Smaller islands, such as Niihau, Nihoa, Kaula, Kahoolawe and Lanai, are not shown here.

Species	**Varieties**	**Habit**	**Habitat**	**Kauai** 5.1 my	**Oahu** 2.75-4.00 my	**Molokai** 1.90-2.10 my	**Maui** 1.00-1.75 my	**Hawaii** 0.00-0.60 my
sparsiflora		Subshrub	Bog	X				
remyi	***remyi***	Shrub	Wet forest	X				
remyi	***hanaleiensis***	Shrub	Wet forest	X				
remyi	***kauaiensis***	Tree	Wet forest	X				
rockii	***rockii***	Shrub to small tree	Wet forest		X			
rockii	***grandifolia***	Small tree	Wet forest		X			
herbstii		Tree	Wet forest		X			
clusiifolia		Shrub	Mesic to wet forest		X			
halemanui		Shrub	Mesic to wet forest	X				
celastroides	***hanapepensis***	Shrub	Mesic forest	X				
deppeana		Subshrub	Mesic forest		X			
eleanoriae		Shrub	Mesic forest	X				
celastroides	***tomentella***	Shrub	Forest		X			
atrococca		Shrub to small tree	Dry to mesic forest	X				
arnottiana		Shrub	Dry forest		X		X	
celastroides	***amplectens***	Shrub	Dry forest	X	X	X	X	X
celastroides	***lorifolia***	Shrub to small tree	Dry forest				X	
multiformis	***multiformis***	Shrub	Dry forest		X		X	
multiformis	***microphylla***	Shrub	Dry forest	X	X	X	X	
olowaluana		Tree	Dry forest				X	X
skottsbergii	***vaccinioides***	Shrub	Scrub			X	X	
kuwaleana		Shrub	Scrub		X			
celastroides	***celastroides***	Shrub	Coastal	X				
celastroides	***stokesii***	Shrub	Coastal	X		X		
celastroides	***kaenana***	Shrub	Coastal		X			
celastroides	***laehiensis***	Shrub	Coastal				X	
degeneri		Subshrub	Coastal	X	X	X	X	X
skottsbergii	***skottsbergii***	Shrub	Coastal		X			
skottsbergii	***audens***	Shrub	Coastal			X		
No. of Taxa				**13**	**14**	**6**	**9**	**3**

Table 4.2. Data sets and parsimony tree characteristics for Hawaiian Chamaesyce phylogenetic analyses. A. individual chloroplast gene regions. B. concatenated cpDNA data set, and nuclear ITS, *LFY* and *G3pdhC* data sets.

A

Data set	*rpl16*	*trnH-psbA*	*trnL*	*trnL-F*	*rpl14-rpl36*	*psbB-psbH*	*atpI-atpH*	*psbD-trnT*
No. terminals	79	79	79	79	79	79	79	79
Aligned length	1373	898	624	455	1002	613	990	2252
Characters analyzed	1363	890	559	418	998	613	962	2171
Variable characters (proportion)	84 (6.2%)	84 (9.4%)	23 (3.7%)	27 (6.5%)	491 (6.9%)	23 (5.2%)	68 (7.1%)	149 (6.9%)
Informative characters (proportion)	34 (2.5%)	26 (2.9%)	6 (0.96%)	4 (0.96%)	26 (2.6%)	9 (1.5%)	19 (2.0%)	41 (1.9%)
No. indels coded	30	37	6	5	12	7	19	43

B

Data set	Concatenated cpDNA	ITS	*LFY*	*G3pdhC* copy 1	*G3pdhC* copy 2-4	*G3pdhC* copy 5-6
No. terminals	79	116	90	23	52	5
Aligned length	8207	706	1447	1063	1030	929
Characters analyzed	7949	706	1387	1063	1030	929
Variable characters (proportion)	513 (6.5%)	177 (25.1%)	405 (29.2%)	126 (11.9%)	171 (16.6%)	148 (15.9%)
Informative characters (proportion)	165 (2.1%)	109 (15.4%)	227 (16.4%)	9 (0.8%)	96 (9.3%)	54 (5.8%)
No. indels coded	147	-	88	12	19	12
No. MP trees	24	24	2922	4	465	1
MP tree length	806	318	868	168	219	176
CI	0.87	0.65	0.63	0.97	0.93	0.95
RI	0.88	0.90	0.73	0.81	0.99	0.87
Nucleotide substitution model selected by AIC	GTR+I+γ	GTR+I+γ	GTR+I+γ	-	-	-

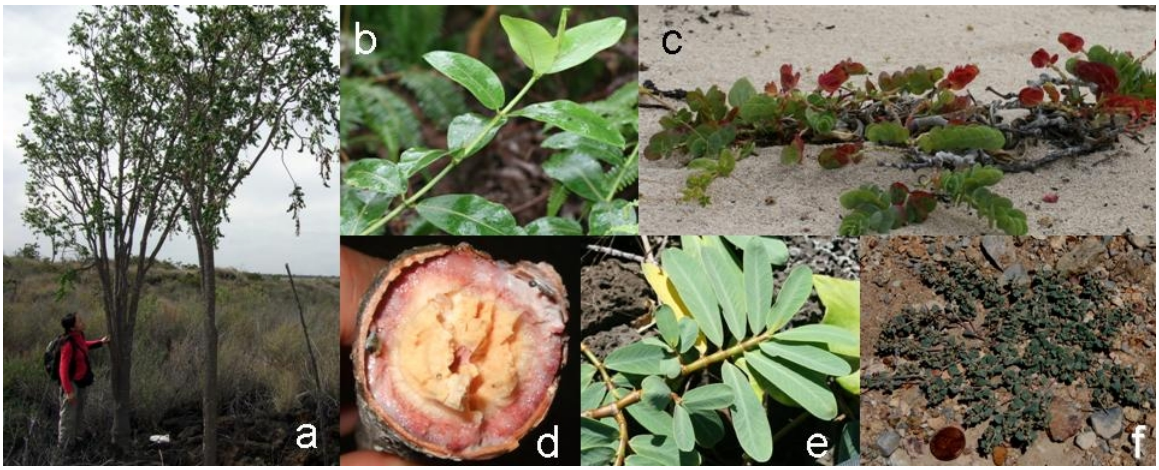


Figure 4.1. Hawaiian Chamaesyce (a-e) and their closely related North American species (f). a. *Euphorbia olowaluana*, a dry forest pioneer species on recently formed lava field, Hawaii; b. *E .remyi var. remyi*, an ascending shrub in wet forest understory, Kauai; c. *E. degeneri*, a prostrate subshrub on sandy beach, Oahu; d. soft and fleshy woody stem of *E. celastroides var. kaenana*; e. *E. celastroides var. kaenana*, a prostrate shrub, Oahu; f. *E. cinerascens*, a small, prostrate perennial herb native to deserts in southern United States and northeastern Mexico (see coin in the lower left corner for scale).

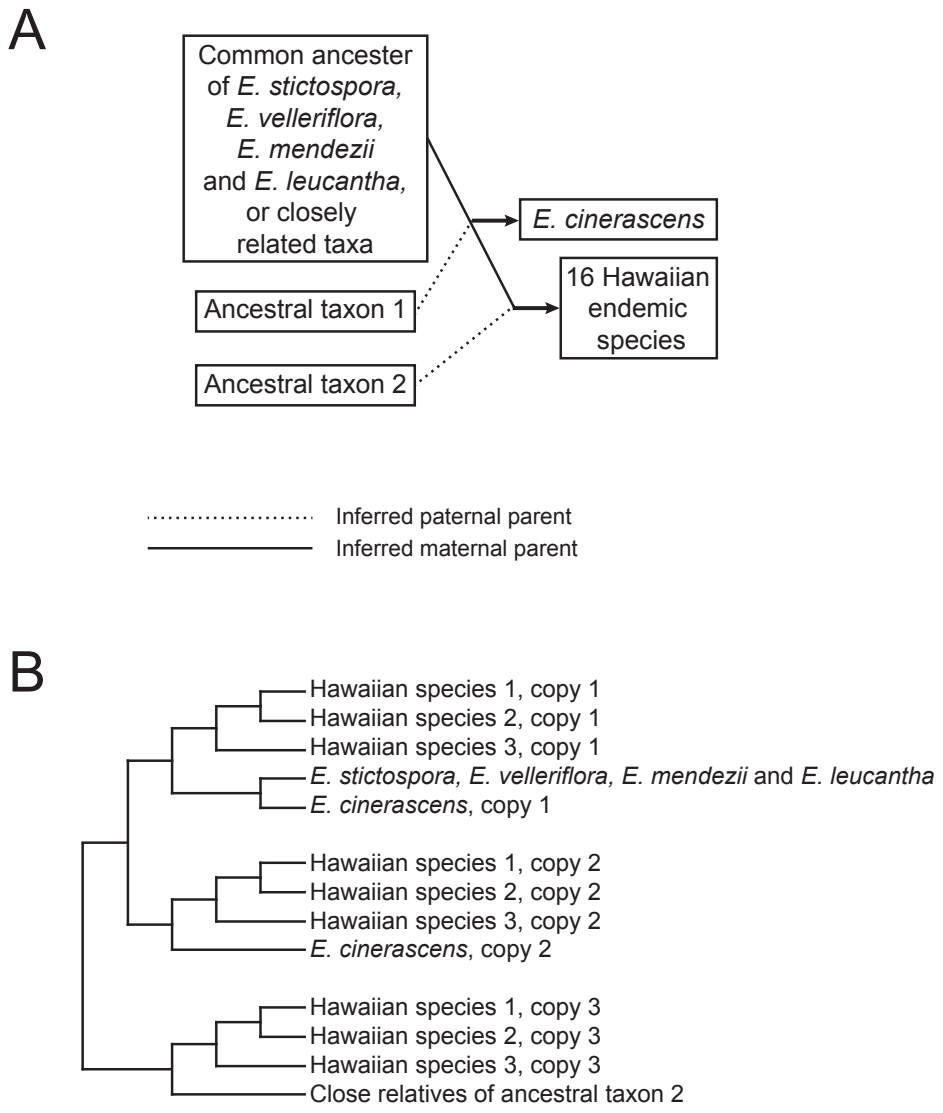


Figure 4.2. Hypothetical allopolyploid origin of Hawaiian Chamaesyce. A. relationships inferred from ITS, cpDNA, and *EMB2765* data sets in Yang and Berry (2011). Arrows go from putative parents toward derived hybrid taxa. The inferred paternal parent is indicated by dotted lines, and maternal parent by solid lines. B. Hypothetical relationships of nuclear low-copy genes given an allopolyploid origin of Hawaiian Chamaesyce.

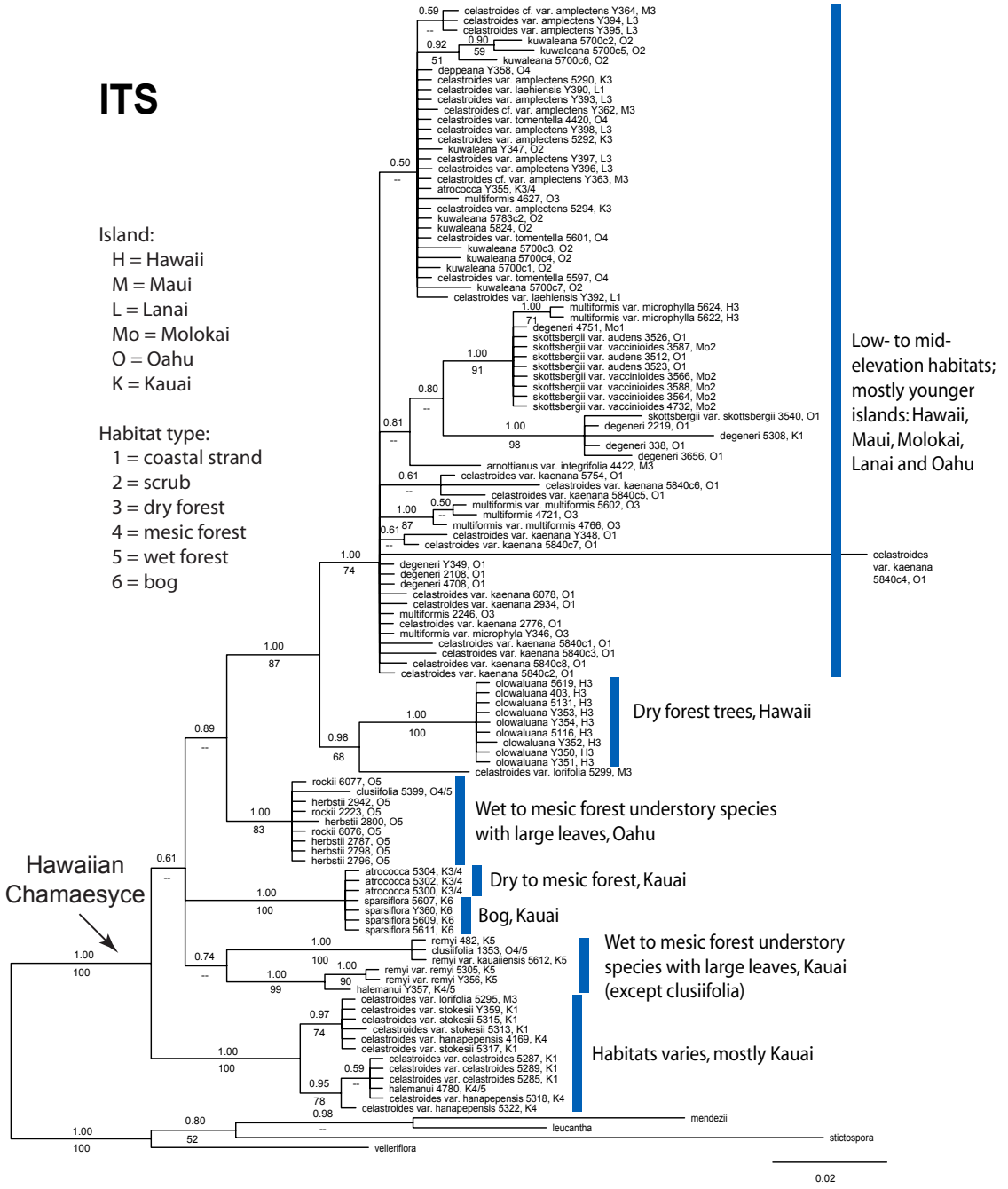


Figure 4.3. Majority rule consensus tree recovered from Bayesian analysis of nuclear ITS data. Numbers above the branches are Bayesian posterior probabilities and numbers below the branches are maximum parsimony bootstrap percentages. Branch length scale is on lower right. Following each taxon name is the DNA accession number, island initials, and vegetation type.

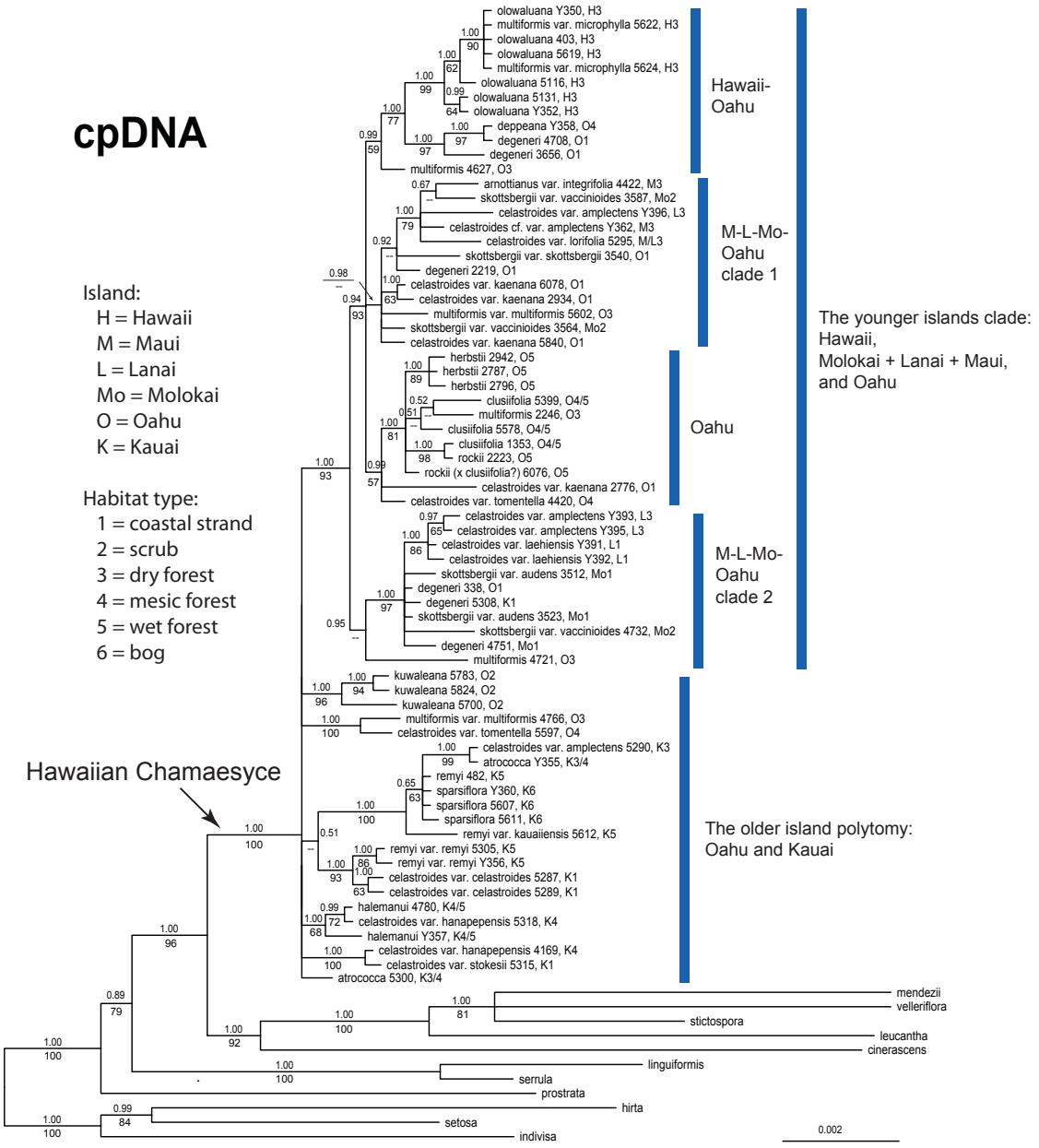


Figure 4.4. Majority rule consensus tree recovered from Bayesian analysis of the chloroplast DNA data (*rpl16* intron + *trnH-psbA* spacer + *trnL* intron + *trnL-F* spacer + *rpl14-rpl36* spacer + *psbB-psbH* spacer + *atpI-atpH* spacer + *psbD-trnT* spacer + indels, cpDNA). Numbers above the branches are Bayesian posterior probabilities and numbers below the branches are maximum parsimony bootstrap percentages. Branch length scale is on lower right. Following each taxon name is the DNA accession number, island initials, and vegetation type.

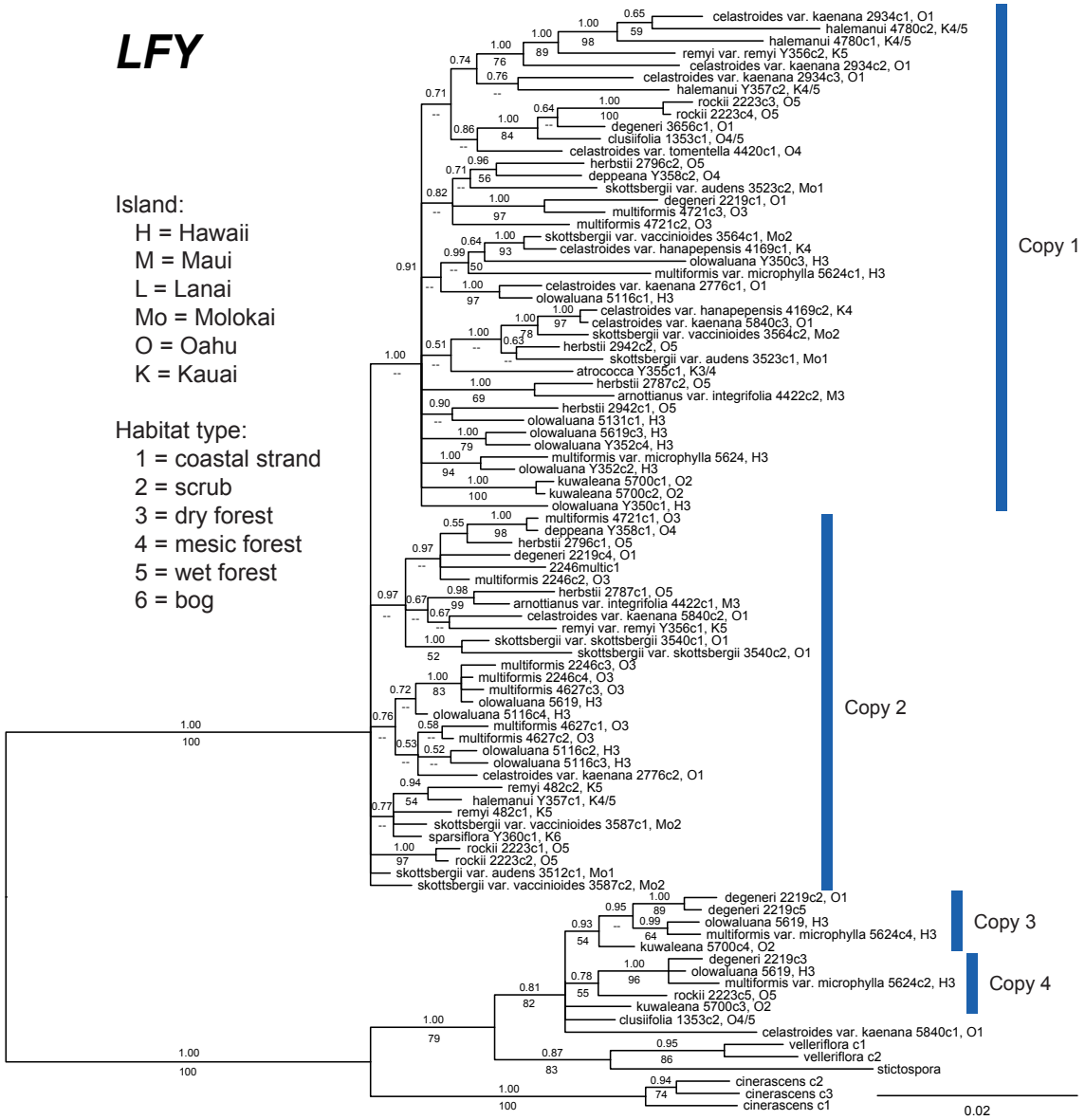


Figure 4.5. Majority rule consensus tree recovered from Bayesian analysis of the *LFY* data. Numbers above the branches are Bayesian posterior probabilities and numbers below the branches are maximum parsimony bootstrap percentages. Branch length scale is on lower right. Following each taxon name is the DNA accession number, island initials, and vegetation type.

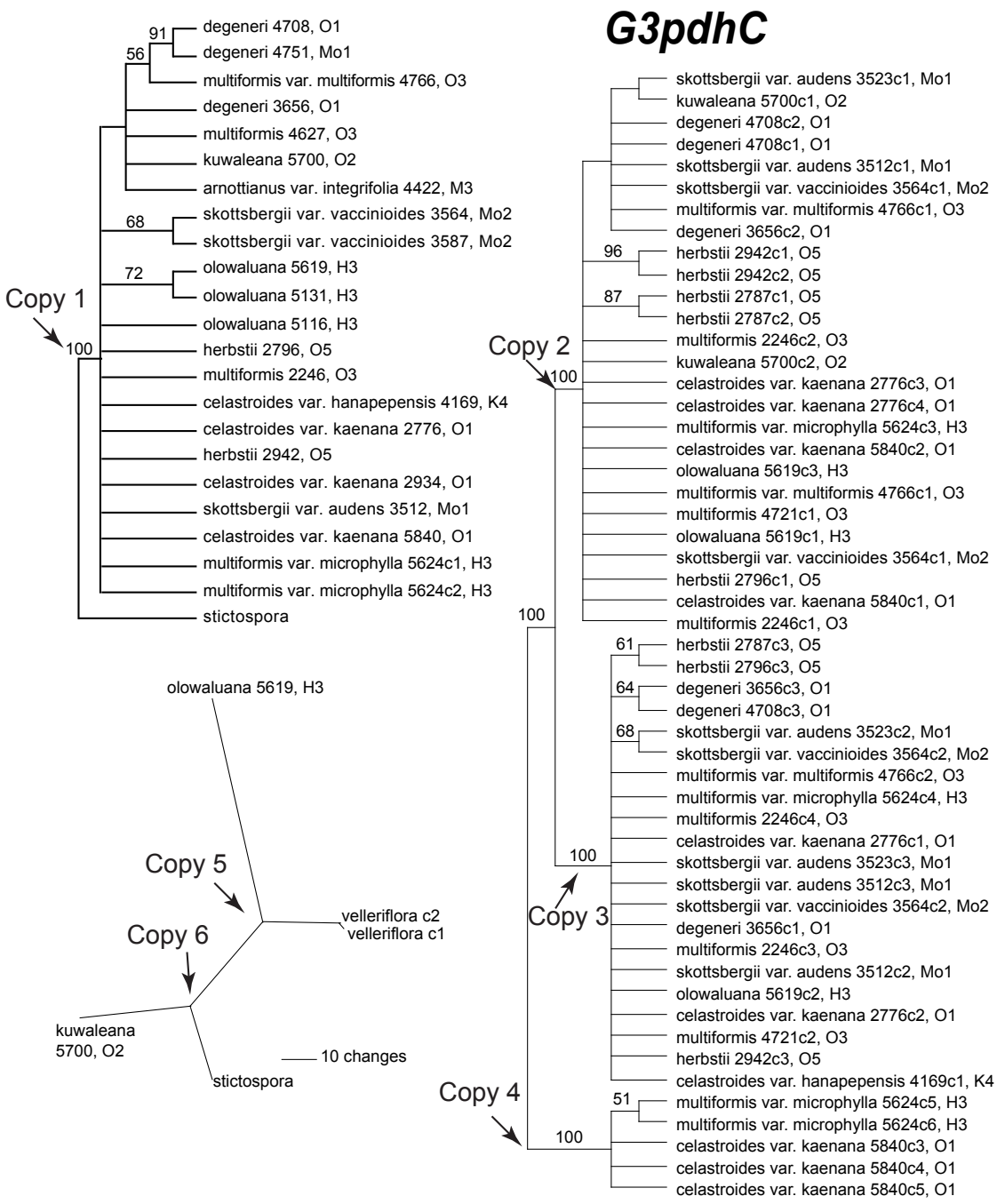


Figure 4.6. Majority rule consensus tree recovered from maximum parsimony analysis of the *G3pdhC* data (copy 1, and copies 2-4). Numbers above the branches are maximum parsimony bootstrap percentages. Following each taxon name is the DNA accession number, island initials, and vegetation type (see Fig. 4.5 for abbreviations). Only one tree was recovered from copies 5 and 6 and therefore the maximum parsimony phylogram is shown, with branch length scale on lower right.

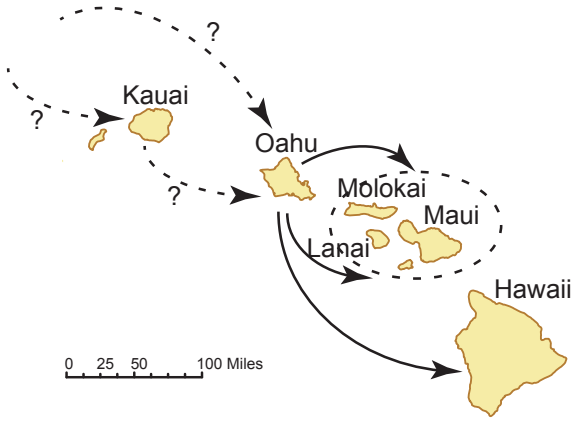


Figure 4.7. Major seed dispersal routes of Hawaiian Chamaesyce inferred from the chloroplast DNA phylogeny. Molokai, Maui and Lanai have been connected by land during the past 1-2 million years and are considered a single island group.

LITERATURE CITED

BACON, C. D., G. J. ALLAN, E. A. ZIMMER, AND W. L. WAGNER. 2011. Genome scans reveal high levels of gene flow in Hawaiian *Pittosporum*. *Taxon* 60: 733-741.

BALDWIN, B. G., D. W. KYHOS, J. DVORAK, AND G. D. CARR. 1991. Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae). *Proceedings of the National Academy of Sciences of the United States of America* 88: 1840-1843.

BALLARD, H. E., AND K. J. SYTSMA. 2000. Evolution and biogeography of the woody Hawaiian violets (*Viola*, Violaceae): Arctic origins, herbaceous ancestry and bird dispersal. *Evolution* 54: 1521-1532.

BARRIER, M., B. G. BALDWIN, R. H. ROBICHAUX, AND M. D. PURUGGANAN. 1999. Interspecific hybrid ancestry of a plant adaptive radiation: Allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotic gene duplications. *Molecular Biology And Evolution* 16: 1105-1113.

BOHLE, U. R., H. H. HILGER, AND W. F. MARTIN. 1996. Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). *Proceedings of the National Academy of Sciences of the United States of America* 93: 11740-11745.

BROWN, J. M., S. M. HEDTKE, A. R. LEMMON, AND E. M. LEMMON. 2010. When trees grow too long: Investigating the causes of highly inaccurate Bayesian branch-length estimates. *Systematic Biology* 59: 145-161.

BURCH, D. 1969. Notes on the Galapagos Euphorbieae (Euphorbiaceae). *Annals of the Missouri Botanical Garden* 56: 173-178.

CARLQUIST, S. 1966. The biota of long-distance dispersal. III. Loss of dispersibility in the Hawaiian flora. *Brittonia* 18: 310-335.

CARR, G. D. 1985. Additional chromosome numbers of Hawaiian flowering plants. *Pacific Science* 39: 302-306.

COMAI, L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* 6: 836-846.

DUNBAR-CO, S., A. M. WIECZOREK, AND C. W. MORDEN. 2008. Molecular phylogeny and adaptive radiation of the endemic Hawaiian *Plantago* species (Plantaginaceae). *American Journal of Botany* 95: 1177-1188.

EDGAR, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797.

EGGENS, F., M. POPP, M. NEPOKROEFF, W. L. WAGNER, AND B. OXELMAN. 2007. The origin and number of introductions of the Hawaiian endemic *Silene* species (Caryophyllaceae). *American Journal of Botany* 94: 210-218.

GIVNISH, T. J., K. C. MILLAM, A. R. MAST, T. B. PATERSON, T. J. THEIM, A. L. HIPP, J. M. HENSS, et al. 2009. Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society B-Biological Sciences* 276: 407-416.

GRIFFIN, P. C., C. ROBIN, AND A. A. HOFFMANN. 2011. A next-generation sequencing method for overcoming the multiple gene copy problem in polyploid phylogenetics, applied to *Poa* grasses. *BMC Biology* 9. doi:10.1186/1741-7007-9-19.

GRIMM, G. W., AND T. DENK. 2010. The reticulate origin of modern plane trees (*Platanus*, Platanaceae): A nuclear marker puzzle. *Taxon* 59: 134-147.
GUTTERMAN, Y., AND S. SHEM-TOV. 1997. Mucilaginous seed coat structure of *Carrichtera annua* and *Anastatica hierochuntica* from the Negev Desert highlands of Israel, and its adhesion to the soil crust. *Journal of Arid Environments* 35: 695-705.
HAMILTON, M. B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8: 521-523.
HEADS, M. 2011. Old taxa on young islands: a critique of the use of island age to date island-endemic clades and calibrate phylogenies. *Systematic Biology* 60: 204-218.
HOWARTH, D. G., AND D. A. BAUM. 2005. Genealogical evidence of homoploid hybrid speciation in an adaptive radiation of *Scaevola* (goodeniaceae) in the Hawaiian Islands. *Evolution* 59: 948-961.
HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.
JORDAN, M. S., AND W. J. HAYDEN. 1992. A survey of mucilaginous testa in *Chamaesyce*. *Collectanea Botanica (Barcelona)* 21: 79-89.
KIM, C., H. SHIN, Y. T. CHANG, AND H. K. CHOI. 2010. Speciation pathway of *Isoëtes* (Isoëtaceae) in East Asia inferred from molecular phylogenetic relationships. *American Journal of Botany* 97: 958-969.
KIM, S. T., AND M. J. DONOGHUE. 2008. Molecular phylogeny of *Persicaria* (Persicarieae, Polygonaceae). *Systematic Botany* 33: 77-86.
KIM, S. T., S. E. SULTAN, AND M. J. DONOGHUE. 2008. Allopolyploid speciation in *Persicaria* (Polygonaceae): Insights from a low-copy nuclear region. *Proceedings of the National Academy of Sciences of the United States of America* 105: 12370-12375.
KOUTNIK, D. L. 1987. A taxonomic revision of the Hawaiian species of the genus *Chamaesyce* (Euphorbiaceae). *Allertonia* 4: 331-388.
LINDQVIST, C., AND V. A. ALBERT. 2002. Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany* 89: 1709-1724.
LORENCE, D. H., AND W. L. WAGNER. 1996. A new species of *Chamaesyce* section *Sclerophyllae* (Euphorbiaceae) from Kaua'i. *Novon* 6: 68-71.
MADDISON, D. R., AND W. P. MADDISON. 2005. MacClade 4.08: an analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.
MAIZEL, A., M. A. BUSCH, T. TANAHASHI, J. PERKOVIC, M. KATO, M. HASEBE, AND D. WEIGEL. 2005. The floral regulator *LEAFY* evolves by substitutions in the DNA binding domain. *Science* 308: 260-263.
MARSHALL, D. C. 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: Lost in the land of long trees. *Systematic Biology* 59: 108-117.
MELZER, S., F. LENS, J. GENNEN, S. VANNESTE, A. ROHDE, AND T. BEECKMAN. 2008. Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. *Nature Genetics* 40: 1489-1492.
MORDEN, C. W., AND M. GREGORITZA. 2005. Population variation and phylogeny in the endangered *Chamaesyce skottsbergii* (Euphorbiaceae) based on RAPD and ITS analyses. *Conservation Genetics* 6: 969-979.

MORDEN, C. W., V. C. CARAWAY, AND T. J. MOTLEY. 1996. Development of a DNA library for native Hawaiian plants. *Pacific Science* 50: 324-335.

MOTLEY, T. J., AND L. RAZ. 2004. Origins, evolution, and systematics of Hawaiian and Pacific *Chamaesyce* (Euphorbiaceae). Botany 2004: Annual Meeting of the Botanical Society of America, Salt Lake City, Utah, USA [online abstract]. Website http://www.2004.botanyconference.org/engine/search/index.php?func=detail&aid=360.

MOYROUD, E., E. KUSTERS, M. MONNIAUX, R. KOES, AND F. PARCY. 2010. LEAFY blossoms. *Trends in Plant Science* 15: 346-352.

MÜLLER, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular Phylogenetics and Evolution* 38: 667-676.

NYLANDER, J. A. A. 2004. MrModeltest, version 2.3 for Mac. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Website: http://www.abc.se/~nylander/.

PEARCY, R. W., AND J. TROUGHTON. 1975. C_4 photosynthesis in tree form *Euphorbia* species from Hawaiian rainforest sites. *Plant Physiology* 55: 1054-1056.

PENFIELD, S., R. C. MEISSNER, D. A. SHOUE, N. C. CARPITA, AND M. W. BEVAN. 2001. *MYB61* is required for mucilage deposition and extrusion in the *Arabidopsis* seed coat. *Plant Cell* 13: 2777-2791.

RAMBAUT, A., AND A. DRUMMOND. 2007. Tracer v1.4. Computer program and documentation distributed by the author, website: http://beast.bio.ed.ac.uk/Tracer [accessed 14 Jul 2010].

RANDELL, R. A., AND C. W. MORDEN. 1999. Hawaiian Plant DNA library II: endemic, indigenous, and introduced species. *Pacific Science* 53: 401-417.

RONQUIST, F., AND J. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572 - 1574.

SAGE, R. F. 2004. The evolution of C_4 photosynthesis. *New Phytologist* 161: 341-370.

SEARS, B. B. 1980. Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. *Plasmid* 4: 233-255.

SHAW, J., E. B. LICKEY, E. E. SCHILLING, AND R. L. SMALL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94: 275-288.

SHAW, J., E. B. LICKEY, J. T. BECK, S. B. FARMER, W. S. LIU, J. MILLER, K. C. SIRIPUN, et al. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

STRAND, A. E., J. LEEBENSMACK, AND B. G. MILLIGAN. 1997. Nuclear DNA-based markers for plant evolutionary biology. *Molecular Ecology* 6: 113-118.

SWOFFORD, D. 2002. PAUP*: Phylogenetic analysis using parsimony (and other methods) 4.0 beta.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.

VOLZ, S. M., AND S. S. RENNER. 2009. Phylogeography of the ancient Eurasian medicinal plant genus Bryonia (Cucurbitaceae) inferred from nuclear and chloroplast sequences. *Taxon* 58: 550-560.

WADA, M., Q. F. CAO, N. KOTODA, J. SOEJIMA, AND T. MASUDA. 2002. Apple has two orthologues of *FLORICAULA/LEAFY* involved in flowering. *Plant Molecular Biology* 49: 567-577.

WAGNER, W. L., AND S. H. SOHMER. 1999. Manual of the flowering plants of Hawai'i. Revised and illustrated ed. University of Hawai'i Press, Honolulu, Hawai'i, USA.

WAGNER, W. L., D. R. HERBST, AND S. H. SOHMER. 1999. Manual of the flowering plants of Hawaii, revised edition with supplement by W. L. Wagner and D. R. Herbst. Bishop Museum Special Publication, vol. 97. University of Hawaii Press, Honolulu, Hawaii, USA.

WEI, X.-X., Z.-Y. YANG, Y. LI, AND X.-Q. WANG. 2010. Molecular phylogeny and biogeography of Pseudotsuga (Pinaceae): Insights into the floristic relationship between Taiwan and its adjacent areas. *Molecular Phylogenetics and Evolution* 55: 776-785.

WILLYARD, A., L. E. WALLACE, W. L. WAGNER, S. G. WELLER, A. K. SAKAI, AND M. NEPOKROEFF. 2011. Estimating the species tree for Hawaiian *Schiedea* (Caryophyllaceae) from multiple loci in the presence of reticulate evolution. *Molecular Phylogenetics and Evolution* 60: 29-48.

YANG, Y., AND P. E. BERRY. 2011. Phylogenetics of the Chamaesyce clade (*Euphorbia*, Euphorbiaceae): Reticulate evolution and long-distance dispersal in a prominent C4 lineage. *American Journal of Botany* 98: 1486-1503.

ZIEGLER, A. C. 2002. Hawaiian natural history, ecology, and evolution, 184-201. University of Hawai'i Press, Honolulu, Hawai'i, USA.

CHAPTER V

CONCLUSIONS

With the advance of molecular techniques, especially the development of sets of standard markers with universal primers readily available, we are now much better equipped to resolve phylogenetic relationships in groups that have been previously considered large and difficult. When we started the *Euphorbia* project five years ago, classification of *Euphorbia* was in considerable disarray. Most references dealt with a particular species, a chemical compound in a species, or at most a regional group, while the most recent comprehensive taxonomic revision was more than a century old. Different authors often referred to the same plant structure using different terms, and some of the primary literature was written in Latin, German, Spanish or French, taking considerable effort to interpret. The only molecular study available at the time (Steinmann and Porter, 2002) sampled only 10% of the species diversity in the genus, and it did not provide much support in deeper nodes. Many authors of regional flora surveys were still debating whether "*Euphorbia*" is one genus, or if it should be divided into several smaller genera instead (e.g., Koutnik, 1984; Ward, 2001).

My dissertation started with resolving the relationships in *Euphorbia* subgenus *Chamaesyce*, a group that is relative accessible due to its New World centered distribution. It was mainly circumscribed by molecular evidence, and its range of distribution and morphological diversity largely covered the spectrum of the entire genus. Combining field collection and observation, molecular tools, and studying herbarium specimens, I identified fifteen clades within the subgenus, each of which is distinctive molecularly, geographically and morphologically. Fifteen sections were therefore designated, and the majority of them were either newly named, or previous circumscriptions were considerably modified. Full descriptions and complete species list were provided, partly with help from our regional collaborators. Much of the

relationships recovered in this study were previously unknown, such as the sister relationship between the central Asian endemic section *Cheirolepidium* with the Australian endemic section *Eremophyton*. It turned out that they actually share many similarities in growth forms, cyathial morphology, leaf serration, and habitat types. The general biogeographical pattern within the subgenus is an Old World origin, with a New World clade sister to an Old World-eastern Brazilian clade, and together they nested in an Old World grade. In addition, relationships between plant architecture and photosynthetic systems were explored. The different modules in a *Euphorbia* plant, and the three organization levels we recognize in its synflorescence, may have provided opportunities for modifications that have led to unique adaptations in the genus.

Among the fifteen sections in subgenus *Chamaesyce*, section *Anisophyllum* contains the only lineage of C_4 plants. Section *Anisophyllum* has about 350 species and a worldwide distribution. Through a complex suite of character switches, including physiology and anatomy (C_4 photosynthesis), seed morphology (sticky surface and small size), and life history (reduced vegetative growth and prolonged reproductive stages), it became specialized to warm, dry, and disturbed habitats. Our well-sampled phylogeny indicates that section *Anisophyllum* originated in subtropical North America, diversified locally into three major clades, and subsequently achieved worldwide distribution through multiple long-distance dispersal events. Incongruence between ITS and chloroplast markers, and cloning of nuclear low-copy gene suggests extensive genetic mixing through reticulate evolution. In addition, long-distance dispersal followed by local adaptation has produced new species and groups with novel adaptations.

One of the most notable cases of such local adaptations is the radiation of woody Hawaiian clade of section *Anisophyllum*. With comprehensive taxon sampling including 15 of the total 16 species in the clade, our cpDNA data set suggests that seed dispersals were generally from older to younger island, although not strictly following a stepping-stone fashion. All nuclear data sets, ITS, *LFY* and *G3pdhC* show increased numbers of copies compared to outgroups, supporting the allopolyploid origin of the Hawaiian clade in section *Anisophyllum*. The reticulate relationship is further complicated by recent hybridizations, as evident from local discordances between ITS and cpDNA. In addition,

we documented for the first time recent, recurrent losses of copies of *LFY* following allopolyploidy, and the loss appears to be non-random.

Although the phylogenetic relationships within *Euphorbia* subgenus *Chamaesyce* has been largely resolved, certain parts still require further investigation. For example, section *Articulofruticosae*, a clade of stem succulents endemic to southern Africa, has gone through a substantial recent radiation. While the section is one of the most molecularly and morphologically well defined in the genus, species delimitation within the section is still very problematic. On the other hand, the New World sections are relatively well studied. However, some of these groups have gone through recent diversification with reticulate history. Similar to the patterns in section *Anisophyllum*, the entirely New World section *Alectoroctonum* shows some major discordance among markers, and further resolving the relationships within this group will require additional molecular as well as taxon sampling. This group is particularly interesting because stem succulents evolved at least three times within it, and CAM photosynthesis has been confirmed by stable isotope ratios in this group. Having a good resolution of its evolutionary history will help us to understand the pre-adaptations for evolving CAM photosynthesis, in a clade that is closely related to the largely C_4 section *Anisophyllum*.

Future work would also investigate the evolution of niche diversification in the subgenus, especially its correlation with the parallel evolution of succulent growth forms and CAM photosynthesis. Another interesting topic would be to investigate the utility of targeted next-generation sequencing to resolve groups of recent and rapid diversification, especially those that are complicated by polyploidy.

LITERATURE CITED

HORN, J. W., B. W. VAN EE, J. J. MORAWETZ, R. RIINA, V. W. STEINMANN, P. E. BERRY, AND K. J. WURDACK. in press. Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). *Molecular Phylogenetics and Evolution*.

KOUTNIK, D. L. 1984. *Chamaesyce* (Euphorbiaceae) - a newly recognized genus in southern Africa. *South African Journal of Botany* 3: 262-264.

STEINMANN, V. W., AND J. M. PORTER. 2002. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* 89: 453-490.

WARD, D. B. 2001. New combinations in the Florida flora. *Novon* 11: 360-365.