Phylogenetics and Morphological Evolution of *Euphorbia* subgenus *Euphorbia*

by

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Dedicated to my Father, who taught me “There is no such thing as can’t.”

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Chapter I

Introduction

Euphorbia is among the largest angiosperm genera, with approximately 2000 species, and it has long been admired for its great diversity of growth forms, including many xerophytic species. Despite its great vegetative diversity, the genus is united morphologically by the possession of a cyathium, a highly reduced inflorescence that resembles a single flower (Steinmann & Porter, 2002). It has recently been established that the genus is composed of four subgenera (Steinmann & Porter, 2002; Bruyns & al., 2006; Bruyns & al., 2011; Horn & al., 2012). Of these four, subg. *Euphorbia* is the largest and most morphologically diverse, and this makes it a particularly good group in which to investigate the causes of morphological evolution. My dissertation focuses on the phylogenetic relationships of subg. *Euphorbia* and its morphological evolution at two phylogenetic scales – within the largest section of the subgenus and across the subgenus as a whole.

Chapter II — The goal of chapter two was to establish a phylogenetic framework for further investigations of the evolution of morphological diversity in subg. *Euphorbia* and to produce a classification for the group. Relationships within this group have been difficult to discern due mainly to homoplasious morphological characters and inadequate taxon sampling in previous phylogenetic studies (White & al., 1941; Steinmann & Porter, 2002; Bruyns & al., 2006; Cacho & al., 2010; Bruyns & al., 2011). Previous studies had established the monophyly of several clades belonging to subg. *Euphorbia*, but in each case the focus on the entire genus or was limited in geographic scope (Steinmann & Porter, 2002; Bruyns & al., 2006; Bruyns & al 2011; Horn & al., 2012). Consequently, a comprehensive phylogeny was still lacking. Because of immeasurable assistance from collaborators, I was able to obtain 317 individual exemplars from 226 species of *Euphorbia* from across its known geographic range. From these accessions I sequenced two chloroplast coding regions (*mat*K and *ndh*F) and the internal transcribed spacer region of the nuclear ribosomal DNA (ITS), for the largest DNA sequence data set of subg. *Euphorbia* to date. These data were used to infer the phylogenetic relationships within the subgenus and, along with morphological and geographic distribution data, to assign 661 species to sections within subg. *Euphorbia*. Using this phylogenetic framework, I discuss patterns of homoplasy in morphological evolution and general patterns of biogeography. Finally, I present a new subgeneric classification of subg. *Euphorbia* comprising 21 sections, nine of them newly described here.

Chapter III — The largest section in subg. *Euphorbia* is sect. *Euphorbia*, the 'spine-shield' euphorbias. The spine-shield euphorbias, so-called because of the hard pad of tissue at each leaf axil that bears several spines, are perhaps the most recognizable of the xerophytic groups within *Euphorbia*. These species, along with the cacti, are a classic example of convergent evolution in distantly related groups. The spine-shield euphorbias are almost exclusively spiny, stem-succulent species, ranging from dwarf shrubs less than 15 cm tall to large trees over 25 m high, although a few geophytes belong to the clade as well. Because of this variation in stature and the section's distribution from Southeast Asia through India and across much of Africa, this group is an excellent system in which to investigate the roles of biogeography and habitat variation in growth form evolution. In this chapter I produced the most comprehensive phylogeny of sect. *Euphorbia* to date and used this to reconstruct the biogeographic history and the ancestral growth forms of the section. I then tested the hypothesis that variation in climatic parameters selected for three dominant growth forms within the spin-shield euphorbias. Our results show that ancestral sect. *Euphorbia* were trees that likely originated in eastern Africa and subsequently spread across Africa and into southern Asia, through a combination of vicariance events at the periphery of, and diversification within, the ancestral range. Our analyses also show that the evolution of different growth forms within subg. *Euphorbia* has been an adaptive response to variation in climatic parameters, but that the dynamic selective landscape defined by these parameters allowed for rapid switching among forms.

Chapter IV — Within *Euphorbia* subg. *Euphorbia* there is a pattern of repeated evolution of species with semi-succulent, leafless, photosynthetic stems (termed ‘pencil-stem’ plants), which suggests that these species have adapted in response to similar selective filters. The strategy of shifting photosynthesis to the stem has been hypothesized to be an adaptation to conserve water in arid habitats. An interesting caveat to this hypothesis, as applied to subg. *Euphorbia* especially in Madagascar, is that the geographic distributions of leafless species overlap with those of leafy species. In this chapter, I tested the hypothesis that the evolutionary loss of leaves and switch to stem-based photosynthesis in *Euphorbia* subg. *Euphorbia* is an adaptation to specific niche parameters related to water availability. Estimates of species climatic niches (temperature and precipitation tolerances) and a relatively dated phylogeny of the subgenus were used to test for differences in these parameters between leafy and leafless species. The results indicate that while there is a significant difference between the ‘climatic niches’ of leafy vs. leafless species in subg. *Euphorbia*, this pattern breaks down among closely related species where further diversification of climatic niches has occurred. This pattern of niche differentiation, subsequent to selection by a common filter of low water availability, suggests a possible explanation for the co-occurrence of the two growth forms.

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Chapter II

Phylogenetics, morphological evolution, and classification of *Euphorbia* subgenus *Euphorbia*

INTRODUCTION

Euphorbia is one of the largest recognized genera of flowering plants, with about 2,000 species. It has a worldwide distribution but is especially diverse in arid or semi-arid regions of the tropics and subtropics. The morphological diversity in this genus includes geophytes, herbs, shrubs, understory and canopy trees, and an array of succulent and xerophytic forms. Despite this vast vegetative variation, the entire genus is united by a distinctive morphological synapomorphy, the cyathium – a pseudanthial inflorescence that looks superficially like a typical dicot flower (Steinmann & Porter, 2002; Horn & al., 2012). This structure is intermediate between a flower and an inflorescence in developmental terms (Prenner & Rudall, 2007) and is comprised of a cup-like involucre that surrounds multiple male flowers (reduced to single stamens) and a single female flower (reduced to a single pistil). From this basic structure various elaborations have evolved, including colorful subtending bracts, cyathial nectary glands with petaloid appendages, and fusion or addition of cyathial glands. Some of these cyathial traits represent synapomorphies for particular clades within the genus. Despite the information provided by the cyathium and its variations, relationships among species within *Euphorbia* based on morphological characters have been shown to be equivocal in many cases (Steinmann & Porter, 2002).

Previous phylogenetic work based on DNA sequence data suggests that the evolution of characters in *Euphorbia,* including growth form, photosynthetic systems, and cyathial form are highly homoplasious, and that the genus has a complex biogeographic history leading to its nearly worldwide distribution (Steinmann & Porter, 2002; Haevermans & al., 2004; Bruyns & al., 2006; Park & Jansen, 2007; Bruyns & al., 2011; Horn & al., 2012; Yang & al., 2012). These evolutionary and biogeographic patterns make *Euphorbia* an ideal system for the study of complex character evolution and adaptation of plants to different environments. Establishing a well-resolved phylogenetic framework for the major clades of the genus is a prerequisite to this research.

Over the past decade, molecular phylogenetic studies have made much progress in understanding the broad scale relationships within *Euphorbia* (Steinmann & Porter, 2002; Bruyns & al., 2006; Park & Jansen, 2007; Bruyns & al., 2011; Horn & al., 2012; Yang & al., 2012). Steinmann & Porter (2002) circumscribed *Euphorbia* as the clade including all species with cyathia and furthermore established the presence of four major clades within the genus. Bruyns & al. (2006) formally recognized these four clades as subgenera: *E.* subg. *Esula* Pers., *E.* subg. *Rhizanthium* (Boiss.) Wheeler, *E.* subg. *Chamaesyce* Raf., and *E.* subg. *Euphorbia*. Horn & al. (2012) analyzed ten gene regions from all three plant genomes to firmly establish that subg. *Esula* is the first clade to diverge, followed by subg. *Rhizanthium*, which is sister in turn to the clade of subg. *Chamaesyce* and subg. *Euphorbia*. With the relatively sparse taxon sampling in all previous phylogenetic studies, many species in *Euphorbia* had not been placed to their corresponding subgenus, and relationships within the subgenera are still incompletely resolved. From an evolutionary and taxonomic standpoint, there is a need to develop a comprehensive sectional classification for each of the subgenera. In this study we attempt to resolve these issues in subg. *Euphorbia*, which is the largest subgenus in *Euphorbia*.

Euphorbia subg. *Euphorbia* contains over 650 species, and it is the most diverse of the four subgenera in terms of cyathial variation, growth form, and habitat (Horn & al., 2012). Variation in cyathial morphology in this subgenus is particularly high and had been the basis for previously recognizing five segregate genera. These segregates include sect. *Monadenium* (including the former genera *Monadenium* Pax + *Synadenium* Boiss. + *Endadenium* L.C. Leach) from eastern tropical Africa, sect. *Crepidaria* (represented by the former genus *Pedilanthus* Necker) from Mexico and the Caribbean, and sect. *Cubanthus* (the former genus *Cubanthus* (Boiss.) Millspaugh) from the Caribbean. Each of these groups displays a unique variation on the basic cyathial form. The genus *Elaeophorbia* was also previously segregated based on the possession of fleshy fruits, a rare character state in *Euphorbia*. Species of subg. *Euphorbia* include herbs, geophytes, woody shrubs, understory and canopy trees, stem succulents that range from dwarf shrubs to candelabriform trees, and the unusual growth form of pencil-stemmed plants (e.g. *E. tirucalli*, Fig. 2.1F). Woody shrubs and trees occur in both xeric and mesic forests of Madagascar and in the New World tropics. Herbaceous species are found in South America, Africa, and Australia. Geophytes have evolved several times in subg. *Euphorbia* and are found in Africa, Madagascar, the Arabian Peninsula, and southern Asia. The species of sect. *Euphorbia* from Africa and Asia are often compared to the cacti of the New World as a classic example of convergent evolution, and they are the prime representatives of stem succulents in subg. *Euphorbia*.

Bruyns & al. (2006) proposed a sectional classification for subg. *Euphorbia* based almost entirely on Old World species, but their relatively small taxonomic sampling did not allow for the establishment of a comprehensive phylogenetic hypothesis and classification. Even with an expanded sampling of mostly African and Arabian species from subg. *Euphorbia* (Bruyns & al., 2011), important groups from Madagascar and the New World were not well represented in a phylogenetic analysis of subg. *Euphorbia*; a much broader sampling of the putative members of subg. *Euphorbia* from across the geographic range of the subgenus is required to gain a global understanding of the evolutionary relationships in this complex group.

To date, the relationships and phylogenetic position of the New World species in subg. *Euphorbia* have not been suitably resolved (Steinmann & Porter, 2002; Bruyns & al., 2006). The 26 New World species in subg. *Euphorbia* included by Zimmerman & al. (2010) formed a grade in their analysis of ITS and *trn*L-*trn*F chloroplast spacer data, but the 11 species sampled by Horn & al. (2012) for their 10-locus study formed a well-supported clade. The placement of the New World members of subg. *Euphorbia* and determining whether they represent a single clade is one of the main aims of this study.

Another key question involves an ecologically and morphologically diverse set of approximately 120 species from Madagascar. In contrast to other well defined Old World groups, such as sect. *Euphorbia* and sect. *Monadenium*, the Madagascan species are the least understood group of species in subg. *Euphorbia* from a phylogenetic standpoint (Steinmann & Porter, 2002; Haevermans & al., 2004; Bruyns & al., 2006; Park & Jansen, 2007; Zimmermann & al., 2010; Horn & al., 2012). The Madagascan species include, among others, the crown-of-thorns complex (*E. milii* and allies) and coraliform, pencil-stemmed trees such as *E. tirucalli* and *E. alluaudii*. There have been two clades of Madagascan species resolved in all previous phylogenetic studies involving subg. *Euphorbia,* but their relationship to each other has not been well resolved. All of the pencil-stemmed species in *Euphorbia* have previously been placed in one group, sect. *Tirucalli*, (e.g. Boissier, 1862; Hassall, 1977), but there is ample evidence now that this previous circumscription of sect. *Tirucalli* does not represent a monophyletic group (Boissier, 1862; Hassall, 1977; Steinmann & Porter, 2002; Haevermans, 2003; Haevermans & al., 2004; Bruyns & al., 2006; Barres & al., 2011; Horn & al., 2012; Yang & al., 2012). Also, there are a number of poorly-studied leafy species, such as *E. pervilleana* from Madagascar, which have been placed in sect. *Tirucalli* by Bruyns & al. (2006), but are likely part of a separate clade (Haevermans & Labat, 2004). Because the position of these species within subg. *Euphorbia* has not been congruent among previous studies, we still do not know if the Madagascan species form a single clade or represent two or more independent radiations on the island.

As part of the worldwide *Euphorbia* Planetary Biodiversity Inventory (PBI; www.euphorbiaceae.org) project, the goals of the present study are to: 1) produce a robust phylogenetic hypothesis of the relationships of all major groups in subg. *Euphorbia* based on a thorough taxonomic and geographic sampling and utilizing multiple regions of DNA sequence data, 2) establish the phylogenetic position of as many members of subg. *Euphorbia* as possible, and 3) create a sectional classification system for the subgenus. We plan to use this phylogenetic framework to address the outstanding phylogenetic questions within subg. *Euphorbia,* as mentioned above. In particular, we aim to determine if the New World species form a monophyletic group and to resolve the relationships of the Madagascan species.

MATERIALS AND METHODS

***Taxonomic sampling* —** A total of 317 accessions representing 226 species of *Euphorbia* were analyzed in this study, including ten outgroup species representing the three other subgenera within the genus. Taxa were selected to obtain a broad and thorough sampling of putative members of subg. *Euphorbia* across its geographic range based on previous molecular phylogenetic studies and the collective knowledge of the collaborators within the *Euphorbia* PBI project. Material for DNA extraction was collected by the authors and by additional PBI collaborators from live plants in the field or in living collections, as well as from herbarium collections. Live tissue was preserved in silica gel prior to extraction. Vouchers of wild specimens were collected as whole or partial plants and deposited at MICH and/or local herbaria (see Appendix). Greenhouse specimens were documented with digital photographs because destructive sampling was discouraged by collections owners. In this case, photo voucher specimens were made using a set of printed photographs from each sampled specimen and deposited at MICH.

***DNA extraction, amplification, and sequencing* —** Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, California, USA) following the manufacturer's instructions with slight modification for herbarium material. DNA was diluted 10--50 times to reduce inhibition of PCR enzymes by secondary compounds. Primer pairs were chosen for this study based on their previous utility in phylogenetic studies of *Euphorbia* or preliminary tests for polymerase chain reaction (PCR) amplification specificity, phylogenetic information content, and absence of long polyA/T regions that may interrupt sequencing reactions (Steinmann & Porter, 2002; Haevermans & al., 2004; Bruyns & al., 2006; Steinmann & al., 2007). The final regions chosen were the cpDNA coding region *mat*K including the partial *trn*K intron, the cpDNA coding region *ndh*F, and the internal transcribed spacer region of the nuclear ribosomal DNA (ITS). All PCR reactions from genomic DNA were carried out using *Ex Taq*™ taq-polymerase and buffer (Takara Bio Inc., Otsu, Shiga, Japan).

The *ndh*F gene was PCR amplified in two pieces: the 5' half was amplified using primers 536F and 1318R (Olmstead & Sweere, 1994), and the 3' half using primers 972F (Olmstead & Sweere, 1994) and 2110Ri (Steinmann & Porter, 2002). The 15 µl PCR mixture contained 1.5 µL 10×*Ex Taq*™ Buffer, 1.2 µL dNTP (2.5 mM), 0.4–0.6 µL of each primer (10 µM), 0.075 µL of 5 units/µL *Ex Taq*™, 2–2.5 µL of diluted template DNA and the remainder of ddH$_2$O. The PCR protocol consisted of an initial denaturing step of 95°C for 2 min followed by 35 cycles of denaturing at 95°C for 45 s, annealing at 53.8°C for 45 s, extension at 65°C for 3 min and then a final extension step at 65°C for 5 min (modified from Shaw & al., 2005). The *mat*K region was amplified using the primers *trn*K570F and *mat*K1710R (Samuel & al., 2005). The 15 µl PCR mixture contained 1.5 µL 10×*Ex Taq*™ Buffer, 1.2 µL dNTP (2.5 mM), 0.5 µL of each primer (10 µM), 0.12 µL of 5 units/µL *Ex Taq*™, 2 µL of diluted template DNA and the remainder of ddH$_2$O. The PCR protocol consisted of an initial denaturing step of 95°C for 2 min followed by 35 cycles of denaturing at 95°C for 45 s, annealing at 57°C for 45 s, extension at 65°C for 5 min and then a final extension step at 65°C for 10 min (modified from Shaw & al., 2005). The ITS region was amplified using the primers ITS-I (Urbatsch & al., 2000) and ITS4 (White & al., 1990). The 15 µl PCR mixture contained 1.5 µL 10×*Ex Taq*™ Buffer, 1.2 µL dNTP (2.5 mM), 0.5 µl of 5M Betaine solution (Sigma-Aldrich, Inc., St. Louis, Missouri, USA), 0.5 µL of each primer (10 µM), 0.12 µL of 5 units/µL *Ex Taq*™, 2 µL of diluted template DNA and the remainder of ddH$_2$O. A touchdown PCR protocol was used for the ITS region with an initial denaturing step at 95°C for 2 min and a final extension at 72°C for 15 min. The intervening 35 cycles each had a 2 min denaturing step of 30 s at 95°C and an extension step of 45 s at 72°C. The annealing temperature was decreased from 60°C to 50°C as follows: 1 cycle at 60°C, 2 cycles at 59°C, 3 cycles at 58°C, 4 cycles at 57°C, 5 cycles at 55°C, 6 cycles at 52°C, and 14 cycles at 50°C.

All PCR products were examined by gel electrophoresis on 1% agarose gels. Specific PCR products were purified using ExoSap-IT® (USB Corporation, Cleveland, Ohio, USA). For weak PCR products, or products with primer dimers, the QIAquick PCR Purification Kit was used. All PCR products were sequenced at the University of Michigan DNA Sequencing Core. ITS and *ndh*F PCR products were sequenced using the amplification primers. To maximize bidirectional reads for the entire length of the *mat*K region the *mat*K80F primer (Samuel & al., 2005) and three custom primers [*mat*K688R (5'-CRA GAT GRA TGG GRT AMG G-3'), *mat*K1850R (CGT CCT CTA TAT AAC TTG CGC G), and *mat*K1387F (CAG TAG GAC ATC CTA TTA GTA AAC CG)] were used in addition to amplification primers for sequencing.

***DNA sequence alignment and Models of Evolution* —** All ABI chromatograms from sequencing were edited and assembled using the Staden software package (v. 2.0; Staden, 1996). All sequences are deposited in GenBank, and species names, vouchers, and GenBank accession numbers are given in the Appendix. Sequences of each region were aligned using the MUSCLE web server at http://www.ebi.ac.uk/Tools/msa/muscle (Edgar, 2004) and adjusted manually using the program BioEdit v. 7.1.3 (Hall, 1999) to maximize the similarity among sequences in the relatively few regions MUSCLE unnecessarily created multiple gaps when fewer gaps could be inferred (Simmons, 2004). Some regions of the partial *trn*K intron flanking the *mat*K gene were difficult to unambiguously align and were excluded from the alignment. In total, seven data matrices were assembled. For the ML analyses all accessions were included to produce matrices for each of the individual regions, the cpDNA regions concatenated, and all regions concatenated (*mat*K, *ndh*F, ITS, cpDNA, and 3-gene matrices, respectively). A reduced taxon set that minimized missing data was used to construct concatenated matrices of the cpDNA (cpDNA-min) and of all regions (3-gene-min) for the Bayesian analyses (see below). Indels were coded as binary characters for the *mat*K and the *ndh*F regions using the simple coding method of Simmons & Ochoterena (2000) as implemented in the program SeqState v. 1.4.1 (Müller, 2005, 2006). ITS indels were not coded because many were part of ambiguously aligned sections that were more common and spread out in the ITS matrix than in the chloroplast datasets. The best-fitting model of sequence evolution for each of the three individual matrices was selected using Modeltest v. 3.07 (Posada & Crandall, 1998), employing the Akaike Information Criterion (AIC). Modeltest chose the GTR+I+γ model of nucleotide substitution for all regions and concatenated data sets except *mat*K, for which the TVR+I+γ model was chosen. However, because MrBayes does not allow this model to be specified, and to avoid potential parameter interactions by modeling among-site rate variation with two parameters (RAxML manual, v 7.0.4), we used the GTR+γ model for all analyses.

***Phylogenetic Inference* —** Separate data partitions were defined for each gene region and character type (DNA or indel), and for codon position within the *ndh*F region. In the alignment of the *mat*K region, which includes the *trn*K intron and many more gaps across the entire matrix, we were unable to identify a reliable orf across sequences. We therefore decided not to treat codon positions as separate partitions in the analysis. Maximum likelihood estimation (MLE) of phylogeny and model parameters was performed using the program RAxML v. 7.2.8 (Stamatakis, 2006). Where applicable, all model parameters except for branch lengths were estimated separately for each partition. Branch lengths were estimated as the average across partitions. Matrices were analyzed using the rapid bootstrap (1000 pseudoreplicates) plus thorough MLE search option [-f a]. Bayesian inference (BI) of phylogeny and model parameters was performed on the cpDNA-min and 3-gene-min matrices using MrBayes v. 3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Six independent MCMC runs of 4 chains each were run for 14.8 x 10^7 generations sampling every 10^4 generations. Stationarity and convergence of the MCMC runs was assessed using the programs Tracer v. 1.5 (Rambaut & Drummond, 2007) and online version of AWTY (http://ceb.csit.fsu.edu/awty, Wilgenbusch & al., 2004). Preliminary analyses were used to determine the appropriate parameters for the MCMC runs with the following results. To achieve swap frequencies of 20–60% between adjacent heated chains, the temperature parameter was set to 0.02. To prevent unrealistically long tree length estimates, the mean of the prior distribution of tree lengths was adjusted as suggested by Marshall (2010) to a final value of 250 [brlenspr = unconstrained: exponential (250)]. Reduced taxa matrices were used because original matrices failed to converge on a single posterior distribution of tree and model parameters. Recent studies have found that Bayesian analyses can be biased by missing data, potentially due to improper branch length estimation (Marshall & al., 2006; Lemmon & al., 2009; Marshall, 2010). These matrices contained approximately 30% missing data due to variation in sequencing success for regions among taxa. In preliminary analyses of both the cpDNA matrix and the 3-gene matrix, each with all taxa included, multiple independent runs appeared to converge on a particular log likelihood (LnL) and tree length (TL) distribution while other runs converged on a markedly different distribution of each parameter. In these cases a higher mean LnL was consistently associated with a lower mean TL. When the proportion of missing data in the matrices was reduced to approximately 10% (including gaps) by removing most taxa without all three regions sequenced, much better performance of the MCMC chains was achieved in terms of chain swap frequencies, mixing of chains, and convergence of all parameters.

***Assessment of Clade Membership* —** Morphological and geographic distribution data were compiled from herbarium collections, live collections, field observations, and the literature for all species included in our molecular data sets as well as any that were previously classified as closely related to these species or suspected to belong in subg. *Euphorbia*. These data were used to determine whether *Euphorbia* species not sampled for molecular sequence data sets belong to subg. *Euphorbia* and to assign these species to clades within the subgenus.

RESULTS

***Individual data sets* —** Summary statistics for each dataset are given in Table 1. The *mat*K data provided the greatest information across all levels of the subgenus, while *ndh*F was less informative, although it did provide support for major clades. The ITS data supported some major clades but provided little resolution regarding relationships among them, and was most informative for closely related groups (Fig. 2.S1 Electronic Supplement). The ML analyses of the individual *ndh*F and *mat*K data sets resulted in trees with some topological incongruence but no instances of well-supported conflict (trees not shown), so these were concatenated for the cpDNA analyses. We found eight instances of topological incongruence between the ITS phylogeny and the concatenated cpDNA phylogeny (Figs. 2.S1 and 2.S2) where both of the alternate topologies had bootstrap support (BS) over 80%. One of these involved the sister relationship of two species in sect. *Monadenium* and the other six the placement of species within sect. *Euphorbia.* Both of these sections are well-supported clades in all analyses. Notable among these incongruences, however, is the placement of *E. abdelkuri* and *E. lacei*. In the ITS tree, *E. abdelkuri* is sister to the rest of sect. *Euphorbia* and *E. lacei* is the next to diverge, while in the cpDNA tree these species are nested within the section close to a group of Indian species that includes *E. antiquorum* and *E. lactea*. Given that the few instances of conflict were within a single strongly supported clade, all data sets were concatenated for the 3-gene analysis.

***Phylogenetic results* —** Figure 2.2 shows the ML tree from the 3-gene data set. This phylogeny is largely congruent with the cpDNA and ITS trees; the few exceptions are noted below (Figs. 2.2, 2.S1—2.S2). The ML tree includes the most complete taxon sampling of subg. *Euphorbia*, so we chose to use this tree as the best estimate of the phylogeny. All data sets recovered a monophyletic subg. *Euphorbia* consisting of four major lineages (Figs. 2.2, 2.S1—2.S3). These clades can be distinguished by their geographic distributions and we refer to them as the Pacific Clade, the New World Clade, Old World Clade I, and Old World Clade II (Fig. 2.2). Support for each of the major lineages is high (BS >99%, posterior probability [PP] >0.95) from all data sets, with the exception of the ITS support for the New World Clade and Old World Clade II (BS 28% and 79%, respectively); however, the relationships among these clades are not well-supported (Figs. 2.2, 2.S1—2.S3). One species from Madagascar in our sampling, *E. mandravioky*, which represents sect. *Pachysanthae*, a newly defined section in subg. *Euphorbia*, is not well supported as part of any of the four major clades by any data set. It is sister to Old World Clade I in the cpDNA (BS 78%) and 3-gene trees (BS <50%) and sister to Old World Clade II in the ITS tree (BS 54%; Figs. 2.2, 2.S1—2.S2). The position of *E. mandravioky* as sister to Old World Clade I has the highest BS and makes the most sense geographically given that Old World Clade I is mainly a Madagascan clade.

Two additional topological incongruences exist between the ITS, cpDNA and 3-gene trees. First, *E. abdelkuri* and *E. lacei* are strongly supported as sister to the rest of sect. *Euphorbia* in the 3-gene tree (BS 93%) or moderately supported as successive sister lineages in the ITS tree (BS 81%). In contrast, their nested position in the cpDNA tree is well-supported (BS

100%), and the relationships at the base of section *Euphorbia* lack strong support. Second, the position of sect. *Rubellae,* from northeast Africa, is incongruent between the concatenated data sets. In the 3-gene tree, sect. *Rubellae* is sister to the rest of Old World Clade II with BS 68%. In the cpDNA tree this section is nested within Old World Clade II (BS = 82%) and sister to the clade of sect. *Monadenium* and sect. *Euphorbia* (BS = 77%). We note that while we chose the 3-gene tree to represent our best estimate of the phylogeny of subg. *Euphorbia*, the cpDNA data resolve the relationships within Old World Clade II with higher support. However, because the *mat*K matrix did not include sect. *Rubellae,* its position in the cpDNA tree is determined by the *ndh*F data only.

Among the MrBayes runs that had converged on the same posterior probability distribution, we discarded those that likely had not converged on the same distribution of trees, based on their substantially different support for individual clades (determined with the AWTY Compare analysis). However, for each data set there were two runs from which the AWTY Compare plots of posterior probabilities for clades were very close to the unity line, indicating that they had converged on the same distribution of trees. These runs were used to determine support for clades. The Cumulative analyses in AWTY for both the cpDNA-min and 3-gene-min data sets indicated that runs that had likely converged reached stationarity by generation $1.2\text{x}10^7$. All prior trees were discarded as burn-in. Although the difference in taxon sampling among inference methods does not allow for detailed comparisons between the Bayesian and ML trees, the topology of the 50% majority-rule consensus tree from the Bayesian analysis was generally congruent with the ML tree (e.g., the same major clades were recovered). The posterior probabilities of major clades and sections in subg. *Euphorbia* are summarized in Fig. 2.S3.

***Taxonomic results* —** The phylogenetic hypothesis presented here along with a review of morphological and geographic distribution data allowed us to assign 661 species to subg. *Euphorbia* and place all of these species to sections. These sections are indicated on Fig. 2.2 and are detailed below in the taxonomic treatment, where we newly define or significantly recircumscribe 11 sections, resulting in a total of 21 sections within subg. *Euphorbia*. The new sections are concentrated in the New World and Madagascar clades.

DISCUSSION

With our greatly increased sampling effort, we confirmed the results of several previous studies, which found that *Euphorbia* subg. *Euphorbia* is a strongly supported monophyletic group (Steinmann & Porter, 2002; Bruyns & al., 2006; Zimmermann & al., 2010; Horn & al., 2012). Our analyses also show that the subgenus is comprised of four strongly supported clades: the New World Clade, the Pacific Clade (sect. *Pacificae*), Old World Clade I, and Old World Clade II, although the relationships among them are not well supported (Fig. 2.2). The lack of resolution along the backbone of the subgenus in this and all previous studies, and the very short branch lengths at this level of the tree (data not shown), suggest that the lack of resolution is not an artifact of marker choice but rather a 'true' signal, which may be the result of a rapid radiation early in the evolutionary history of the subgenus. In addition, by comparing our results with those of Horn & al. (2012), we do not see an improvement of the support values in this part of the phylogeny through an increase in taxon sampling. Below we discuss significant morphological characters within each major clade and then discuss general patterns of morphological evolution within the subgenus.

New World Clade — All species from the New World shown here to belong to subg. *Euphorbia* are included in a single clade that is highly supported in all but the ITS analysis (Figs. 2.2, 2.S1—2.S3). The geographic distribution of species in subg. *Euphorbia* (Fig. 2.2) suggests that there has been a split between New World and Old World clades early in the history of subg. *Euphorbia*, although confidence in this interpretation is limited by the low support for the relationships among the Old World clades and the uncertain position of sect. *Pachysanthae*. New World species have been placed in subg. *Euphorbia* in previous studies (Steinmann & Porter, 2002; Bruyns & al., 2006; Zimmermann & al., 2010), but our sampling and sequence data are the first to show the extent of the New World lineage and to demonstrate its monophyly. The New World Clade consists of 11 highly supported clades or single species which we treat here as sections. They range in size from a single species in sect. *Lactifluae* to 36 species in sect. *Nummulariopsis*. The branches that connect the New World sections are very short relative to those that subtend each of them, and accordingly there is poor resolution of the sectional relationships within the New World Clade. As with the backbone of the subgenus, this pattern is the same as that found by Horn & al. (2012) in their species-poor but marker-intensive phylogeny; thus this pattern is not likely to be an artifact of taxon sampling or marker choice, but rather a reflection of the history of this group. The New World Clade is where our results differ most from previous work. Five of the 11 sections that we recognize here have been previously described and remain largely unchanged in our circumscription, but the remaining six are new sections or are significant recircumscriptions of previously named groups reflecting our phylogenetic results.

A notable section we recognize among the New World species is sect. *Euphorbiastrum*, which is circumscribed here as an unusually diverse group of species in terms of their growth forms. At the base of this clade are two species, *E. laurifolia* and *E. cestrifolia*, which are both small trees that occur in open, dry scrubland in the northern to central Andes. The next diverging species is *E. weberbaueri*, which is very different from the previous two in being a semi-succulent pencil-stem shrub growing in cactus-scrub vegetation of Peru and Ecuador. This is followed by *E. dussii*, which is a rare shrub from moist forests of Martinique and St. Lucia in the Lesser Antilles. Steinmann & al. (2007) hypothesized that *E. dussii* was part of sect. *Cubanthus*, but our data place it instead well nested within sect. *Euphorbiastrum*. This indicates that *E. dussii* and sect. *Cubanthus* represent two separate introductions of subg. *Euphorbia* into the West Indies. *Euphorbia pteroneura* is another semi-succulent species in this clade, with green stems resembling those of *E. weberbaueri*, but it is an herb from southern Mexico with fully developed, drought-deciduous leaves. Sister to *E. pteroneura* is *E. hoffmanniana*, a large shrub from Costa Rica. Despite the heterogeneous morphology of these species, their monophyly is highly supported by our molecular sequence data (BS 93%; Fig. 2.2A). The relationship of this clade to other species in the New World Clade, however, has very little support. This section is a fine-scale example of the lability of growth form evolution in *Euphorbia* and of the potential this group holds for studying the drivers of such evolutionary patterns.

Section Pacificae (= Pacific Clade) — This clade was originally recovered by Steinmann and Porter (Steinmann & Porter, 2002), although they only included three species. Based on our results (Fig. 2.2) as well as previous treatments (Hassall, 1977; Forster, 1994), sect. *Pacificae* consists of 12 species. Nine of these species form a complex related to *E. plumerioides,* and are all shrubs to small trees that grow in highland forests or coastal headlands in the southwestern Pacific area, New Guinea, and tropical Australia. Three other subtropical Australian species are included in sect. *Pacificae,* namely *E. boophthona*, *E. stevenii* (both herbs), and *E.*

sarcostemmoides (a pencil-stem shrub). Zimmermann & al. (2010) included two accessions of *E. boophthona* in their analysis, one from Steinmann & Porter (2002) and another from their own collection. Their collection was placed in subg. *Chamaesyce,* while the Steinmann collection was placed in sect. *Pacificae*. Our own third accession is strongly supported as part of sect. *Pacificae* and thus confirms the results of Steinmann & Porter (2002) and Horn & al. (2012). This species is distinct from all other members of sect. *Pacificae* in having four cyathial glands and seeds with a pronounced caruncle, while the rest of the species have five glands and ecarunculate seeds. The cpDNA and 3-gene data sets resolve sect. *Pacificae* as sister to the New World Clade, but the ITS data place it sister to Old World Clade I. Both relationships have poor BS (≤ 55%), but the clade itself is highly supported in all analyses regardless of taxon sampling or sequence data used (BS 100%, PP > 95; Figs. 2.2, 2.S1—2.S3). Sect. *Pacificae* has a unique geographic distribution within *Euphorbia* (Fig. 2.2), occurring mainly in Papua New Guinea and Australia but also on islands of Malesia, the Philippines, Melanesia, and Hawaii.

***Old World Clade I* —** The third major clade recovered in our study, which we refer to as Old World Clade I, is distributed mainly across Madagascar, but with several species in Africa and the Arabian Peninsula. In the 3-gene and cpDNA analyses (Figs. 2.2B, 2.S2), this clade is sister to the remaining Old World species, although in the ITS tree it is sister to the Pacific Clade (Fig. 2.S1). Neither of these placements is strongly supported, but the geographical distribution of Old World Clade I supports the results of our combined analyses (Fig.2.2B). The exclusion of this group, with high support (BS 100%), from the rest of the Madagascan species (mostly placed in Old World Clade II) suggests that there have been at least two, and possibly three, independent introductions to Madagascar within subg. *Euphorbia*.

Old World Clade I contains several growth forms and a diversity of cyathium and fruit characters, but it can be distinguished from other clades by the prevalence of dioecy in most species. This reproductive system has not been conclusively documented in all species, but it appears to be a morphological synapomorphy for the clade. We recognize two sections in Old World Clade I, the newly described sect. *Pervilleanae* and the substantially recircumbscribed sect. *Tirucalli*. Section *Tirucalli*, as circumscribed here, includes only pencil-stemmed species that have photosynthetic, semi-succulent branches that are typically long relative to their diameter and usually bear rudimentary, caducous leaves (Fig. 2.1F; Horn & al., 2012). This growth form is exemplified by *E. tirucalli*, the milkbush tree, which is commonly planted in the tropics but is likely native to southern Africa or Madagascar. *Euphorbia tirucalli* has been the basis for a group at various ranks that has included all or most of the pencil-stemmed species in *Euphorbia*. Previous work has shown that this was an artificial grouping and that the pencil-stemmed habit has evolved independently multiple times, and in each subgenus of *Euphorbia* (Steinmann & Porter, 2002). The true relatives of *E. tirucalli* are a group of 23 species occurring mostly in Madagascar, but with several species in Africa and the Arabian Peninsula (Fig. 2.2). The various groups of pencil-stemmed species in *Euphorbia* are often difficult to distinguish morphologically. In contrast, the species of sect. *Tirucalli* are well-characterized as dioecious shrubs or trees, with alternate branches that can appear whorled, conspicuous pubescence (especially on the ovaries), female flowers with calyx lobes, and carunculate seeds. This combination of characters can usually serve to tell all species of sect. *Tirucalli* from pencil-stemmed species belonging to other clades.

***Old World Clade II* —** The remaining Old World species in subgenus *Euphorbia* comprise Old World Clade II, which is divided into four sections. The size of these sections varies from over 350 species in sect. *Euphorbia* to three species in sect. *Rubellae*. Species in Old World Clade II are distributed in Africa, Asia, and Madagascar, with the majority occurring in warm, arid climates. Spines of various form, but generally considered of stipular origin (White & al., 1941), are quite common in this clade, as are succulent stems (Fig. 2.1C, G, M--P). Growth forms also vary greatly among the sections of Old World Clade II and include geophytes, pencil-stemmed shrubs and trees, leafy shrubs and trees, spiny xerophytic shrubs, and various kinds of stem succulents (Fig. 2.1B--E, M, Fig. 2.2B--C).

Support for Old World Clade II and for each of the four subclades is high (BS = 100%, PP = 1.0). However, only the cpDNA analysis resolved the relationships among the subclades with good support (BS >80%, Fig. 2.S2). Several nodes differ between the ITS tree and the cpDNA tree (though with low BS from ITS), and these are likely the cause of the low BS support in the 3-gene tree. First, sect. *Rubellae* is inferred as sister to the rest of Old World Clade II in the ITS tree (Fig. 2.S1), but as sister to sect. *Monadenium* + sect. *Euphorbia* in the cpDNA tree (Fig. 2.S2). Second, the sister clade of sect. *Euphorbia* is the clade of sections *Denisophorbia, Deuterocalli,* and *Goniostema* in the ITS tree, but it is sect. *Monadenium* in the cpDNA tree. Horn & al. (2012), who compiled the largest data set in terms of sequence data, found the same sister relationship for sect. *Euphorbia* as in our 3-gene tree (Fig. 2.2). However, they did not include sect. *Rubellae,* so we cannot draw comparisons about the overall topology of Old World Clade II. For simplicity, we discuss the groups within Old World Clade II referring to the topology in Fig. 2.2.

Section *Rubellae,* represented by *E. rubella* and *E. brunellii,* is a group of three species from northeastern Africa and is the only clade in subg. *Euphorbia* comprised entirely of geophytic species. Aside from their habit, the most distinctive feature of this group is the 4 + 2 arrangement of the cyathial glands: four oblong-elliptic glands of equal size and two smaller, orbicular ones that are apparently derived from splitting of the original fifth gland (Gilbert, 1987). These species were formerly included in subg. *Lacanthis* (= sect. *Goniostema*), but Haevermans & al. (2004) showed that the species of sect. *Rubellae* are not part of sect. *Goniostema*, which is now an entirely Madagascan group. Our results support their hypothesis of relationships.

Following sect. *Rubellae* in Old World Clade II is a highly supported clade (BS 100%) consisting of three sections endemic to Madagascar: sect. *Goniostema*, sect. *Denisophorbia*, and sect. *Deuterocalli* (Fig. 2.2B). In our ITS analysis, these sections form monophyletic groups with 100% BS for sections *Denisophorbia* and *Deuterocalli*, and low support (BS 56%) for sect. *Goniostema* (Fig. 2.S1), but the plastid data does not resolve the relationships of these three groups well (Fig. 2.S2). Consequently, sect. *Goniostema* forms a grade in the 3-gene analysis, although the other two sections are monophyletic and still have 100% BS (Fig. 2.2B). The grade pattern of sect. *Goniostema* in the 3-gene analysis (Fig. 2.2B) is due to the placement of several species (*E. iharanae*, *E. neohumbertii*, *E. alfredii*, *E. geroldii*, *E. francosii* and *E. viguieri*) outside the core of sect. *Goniostema* in the analysis of the plastid data set (Fig. 2.S2). Horn & al. (2012) included *E. iharanae* and *E. neohumbertii* in their analysis and also found these two species to form a clade outside the core of sect. *Goniostema*. *Euphorbia iharanae* and *E. neohumbertii* represent a group of species from northern Madagascar that share the well-developed, colorful cyathophylls of the rest of sect. *Goniostema*. Several species in this northern group are unarmed trees or small shrubs from the rainforest of northeastern Madagascar (e.g., *E geroldii*), while others are succulent shrubs or monocauls that occur in dry habitats of northern Madagascar. The latter group has bristly spines (e.g., *E. viguieri, E. iharanae*) or small glandular combs (e.g., *E. alfredii*) in vertical rows along the stem (Fig. 2.1M). Some are also distinguished by erect cyathophylls that envelop the cyathia and resemble a floral tube (Fig. 2.1L). These species have previously been placed in sect. *Goniostema* based on morphology and distribution, and despite their placement outside the core of the section in our concatenated analyses, they are part of a monophyletic sect. *Goniostema* in our ITS tree (Fig. 2.S1). Moreover, analyses by Aubriot & Haevermans (in prep.) of two nuclear and six chloroplast regions, with comprehensive sampling of sections *Deuterocalli, Denisophorbia,* and *Goniostema*, produce a well-resolved phylogeny that supports the monophyly of the three sections in Old World Clade II and firmly places these species within sect. *Goniostema*.

One of the most morphologically distinct groups within Old World Clade II is sect. *Monadenium*, which is distributed across eastern, central, and southern Africa. This is a highly supported clade (BS 100%, Fig. 2.2B) of about 90 species that are easily recognized morphologically by the fusion of the cyathial glands into a horseshoe-shaped rim, or less commonly into a complete ring. This unique cyathial morphology was the reason this group was formerly segregated from *Euphorbia*, but our results and those from previous studies have confirmed that this section is well nested within subg. *Euphorbia* (Steinmann & Porter, 2002; Bruyns & al., 2006; Zimmermann & al., 2010; Horn & al., 2012). Species of sect. *Monadenium* are particularly diverse in terms of growth form and include geophytes, succulent and non-succulent shrubs, as well as trees, vines, and one pencil-stem species (Fig. 2.2B).

The largest clade in subg. *Euphorbia* and perhaps the best known is sect. *Euphorbia* – the “spine-shield” euphorbias (Figs. 2.1C--D, G, N). This group is a well-defined clade of over 340 species distributed mostly in Africa but also across southern and southeast Asia and into Indonesia. The defining character for this clade is the spine-shield – a horny pad of tissue subtending or surrounding each leaf base that typically bears two or four spiny outgrowths (Figs. 2.1G, N). These outgrowths are usually interpreted as a pair of stipular spines and a pair of prickles (Carter, 1994). The vast majority of species in sect. *Euphorbia* have spine-shields, but they have been lost in a few species, such as *E. abdelkuri* and *E. piscidermis*. Most species in this section are stem succulents that vary in size from prostrate dwarf shrubs to large candelabriform trees, and have angled, winged, or tuberculate stems and minute, caducous leaves. However, there are also geophytes and large trees that have well developed leaves. Several geophytic species from India, including *E. fusiformis*, *E. nana* (Fig. 2.1D), and *E. meenae*, are now known to belong to sect. *Euphorbia.* There are no obvious characters placing them in sect. *Euphorbia* because they lack above-ground stems. However, DNA sequence data from previous studies confirm that they are indeed part of this section (Steinmann & Porter, 2002; Bruyns & al., 2006; Zimmermann & al., 2010).

Growth form evolution — *Euphorbia* subg. *Euphorbia* contains the full range of growth form diversity that is present within the genus (Figs. 2.1A--F, 2.2). Moreover, our phylogenetic results confirm the conclusion of Horn & al. (2012) that specialized growth forms have evolved independently, and repeatedly, within the subgenus. Horn & al. (2012) inferred the common ancestor of subg. *Euphorbia* to be a shrub or tree with alternate leaves and terminal cyathia. This growth form (exclusive of the cyathial position) is mostly retained in sections *Pachysanthae, Pervilleanae*, and *Denisophorbia* in Madagascar, as well as in most sections of the New World Clade. The herbaceous habit has evolved four times in subg. *Euphorbia*: in the clade of sect. *Portulacastrum* + sect. *Nummulariopsis*, in *E. pteroneura* of sect. *Euphorbiastrum*, in sect. *Monadenium*, and in several members of sect. *Pacificae*. Geophytic species are present in all sections of Old World Clade II and comprise the entire sect. *Rubellae*. The one geophyte from sect. *Monadenium* in our study, *E. neogracilis*, is sister to the rest of the section in our cpDNA and 3-gene analyses. The geophytes in sect. *Goniostema* and sect. *Euphorbia* have been shown to be well nested within those clades. The phylogenetic position of these geophytic species suggests that geophytes have evolved independently multiple times in Old World Clade II (Haevermans & al., 2004; Zimmermann & al., 2010; Bruyns & al., 2011). Similarly, the pencil-stem tree/shrub habit appears to have evolved at least eight times in subg. *Euphorbia*: four times in the New World Clade, twice in Old World Clade I, and twice in Old World Clade II (Fig. 2.2).

Highly succulent, photosynthetic stems are present in sect. *Euphorbia* and in sect. *Monadenium*. While both of these sections contain a range of growth forms, all but a few species in sect. *Euphorbia* have spiny, succulent stems. The exceptions include the geophytes mentioned above, and some leafy trees such as *E. drupifera*. Species range from trees up to 25 m tall, such as *E. cussonioides,* to dwarf shrubs only a few centimeters high, such as *E. decidua*. While most species have minute, caducous leaves, some produce quite large, persistent ones (e.g., *E. neriifolia* and *E. royleana* from India). Because these species are nested well within sect. *Euphorbia,* the presence of persistent leaves appears to be a reversal to the ancestral state for the subgenus (Horn & al., 2012). Growth form evolution in sect. *Monadenium* involves changes in habits rather than changes in size. This section contains the entire range of growth forms found in the subgenus, and the stem succulent species make up only a few of the total. While the stems of some species are succulent and green, the loss of functional leaves is much less pronounced in sect. *Monadenium*, and most species produce large, succulent leaves.

The switch to stem-based photosynthesis, as has happened in sect. *Euphorbia* and in all pencil-stemmed species, putatively involves several steps, including an increase in the stem stomatal density, delaying or abandoning bark initiation, increasing the volume of cortex for water storage, and the development of the Crassulacean Acid Metabolism (CAM) photosynthetic system (Nobel, 1988; Edwards & Donoghue, 2006). Given the frequency with which stem-based photosynthesis has evolved in subg. *Euphorbia* (Fig. 2.2), it seems that these are relatively "easy" evolutionary transitions to make in this group. In the Cactaceae, Edwards & Donoghue (2006) showed that *Pereskia* species, which are the closest relatives to the core cacti, have high water use efficiency and some level of CAM photosynthesis. They suggested that this was preadaptive for the transition to the succulent-stemmed cactus life form. CAM has been inferred in most stem photosynthetic species in subg. *Euphorbia,* but the close relatives of the pencil-stem species are all C3 plants (Horn & al., 2011), suggesting that CAM is not a prerequisite for the evolution of highly succulent, photosynthetic stems.

***Diversity of cyathial morphology* —** While most species in subg. *Euphorbia* have relatively unspecialized radially symmetric cyathia (with five, usually yellow glands that lack appendages, and with small, inconspicuous cyathophylls), some of the showiest and morphologically most derived forms of cyathia in the genus are also found within this subgenus (Fig. 2.1G--L). For example, all species in sect. *Monadenium* possess cyathia with nectar glands fused either into a horseshoe shape or a full circle, as well as cyathophylls that are more or less fused dorsally, resulting in bilateral symmetry (Fig. 2.1J).

Section *Crepidaria* contains another striking example of fusion of cyathial parts and a shift to bilateral symmetry (Fig. 2.1I). In this section, the nectar glands are positioned on one side of the involucre and are enveloped in a spur formed by the fusion of petaloid gland appendages. This is perhaps the greatest deviation from the general form of the open, actinomorphic cyathium that resembles a typical dicot flower (Fig. 2.1G). This cyathial form in sect. *Crepidaria* is often associated with hummingbird pollination (Dressler, 1957) and represents a unique syndrome within *Euphorbia*. Cacho & al. (2010) suggested that the nectar spur was a key innovation that led to a rapid radiation in sect. *Crepidaria*. They noted that high morphological divergence relative to the low sequence variation among species suggested a rapid radiation after the evolution of nectar spurs, especially when coupled with the long branch leading to the extant members of the clade (found in our study and Bruyns & al., 2006; Zimmermann & al., 2010; Bruyns & al., 2011). However, they were unable to find unique support for the key innovation hypothesis except when assuming an outgroup of only one species. Our results do not resolve the sister relationship of sect. *Crepidaria,* but place it most closely to clades that would also be unlikely to support the key innovation hypothesis.

Well-developed cyathophylls occur in sect. *Goniostema,* and this is a morphological synapomorphy for the clade (Fig. 2.1H). Most species in sect. *Goniostema* have cyathophylls that become brightly colored (usually red or yellow) and can be spreading, or in a few species (e.g., *E. neohumbertii, E. iharanae*) envelop the cyathium completely, mimicking a pseudofloral tube (Fig. 2.1L). Effects on pollination are unknown, but the change in shape in these "tubular" cyathia presumably affects the mechanism and/or effort required to access the nectar and could be associated with more specialized pollinators.

CONCLUSIONS

This study represents the most taxonomically and geographically comprehensive phylogenetic study of *Euphorbia* subg. *Euphorbia* to date. Sequence data from multiple regions representing the nuclear and plastid genomes allowed us to infer phylogenetic relationships across the subgenus, and they support four major lineages within the subgenus that can be characterized by their distributions. Our results clearly establish the monophyly of all New

World species in subg. *Euphorbia*, define the species composition of the New World Clade, and suggest an early split between the Old World and New World early in the history of the subgenus. Our phylogeny also supports the hypothesis of at least two independent lineages in Madagascar within subg. *Euphorbia*. Included in these is sect. *Tirucalli*, which is recircumbscribed as the clade of pencil-stemmed species within subg. *Euphorbia* that includes *E. tirucalli*. Also included in these Madagascan clades are three sections of leafy shrubs and trees (sect. *Denisophorbia*, sect. *Pervilleanae*, and sect. *Pachysanthae*) that will require more thorough study to better understand their circumscriptions and the delimitations of species within them.

Consistent with the overall evolutionary lability of growth form in *Euphorbia* as a whole, several of the sections in subg. *Euphorbia* contain a wide range of growth forms for their small size. The pencil-stem growth form is particularly homoplasious within the subgenus, just as it is across the genus as a whole. Utilizing the phylogenetic framework established in this study, future studies will focus on potential drivers of the evolution of the unusual pencil-stem growth form as well as patterns of geographical and morphological differentiation in the large spine-shield clade, sect. *Euphorbia.*

TAXONOMIC TREATMENT

Altogether, 661 species are recognized in the subgenus. For each section listed below, we provide a list of their component species. Those species appearing in ***boldface-italics*** have been sampled molecularly in this or in previous studies, whereas those appearing only in *italics* are inferred to belong to the section based on their morphology and distribution. A complete database of names and synonyms with additional information is available online at the Tolkin website (http://app.tolkin.org/projects/72/taxa, Riina & Berry, 2012).

Euphorbia **L. subg.** ***Euphorbia*** − Lectotype (Millspaugh 1909: 306): *Euphorbia antiquorum* L.

= *Euphorbia* subg. *Tithymalus* Pers., Syn. Pl., part 2(1): 6. 1806. − Type: *Euphorbia antiquorum* L.

As the largest and most diverse of the four subgenera of *Euphorbia*, subg. *Euphorbia* is probably the most difficult to characterize morphologically. This clade has the greatest number of succulent and spiny species. Subg. *Rhizanthium* also has a significant number of spiny, succulent species, but the spines in that group are generally of peduncular origin, whereas in subg. *Euphorbia* they are either stem enations or more complex 'spine-shields' that may be of stipular origin (Fig. 2.1; Carter, 1994). Many of the succulent, spiny species are leafless, but members of both sects. *Euphorbia* and *Goniostema* can also be both leafy and spiny. There are a number of evergreen or deciduous tree species in subg. *Euphorbia* in both Madagascar and the New World, and there are several lineages or species that show leafless and spineless pencil-stem habits. There are rather few truly herbaceous species in the subgenus, and a limited number of geophytes occur in at least four Old World clades.

Reproductively, subg. *Euphorbia* is notable for its wide variations of the cyathium, particularly in gland number, shape, and degree of fusion (Fig. 2.1G--L). The glands generally lack appendages, but there are several small groups with short horns, finely divided rims, or even finger-like processes (Fig. 2.1K). The bracts immediately subtending the cyathia, as opposed to the dichasial bracts subtending or along the rays of the synflorescence, are showy and diagnostic in several groups (Fig. 2.1H, L). In our descriptions below we use the term ‘cyathophylls’ to refer to these subcyathial bracts in all groups. The larger sections have bisexual cyathia, but dioecy is characteristic of some of the smaller sections. Similarly, caruncles are present in some groups, but absent in others.

Although certain sections of subg. *Euphorbia* can be readily distinguished by their specialized cyathial features (e.g., sects. *Crepidaria* and *Monadenium*), many sections have a similar, basic cyathium, and habit types have been shown to be particularly homoplasious in the subgenus. This makes construction of a morphologically based key to the sections problematic; however, since major clades in the subgenus are well circumscribed geographically, the most straightforward approach is to first break the subgenus into these more manageable groups.

Key to the sections of subg. *Euphorbia*

1a. Old World natives... 2

1b. New World natives... 13

2a. Madagascar and Indian Ocean islands... 3

2b. Other Old World and Pacific areas outside of Madagascar and the Indian Ocean... 9

3a. Stipules spiny, papyraceous or comb-like, more or less branched, diversely colored; cyathia always bisexual; cyathophylls well developed (except *E. boissieri*); seeds verrucose; caruncle absent... sect. ***Goniostema*** **(17)**

3b. Stipules glandular or punctiform, never spiny or papyraceous; cyathia unisexual or bisexual; cyathophylls developed or reduced; seeds smooth; caruncle present or absent... 4

4a. Plants essentially leafless (leaves very small, soon caducous); young stems succulent and photosynthetic... 5

4b. Plants with well-developed leaves, caducous or persistent; young stems not succulent or photosynthetic... 7

5a. Reduced leaves when present more or less lanceolate; petiole base surrounded by a glandular or waxy ring (possibly of stipular origin, but clearly visible after the leaves have fallen off)... sect. ***Deuterocalli*** **(18)**

5b. Reduced leaves when present variable in shape, but not lanceolate; stipules extremely reduced, located on each side of the leaf insertion (visible or not after the leaves have fallen off)... 6

6a. Twigs glabrous; synflorescences terminal; female flower without a calyx; capsule 1- or 2-locular; seeds wider than long, ecarunculate... sect. ***Pervilleanae*** **(14)** (*E. intisy*)

6b. Young twigs more or less tomentose; synflorescences subterminal; female flower with a rudimentary calyx; capsule 3-locular; seeds longer than wide, with a well-developed caruncle... sect. ***Tirucalli*** **(in part) (15)**

7a. Shrubs or trees with sympodial branching, the leaves clustered at nodes connected by slender leafless shoots; cyathia unisexual (rarely bisexual); capsule 3-locular, smooth; seeds cylindrical, smooth... sect. ***Denisophorbia*** **(19)**

7b. Shrubs or trees with monopodial growth, the leaves regularly spaced or else clustered at the twig apex; cyathia bisexual; capsule 1-or 2-locular, ornamented or not; seeds globose, smooth or sculptured... 8

8a. Shrubs or trees with thick coriaceous leaves; cyathium shallowly bowl-shaped, with large stipitate glands (> 5 mm wide); fascicles of male flowers sheathed by their bracteoles; capsule erect, apparently largely indehiscent, more or less fleshy, the surface smooth or wrinkled, seeds 1 or 2... sect. ***Pachysanthae*** **(13)**

8b. Shrubs or trees with soft and thin leaves; cyathium cup-shaped with sessile glands; male flowers not sheathed by their bracteoles; capsule pendulous, dehiscent, winged or with spiny appendages, seeds usually 2... sect. ***Pervilleanae*** **(14)**

9a. Natives of Australia and Pacific Islands including New Guinea... sect. ***Pacificae***

9b. Natives of Africa, Arabia, and Asia... 10

10a. Plants mostly dioecious, essentially leafless (leaves very small, soon caducous), pencil-stemmed shrubs or trees; young stems succulent and photosynthetic, usually terete, more or less tomentose, spines absent... sect. ***Tirucalli*** **(in part) (15)**

10b. Plants monoecious geophytes, shrubs, or trees either with well developed, caducous or persistent leaves and non-photosynthetic stems, or else leafless and succulent with photosynthetic stems, but then usually with spine-shields or stipular spines... 11

11a. Geophytes with free cyathophylls and cyathia with 4 similar glands and a fifth one divided into two parts; seeds carunculate; northeast Africa... sect. ***Rubellae*** **(16)**

11b. Plants varied in habit, but if geophytic then with glands fused into a horseshoe shape and with carunculate seeds, or else with highly reduced cyathophylls, 5 similar glands, and ecarunculate seeds; widespread (Africa, southern/eastern Asia)... 12

12a. Stems spineless or with spines of stipular origin, but no spine-shields; cyathophylls usually (partly) fused on one side; cyathia with one large horseshoe-shaped or circular gland; seeds carunculate... sect. ***Monadenium*** **(20)**

12b. Stems usually succulent, angled or tuberculate, generally with distinctive spine-shields composed of a horny pad and associated spines; cyathophylls reduced, scale-like; cyathia actinomorphic, with 5 similar glands; seeds ecarunculate ... sect. ***Euphorbia*** **(21)**

13a. Cyathia strongly zygomorphic, the glands hidden in a spur-like extension of the cyathial involucre... sect. ***Crepidaria*** **(10)**

13b. Cyathia actinomorphic, or if slightly zygomorphic, the glands evident on the surface or edge of the involucre...14

14a. Leafless stem-succulent shrubs, stems with 4--6 angles or ridges... 15

14b. Leafy herbs, shrubs, or trees (sometimes deciduous), stems terete or rarely angled... 16

15a. Cyathial glands with two horns; eastern Brazil... sect. ***Brasilienses*** **(12)**

15b. Cyathial glands without horns; Ecuador and Peru... sect. ***Euphorbiastrum*** **(6)** (*E. weberbaueri*)

16a. Cyathia arranged in a tight synflorescence with the subtending bracts greenish and successively overlapping each other like shrimp scales... sect. ***Stachydium*** **(11)**

16b. Cyathia laxer and not subtended by overlapping greenish bracts... 17

17a. Herbs... 18

17b. Shrubs or trees... 20

18a. Annuals, the petioles longer than the blades; cyathia solitary and axillary, the glands each with 3--5 digitate appendages... sect. ***Portulacastrum*** **(8)**

18b. Perennials, the petioles generally shorter than the blades; cyathia in dichasia or pleiochasia, the glands without appendages or else with two horns... 19

19a. Stems terete; pistillate flowers with evident perianth lobes; southern South America and southeastern United States... sect. ***Nummulariopsis*** **(9)**

19b. Stems ridged; pistillate flowers without evident perianth lobes; Mexico and Guatemala... sect. ***Euphorbiastrum*** (*E. pteroneura*)

20a. Shrubs to small trees of moist evergreen tropical forests; leaves very large, 25--40 x 5--12 cm, persistent... sect. ***Mesophyllae*** **(5)**

20b. Shrubs or trees of drier, more subtropical habitats; leaves smaller than above, persistent or deciduous... 21

21a. Densely branched bushes; cyathia subtended by numerous (4--6) creamy-yellow, leafy cyathophylls; edge of Atacama Desert in northern Chile... sect. ***Lactifluae*** **(4)**

21b. Laxly branched shrubs or single-trunked trees; cyathia usually subtended by 2 or 3 cyathophylls; from Andean Peru north to Mexico and the Caribbean... 22

22a. Cyathophylls red (green in *E. cubensis*); cyathial glands 1--8, on the rim of the involucre or on its outer wall; Caribbean islands (Bahamas, Hispaniola, Cuba, Jamaica)... sect. ***Cubanthus*** **(3)**

22b. Cyathophylls, when present, whitish or yellowish; cyathial glands 4 or 5, on the rim of the involucre; Mexico to Peru (one species, *E. dussii*, from St. Lucia in the Caribbean)... 23

23a. Shrubs or small trees to ca. 5 m tall; cyathia subtended by greenish or inconspicuous cyathophylls; Costa Rica to Peru and St. Lucia (Caribbean)... sect. ***Euphorbiastrum*** **(6)**

23b. Small to large trees 5--25 m tall; cyathia subtended by 2 or 3 whitish or yellowish cyathophylls; Mexico...24

24a. Trees to 12 m tall with rugose to furrowed bark; capsule exserted from the involucre, seeds carunculate... sect. ***Calyculatae*** **(7)**

24b. Trees to 25 m tall with coppery, exfoliating bark; capsule included within or barely exserted from the involucre, seeds ecarunculate... sect. ***Tanquahuete*** **(2)**

1. *Euphorbia* sect. *Pacificae* Dorsey, **sect. nov.** – Type: *Euphorbia plumerioides* Teijsm. ex Hassk.

Monoecious or dioecious herbs, shrubs, or small trees. Stems woody to fleshy, terete, glabrous. Stem leaves alternate, those on fertile branches opposite in *E. boophthona*, persistent to caducous, thin to fleshy, usually petiolate; stipules absent. Synflorescences subterminal, much-branched dichasia or solitary, axillary cyathia; cyathia pedunculate; glands (4)5(--11), green, ovate-elliptic, with short marginal processes. Capsule (sub)globose; seeds oblong to ovoid (cylindrical in *E. boophthona*), carunculate (*E. boophthona*) or ecarunculate.

Species included (11). – ***E. boophthona*** C.A. Gardner, *E. brassii* P.I. Forst., *E. buxoides* Radcl.-Sm., ***E. haeleeleana*** Herpst, *E. heyligersiana* P.I. Forst., *E. indistincta* P.I. Forst., *E. kanalensis* Boiss., *E. norfolkiana* Boiss., ***E. plumerioides*** Teijsm. ex Hassk., ***E. sarcostemmoides*** J.H. Willis, ***E. stevenii*** F.M. Bailey.

Distribution and habitat. – Australia, Indonesia, New Guinea, Philippines, and Pacific Islands (Norfolk Island, Fiji, Solomon Islands, Hawaiian Islands); the Australian herbaceous or pencil-stemmed species occur in dry interior or coastal habitats, whereas the remaining species occur in tropical or subtropical moist forests or scrub.

Section *Pacificae* is a novel grouping that has a unique southern Pacific distribution within *Euphorbia*. It contains the *E. plumerioides* complex (Forster, 1994), a group of eight or nine shrubby species, which is most divere in New Guinea. Whether *E. euonymoclada* Croizat belongs here or not is uncertain and will require obtaining molecular data and more specimens of this rarely collected species; it differs from the other members of the *E. plumerioides* complex in its well-developed stipules and single-cyathiate synflorescence. Three quite different-looking species that are endemic to Australia are also included in sect. *Pacificae,* namely *E. boophthona*, *E. stevenii*, and *E. sarcostemmoides*. The first two are herbs, whereas *E. sarcostemmoides* is a leafless pencil-stemmed shrub. *Euphorbia boophthona* is morphologically anomalous here, since it has four cyathial glands and seeds with a pronounced caruncle. An unusual species belonging to this group is *E. haeleeleana*, which is endemic to several of the Hawaiian Islands; it is a tree with large, woody fruits and cyathia with up to 11 glands.

2. ***Euphorbia*** **sect.** ***Tanquahuete*** V.W. Steinm. & Dorsey, sect. nov. – TYPE: *Euphorbia tanquahuete* Sessé & Moc.

Trees to 25 m tall, trunk to 50 cm d.b.h. Stems round in cross section, glabrous to pubescent, bark smooth and exfoliating into thin papery sheets. Leaves spirally arranged, drought-deciduous; stipules glandular, minute, lateral at the base of the petiole; petiole well-defined; blade elliptic, base attenuate, apex acute, obtuse, mucronulate or apiculate, margin entire, glabrous to pubescent, pinnately nerved. Synflorescence axillary, originating from leafless nodes of older stems, rays 3–9, dichasia 1-branched; cyathophylls 2, white to yellow; involucres on a thick peduncle that becomes woody in fruit; glands 5, patelliform, situated on the rim of the involucre, without appendages; gynophore terminating in 3 short triangular calyx-like lobes; ovary glabrous, styles connate at the base, shortly swollen-clavate, apex inconspicuously bifid to lobed. Capsule included within or barely exserted from the involucre, dry or with a slightly fleshy mesocarp; seeds broadly ovoid to subglobose, rounded to slightly quadrangular in cross section, base and apex rounded, smooth, ecarunculate.

Distribution and habitat. – Central-western to southern Mexico; subtropical deciduous forests in montane canyons and lava flows; near sea level to 2100 m.

Species included (2). – E. lundelliana Croizat, ***E. tanquahuete*** Sessé & Moc.

Euphorbia sect. *Tanquahuete* contains two tree species that are by far the largest members of *Euphorbia* in the New World, and among the largest in the genus. *Euphorbia lundelliana* is poorly known, but is placed here because of its arborescent habit, leaves similar to *E. tanquahuete*, large fruits, and smooth, ecarunculate seeds.

3. *Euphorbia* sect. *Cubanthus* (Boiss.) V.W. Steinm. & P.E. Berry, Anal. Jard. Bot. Madrid 64: 123-133. 2007. ≡ *Pedilanthus* sect. *Cubanthus* Boiss. in DC., Prodr. 15(2): 7 1862. ≡

Cubanthus (Boiss.) Millsp., Publ. Field Mus. Nat. Hist., Bot. Ser. 2: 371. 1913. – Type: *Pedilanthus linearifolius* Griseb. (= *Euphorbia scutiformis* V.W. Steinm. & P.E. Berry).

= *Adenorima* Raf., Fl. Tell. 4: 112. 1838 ('1836'). ≡ *Euphorbia* sect. *Adenorima* (Raf.) G.L. Webster, J. Arnold Arbor. 48: 407. 1967. – Type: *Adenorima punicea* (Sw.) Raf. (≡ *Euphorbia punicea* Sw.).

Monoecious shrubs to small trees; stems soft-wooded, somewhat fleshy, terete. Leaves alternate, spirally arranged, clustered toward branch apices, membranaceous to slightly coriaceous, entire, sessile; stipules minute, glanduliform, caducous or apparently absent. Cyathia in terminal dichasia; cyathophylls generally red; glands 1--8, situated on the rim of the involucre or on its outer wall, green, yellow or reddish, appendages lacking; ovary and fruit smooth, glabrous, usually subtended by a three-lobed, calyx-like structure; seeds ecarunculate.

Section *Cubanthus* is the only section within subg. *Euphorbia* with a strictly Caribbean distribution. The cyathial glands are distinctive in subsect. *Moa* in being usually placed on the outer wall of the involucre (rather than on the rim) and in one species, *E. umbelliformis*, reduced to a single gland.

3a. *Euphorbia* subsect. *Cubanthus* V.W. Steinm. & P.E. Berry, Anal. Jard. Bot. Madrid 64: 123-133. 2007. − Type: *Pedilanthus linearifolius* Griseb. (= *Euphorbia scutiformis* V.W. Steinm. & P.E. Berry).

Leaves narrowly spathulate, clustered at branch apices, those subtending the cyathia not distinct; cyathophylls red but less intensely so than in sect. *Moa* (green in *E. cubensis)*; glands usually on the outer wall of the involucre.

Distribution and habitat. – Caribbean (Bahamas, Cuba, Jamaica); scrub forest mostly on limestone, sea level to 500 m.

Species included (6). – ***E. cubensis*** Boiss.*,* ***E. gymnonota*** Urb.*, E. millspaughii* V.M. Steinm. & P.E. Berry*,* ***E. punicea*** Sw.*, E. scutiformis* V.W. Steinm. & P.E. Berry*,* ***E. umbelliformis*** (Urb. & Ekman) V.W. Steinm. & P.E. Berry.

3b. *Euphorbia* subsect. *Moa* V.W. Steinm. & P.E. Berry, Anal. Jard. Bot. Madrid 64: 123-133. 2007. − Type: *Euphorbia helenae* Urb.

Leaves elliptic to oval, regularly spaced along stems, those subtending the cyathia bright red and distinct in shape. Cyathophylls showy, red; involucre red; glands yellow, situated on involucre rim; male flowers red except for anthers; female flower red.

Distribution and habitat. – Caribbean (eastern Cuba); scrub and forests on serpentine soils, sea level to 800 m.

Species included (3). – ***E. helenae*** Urb.*,* ***E. munizii*** Borhidi, ***E. podocarpifolia*** Urb.

4. *Euphorbia* sect. *Lactifluae* Dorsey & V.W. Steinm., **sect. nov.** − Type: *Euphorbia lactiflua* Phil.

Xerophytic shrubs, to 2.5 m tall, but mostly 1 m or less, soft-wooded and highly branched, bark grayish to red-brown, not exfoliating, drought deciduous; stems rounded in cross-section; whole plant glabrous. Leaves well developed, spirally arranged, loosely arranged on long shoots or highly congested on short shoots; stipules absent or represented by minute punctiform glands less than 0.1 mm in diameter at the base of the petiole; petiole short and inconspicuous; blade linear to narrowly elliptic, base attenuate, apex acute to apiculate, margin entire, secondary venation not evident. Synflorescences terminal on short lateral shoots, sometimes terminal on main branches; rays 3 or 4, dichasial bracts and cyathophylls showy, numerous (3--6), pale yellow; cyathia 2(3), sessile, glands 5(6), transversely oblong, situated on the rim of the involucre, without appendages; gynophore terminating in 3 inconspicuous, rounded calyx-like lobes; ovary glabrous, styles united into a short column ca. 1/6 their length, apex emarginate to bifid. Capsule barely exserted from the involucre; seeds plumply obloid, rounded in cross-section, base and apex rounded, smooth, with a conspicuous, hooded caruncle.

Distribution and habitat. – Northern Chile, rocky areas of Atacama Desert from the region of Antofagasta south to the vicinity of Copiapó; nearly sea level to 700 m.

Species included (1). – ***E. lactiflua*** Phil.

Euphorbia lactiflua is distinctive in its swollen branches and showy, yellowish cyathophylls. Although it was previously treated together with a number of leafless Old World succulents in sect. *Tirucalli* Boiss. (Boissier, 1862), all molecular phylogenetic analyses show that section *Lactifluae* is an isolated lineage within the New World Clade of subg. *Euphorbia*.

5. ***Euphorbia*** **sect.** ***Mesophyllae*** V.W. Steinm. & Dorsey, **sect. nov.** − Type: *Euphorbia sinclairiana* Benth.

Mesophytic shrubs or small trees to 5 m tall, little branched, bark not exfoliating. Stems thick and fleshy, rounded in cross-section, glabrous. Leaves large (25–40 x 5–12 cm), obovate to oblanceolate, loosely and spirally arranged, pinnately veined, margin entire; stipules well-developed, lateral at the base of the petiole, ovate to triangular, caducous and leaving a conspicuous calloused pad; petiole distinct, less than 1/10 the length of blade,. Synflorescence terminal (sometimes appearing axillary), often long-pedunculate; cyathophylls 2, foliaceous or scale-like and highly reduced; cyathia on short peduncles, glands 5, circular to transversely oblong, situated on the rim of the involucre, without appendages; gynophore terminating in 3 triangular calyx-like lobes; ovary glabrous, styles united into a slender column 4/5–5/6 their length, apex emarginate to bifid. Capsule included within or barely exserted from the involucre, deeply 3-lobed, smooth or reticulately ridged; seeds globose to ovoid, rounded in cross-section, base truncate with a circular depression, apex mucronulate, smooth, ecarunculate.

Distribution and habitat. – Mexico (Chiapas), Costa Rica, Nicaragua, Panama, Colombia, Peru, Brazil (Acre); understory of wet, lowland forests but also extending to higher elevations in cloud forest, from near sea level to ca. 800 m

Species included (1). – ***E. sinclairiana*** Benth.

Bernal & al. (2006) provided a discussion of the morphological variation within this species and concluded that *Euphorbia elata* is best treated as a synonym of *Euphorbia sinclairiana*. Three other species similar to *E. sinclairiana* have been described (*E. capansa* Ducke, *E. tessmannii* Mansf., and *E. valerii* Standl.). We treat them here as a single widespread species that varies in characters such as inflorescence length and persistence of the cyathophylls, but is characterized by the extremely distinctive habit and large, oblanceolate leaves, as well as growing in wet forest understoreys.

6. *Euphorbia* sect. *Euphorbiastrum* Boiss. in DC., Prodr. 15(2): 10, 99. 1862. ≡ *Euphorbiastrum* Klotzsch & Garcke in Klotzsch Monatsb. Akad. Berlin 1859: 252. – Lectotype (Wheeler, 1943: 467): *Euphorbiastrum hoffmannianum* Klotzsch & Garcke (= *Euphorbia hoffmanniana* (Klotzsch & Garcke) Boiss.).

= *Euphorbiodendron* Millsp., Praen. Baham. II. Field Mus. Pub. Bot., 2: 289-322. 1909. – Lectotype (Wheeler, 1943: 467): *Euphorbia laurifolia* Juss.

= *Euphorbia* sect. *Pteroneurae* A. Berger, Sukk. Euph. 28. 1906: − Type: *Euphorbia pteroneura* A. Berger.

Herbs, shrubs or small trees; branches woody with bark or else green and succulent (then without leaves or with only rudimentary ones, and stems 4--6-ridged). Leaves oblong to rhombic or scale-like and then caducous, stipules present (glandular and surrounding the leaf scar) or absent. Synflorescences axillary or terminal, subsessile to pedunculate; cyathia subsessile to pedunculate; glands 4 or 5, appendages lacking; cyathophylls minute to longer than cyathium; seeds with a tiny caruncle.

Distribution and habitat. – Northern and central Andes of South America (Venezuela, Colombia, Ecuador, Peru), to southern Mexico and West Indies (Windward Islands); montane scrub and forest edges, lowland moist forests.

Species included (6). – ***E. cestrifolia*** Kunth, ***E. dussii*** Krug & Urb. ex Duss, ***E. hoffmanniana*** (Klotzsch & Garcke) Boiss., ***E. laurifolia*** Juss. ex Lam., ***E. pteroneura*** A. Berger, ***E. weberbaueri*** Mansf.

Multiple states from several morphological characters are present in this section, and the group is not easily characterized morphologically. However, as noted in Steinmann & Porter (2002), several morphological characters support the close relationship of these species, including the caruncular seeds and the well-developed, complex glandular stipules in *E. weberbaueri* and *E. cestrifolia*.

7. *Euphorbia* sect. *Calyculatae* V.W. Steinm & Dorsey, **sect. nov**. − Type: *Euphorbia calyculata* Kunth

Trees or shrubs to 12 m tall, trunk to 20 cm d.b.h. Stems round in cross-section, glabrous or sparsely pubescent, bark rugose to furrowed. Leaves spirally arranged, loosely so (*E. xylopoda*) or congested at the ends of the branches (*E. calyculata*), drought-deciduous; stipules present, glanduliform-conical, lateral at the base of the petiole; blade elliptic, base attenuate, apex acute or obtuse, margin entire, pinnately veined. Synflorescences subterminal with 3--5 rays and dichasia 2--4 times branched (*E. calyculata*) or cyathia solitary and terminal at the ends of the main branches and subtended by three prominent white cyathophylls (*E. xylopoda*); cyathia pedunculate, glands 5, transversely elliptic to oblong, situated on the rim of the involucre, without appendages; gynophore terminating in 3 triangular calyx-like lobes; ovary glabrous, styles united into a column for 1/5 to 2/3 their length, swollen and emarginate at the apex. Capsule exserted from the involucre; seeds obloid, slightly angled in cross-section, base and apex rounded, smooth, carunculate.

Distribution and habitat. – Mexico (Jalisco, Michoacán, Guanajuato, Guerrero, Mexico, Oaxaca, Puebla); deciduous montane woodlands, oak and pine-oak forest, sometimes on lava flows, 1600–2300 m.

Species included (2). – ***E. calyculata*** Kunth, ***E. xylopoda*** Greenm.

Euphorbia sect. *Calyculatae* contains two closely related but morphologically very distinct species. Whereas *E. calyculata* has terminal pseudopleiochasial synflorescences typical of many groups of *Euphorbia*, the cyathia of *E. xylopoda* are solitary and terminal at the tips of the main branches, an uncommon condition in the genus. Steinmann & al. (2007) noted morphological similarities between *E. calyculata* and sect. *Cubanthus,* particularly in the leaf arrangement and calyx-like structure, but a close relationship between these sections is not supported by our data.

8. *Euphorbia* sect. *Portulacastrum* Boiss. in DC., Prodr. 15(2): 9, 69. 1862. – Lectotype (Wheeler, 1943: 481): *Euphorbia pentlandii* Boiss.

Monoecious, annual, procumbent, pubescent herbs. Leaves sparse, entire, ovate to orbicular, petioles as long as blade or longer. Cyathia axillary, solitary, sessile or shortly pedunculate; glands 4, with appendages 3--5-digitate; styles undivided. Capsule depressed ovoid, strongly 3-sulcate; seeds transversely grooved, ecarunculate.

Distribution and habitat. – Bolivia and Chile; montane habitats.

Species included (2). – ***E. germainii*** Boiss., *E. pentlandii* Boiss.

This is a rarely collected section that differs from its sister clade, sect. *Nummulariopsis*, in consisting of annual herbs without pistillate calyces and with digitate cyathial gland appendages.

9. *Euphorbia* sect. *Nummulariopsis* Boiss. in DC., Prodr. 15(2): 9, 71. 1862. – Type: *Euphorbia peperomioides* Boiss.

= *Euphorbia* sect. *Tithymalus* subsect. *Inundatae* G.L. Webster, J. Arnold Arb. 48: 400. 1967. – Type: *Euphorbia inundata* Torr.

Monoecious or dioecious herbs, usually perennial, often with a fleshy rootstock. Stems terete, ascending or prostrate. Leaves alternate or sometimes opposite on vegetative stems, entire to serrate, opposite on fertile rays, stipules glandular, minute to elongate. Cyathia solitary and axillary or in terminal pseudopleiochasia, glands 4--5(7), without appendages or sometimes 2-

horned; female flower with well-developed perianth lobes; ovary globose; seeds smooth to variously sculptured, ecarunculate.

Distribution and habitat. – Southeastern U.S.A., southern South America (Argentina, Bolivia, Brazil, Chile, Paraguay, Peru, Uruguay); high montane habitats, grasslands, and sand dunes (South America), pine woodlands and sandy areas (U.S.A.), sea level to ca. 3500 m.

Species included (37). – E. araucana Phil., *E. boerhaavioides* Rusby, *E. burkartii* Bacigalupo, ***E. caespitosa*** Lam., *E. chamaeclada* Ule, *E. collina* Phil., *E. copiapina* Phil., *E. cordeiroae* P. Carrillo & V.W. Steinm., *E. correntina* Parodi, *E. cymbiformis* Rusby, *E. duriuscula* Pax & K. Hoffm. ex Herzog, ***E. eanophylla*** Croizat, ***E. elodes*** Boiss., ***E. elquiensis*** Phil., *E. floridana* Chapm., *E. guachanca* Haenke in Azara, *E. guaraniorum* P. Carrillo & V.W. Steinm., *E. hieronymi* Subils, *E. hinkleyorum* I.M. Johnst., *E. huanchahana* (Klotzsch & Garcke) Boiss., *E. inundata* Torr. ex Chapm., *E. macraulonia* Phil., *E. ovalleana* Phil., *E. pampeana* Speg., ***E. papillosa*** A. St.-Hil., *E. paranensis* Dusén, *E. pedersenii* Subils, ***E. peperomioides*** Boiss., *E. porphyrantha* Phil., ***E. portulacoides*** L., *E. raphanorrhiza* (Millsp.) J.F. Macbr., *E. raphilippii* Oudejans, *E. rhabdodes* Boiss., ***E. rosescens*** E.L. Bridges & Orzell, *E. schickendantzii* Hieron., ***E. stenophylla*** (Klotzsch & Garcke) Boiss., ***E. telephioides*** Chapm., ***E. thinophila*** Phil.

Most species in this section are confined to southern South America, but a group of four species are disjunct to the southeastern United States and were formerly treated as subsect. *Inundatae* G.L. Webster (Bridges & Orzell, 2002). Here this group is strongly supported as sister to the South American species. Both groups share the uncommon feature of a relatively well-developed calyx on the female flower. The species from the southeastern United States are functionally dioecious, a feature so far shared only with *E. hieronymi* among the South American species (Bridges & Orzell, 2002).

10. *Euphorbia* sect. *Crepidaria* (Haw.) Baill., Étude Gén. Euphorb. 284. 1858. ≡ *Crepidaria* Haw., Syn. Pl. Succ. 136. 1812. – Lectotype (Wheeler, 1939: 44): *Crepidaria myrtifolia* (Miller) Haw. (= *Euphorbia tithymaloides* L.).

= *Pedilanthus* Necker ex Poiteau, Elem. Bot. 2: 354. 1790. ≡ *Pedilanthus* sect. *Eupedilanthus* Boiss. in DC., Prodr. 15(2): 4. 1862. ≡ *Tithymaloides* sect. *Pedilanthus* Gomez de la Maza, Fl. Haban. 154. 1897. ≡ *Tithymaloides* sect. *Eutithymalodes* Kuntze in Post & Kuntze, Lex. Gen. Phan. 562. 1904. – Lectotype (Millspaugh, 1909: 300): *Pedilanthus tithymaloides* (L.) Poiteau (= *Euphorbia tithymaloides* L.).

= *Tithymalus* Mill., Gard. Dict. ed. 2, 3. 1754. – Type: *Tithymalus myrtifolius* Mill. (= *E. tithymaloides* L.).

= *Tithymaloides* Ortega, Tab. Bot. Tournefort., ed. 2. 28. 1783. – Lectotype (Wheeler, 1939: 45): *Tithymaloides myrtifolium* (L.) Kuntze (=*Euphorbia tithymaloides* L.).

= *Venitenatia* Tratt., Gen. Pl. 86. 1802. – Type: *Venitenatia bracteata* (Jacq.) Tratt. (≡*Euphorbia bracteata* Jacq.).

= *Diadenaria* Klotzsch & Garcke, Monatsb. Akad. Berlin. 254. 1859. ≡ *Tithymaloides* sect. *Diadenaria* (Klotzsch & Garcke) O. Kuntze, in Post & Kuntze, Lex. Gen.Phan. 562. 1904. – Lectotype (Wheeler, 1939: 44): *Diadenaria pavonis* Klotzsch & Garcke (= *Euphorbia bracteata* Jacq.).

= *Hexadenia* Klotzsch & Garcke, Monatsb. Akad. Berlin 253. 1859. – Type: *Hexadenia macrocarpa* (Benth.) Klotzsch & Garcke (= *Euphorbia lomelii* V.W. Steinm.).

Monoecious shrubs to small trees. Stems succulent or not. Leaves alternate, minute and scalelike to large and elliptic, deciduous to persistent, entire; petiole short to absent, stipules small, caducous. Synflorescences terminal or axillary; cyathia zygomorphic, cyathophylls red to green, spreading or not, variously shaped, glands 2--6, appendiculate, situated on dorsal side of involucre and usually enclosed in a spur-like extension of the involucre tube; seeds ecarunculate.

Distribution and habitat. – Mexico, Central America, West Indies, northern South America, southeastern U.S.A. (Florida); desert scrub, as well as dry and wet tropical forests, sea level to ca. 800 m

Species included (15). – ***E. bracteata*** Jacq., ***E. calcarata*** (Schltdl.) V.W. Steinm., ***E. coalcomanensis*** (Croizat) V.W. Steinm., ***E. colligata*** V.W. Steinm., ***E. conzattii*** V.W. Steinm., ***E. cymbifera*** (Schltdl.) V.W. Steinm., ***E. cyri*** V.W. Steinm., ***E. diazlunana*** (J. Lomelí & Sahagun) V.W. Steinm., *E. dressleri* V.W. Steinm., ***E. finkii*** (Boiss.) V.W. Steinm., ***E. lomelii*** V.W. Steinm., ***E. peritropoides*** (Millsp.) V.W. Steinm., ***E. personata*** (Croizat) V.W. Steinm., ***E. tehuacana*** (Brandegee) V.W. Steinm., ***E. tithymaloides*** L.

Section *Crepidaria* corresponds to the former genus *Pedilanthus* and is a collection of fifteen species from a wide range of habitats. The highly modified, zygomorphic cyathia in which a nectar spur is constructed of both involucre and gland appendage tissue easily distinguish this group (Dressler, 1957).

11. *Euphorbia* sect. *Stachydium* Boiss. in DC., Prodr. 15(2): 9, 65. 1862. – Lectotype (Wheeler, 1943: 481): *Euphorbia comosa* Vell.

Monoecious herbs or shrubs; stems green, terete, laxly to densely branched. Leaves ovate to linear-lanceolate, petiolate or sessile, drought deciduous (except *E. comosa*); synflorescenses monochasial spikes; cyathia sessile, solitary, subtended by a foliaceous bract, which overlaps with those of the other cyathia, glands 4(5), elliptic, green-yellow, appendages lacking. Capsules elongate, glabrous, pedicillate, recurved or erect; seeds tetragonous, tuberculate, carunculate.

Distribution and habitat. –Northern South America to eastern Brazil; low deciduous forest and thorn scrub on sandy or loose stony soils or rocky outcrops; 200--1200 m.

Species included (6). – ***E. comosa*** Vell., ***E. gollmeriana*** Klotzsch ex Boiss., ***E. heterodoxa*** Mull.-Arg., *E. invaginata* Croizat, ***E. lagunillarum*** Croizat, *E. vervoorstii* Subils.

12. *Euphorbia* sect. *Brasilienses* V.W. Steinm. & Dorsey, **sect. nov.** – Type: *Euphorbia phosphorea* Mart.

Monoecious, xerophytic, stem-succulent shrubs to 6 m tall, highly branched. Stems ascending, with a persistent green epidermis, 4--6 angles descending from leaf scars, waxy. Leaves alternate, highly reduced and quickly deciduous, ovate, apex acute, margin entire, secondary venation not evident. Cyathia axillary, solitary or few in dense clusters, subsessile, glands 4--5(--7), transversely oblong, slightly cup-shaped with two horns either spreading or ascending, red or yellow, situated on the rim of the involucre; gynophore terminating in 3 triangular calyx-like lobes; ovary glabrous, styles united most of their length into a slender column, undivided. Capsule exserted from the involucre, green, red, or yellow and red, glabrous, 3-lobed; seeds obloid, rounded to weakly quadrangular in cross-section, base and apex rounded, smooth to minutely papillate, with a minute discoid caruncle.

Distribution and habitat. – Brazil (Espírito Santo, Bahia, Minas Gerais, Paraíba and Pernambuco); rocky uplands (campos rupestres), grasslands, thorn scrub, and tropical deciduous forest; 300–1250 m.

Species included (4). – ***E. attastoma*** Rizzini, *E. holochlorina* Rizzini, ***E. phosphorea*** Mart., ***E. sipolisii*** N.E. Br.

This is the only New World section comprised entirely of stem-succulent species. The group is easily recognized by this habit and the colorful (dark red or yellow), horned cyathial glands (Fig. 2.1K). The distinctions among species are mainly of gland color and number of stem angles (Rizzini, 1987; Eggli, 1994).

13. *Euphorbia* sect. *Pachysanthae* X. Aubriot & Haev., **sect. nov.** – Type: *Euphorbia pachysantha* Baill.

Shrubs to large trees (2--20 m), "bottle-shaped" and unbranched at the base, mostly monoecious. Stems succulent toward the apices; bark gray, smooth to wrinkled. Leaves spirally arranged, grouped at the apices of the branches, obovate to lanceolate, thick (except in *E. pachysantha*); stipules reduced to two small glands. Synflorescence subterminal, 1 or 2--4 cyathia at the apex of the twigs; cyathophylls 2, well-developed, green-yellow, soon deciduous; glands 5, stipitate, elliptic to reniform, exappendiculate, green-yellow. Capsule indehiscent or tardily dehiscent (dehiscence line present though dehiscence not observed), erect or pendant, 3-locular or more commonly 2-locular or 1-locular by abortion, smooth to slightly wrinkled, green when young, 1.5--4 cm diam.; seeds 1--3, smooth, globular, ecarunculate.

Distribution and habitat. – Madagascar; in a variety of habitats, such as thickets in xeric bush of the southeast, tsingy (karst) formations in the north, remnant forests in the high plateaus in the northwest, and rainforest relicts of the central east.

Species included (4) – *E. mananarensis* Leandri, ***E. mandravioky*** Baill., ***E. pachysantha*** Baill., *E. pirahazo* Jum.

The large, uni- or bilocular fruits of this section are quite rare in *Euphorbia* and their presence suggests a close relationship to sect. *Pervilleanae*. Although rarely collected, the fruits of *E. pachysantha* are apparently fleshy and indehiscent. There are two additional species from northern Madagascar awaiting publication by Aubriot et al. (in prep.)

14. *Euphorbia* sect. *Pervilleanae* Haev. & X. Aubriot, **sect. nov**. – Type: *Euphorbia pervilleana* Baill.

Usually dioecious shrubs to trees, non-succulent but with a water storing rootstock; bark smooth to exfoliating. Leaves alternate to subopposite, shape variable (linear, lanceolate, elliptic to obovate, scale-like for *E. intisy*); stipules reduced to black gland-like dots. Synflorescences terminal, male synflorescences bearing a larger number of cyathia; cyathophylls green, inconspicuous, similar to dichasial bracts; glands 4--6, elliptic to bilabiate. Capsule functionally 2(3)-locular, dehiscent or indehiscent, erect, smooth to pubescent, usually bearing ornaments such as wings, spines, or tubercles; seeds (1)2(3), large (5--10 mm diam.), chestnut-shaped, smooth or tuberculate, ecarunculate.

Distribution and habitat. – Widespread across Madagascar.

Species included(7). – *E. adenopoda* Baill., *E. analamerae* Leandri, ***E. intisy*** Drake, ***E. pervilleana*** Baill., ***E. randrianjohanyi*** Haev. & Labat, ***E. rauhii*** Haev. & Labat, ***E. tetraptera*** Baker.

This group was formerly included in sect. *Denisophorbia*, to which it is not closely related. The leafless *E. intisy* was previously included in subg. *Tirucalli* but was shown to be misplaced there (Haevermans, 2003), and this is confirmed here (Fig. 2.2B).

15. *Euphorbia* sect. *Tirucalli* Boiss. in DC., Prodr. 15(2): 10, 94. 1862. ≡ *Euphorbia* subsect. *Tirucalli* (Boiss.) Benth. & Hook.f., Gen. Pl. 3(1): 260. 1880. ≡ *Euphorbia* subg. *Tirucalli* (Boiss.) S. Carter, Kew Bull. 40: 823. 1985. – Type: *Euphorbia tirucalli* L.

= *Arthrothamnus* Klotzsch & Garke, Monatsb. Akad. Berlin 1859: 281. 1859, non Rupr. 1848. ≡ *Euphorbia* sect. *Arthrothamnus* (Klotzsch & Garcke) Boiss. in DC., Prodr. 15(2): 10, 74. 1862. – Lectotype (Millspaugh, 1909: 306): *Arthrothamnus tirucalli* (L.) Klotzsch & Garcke (=*Euphorbia tirucalli* L.).

= *Euphorbia* sect. *Armatocalli* Croizat, Webbia 27(1): 180. 1972. – Type: *Euphorbia stenoclada* Baill.

Usually dioecious shrubs to trees. Stems more or less succulent, with alternate or subverticillate branching (dichotomous in *E. carunculifera*), terete (some species have more or less flattened apices, in *E. stenoclada* sterile twig apices turn into spiny processes), bark much delayed, fissured, detaching in plates (smooth), gray-brown. Leaves alternate, early caducous, variable in size and shape depending on the species but never well-developed; pubescence more or less developed (potentially present on every part of the plant); stipules highly reduced, glandular or absent. Synflorescences subterminal (terminal), composed of one or more lateral dichasia, only the terminal cyathia functional; male synflorescences with many more cyathia than the female ones, cyathophylls green, inconspicuous, similar to dichasial bracts; cyathia usually unisexual, glands 5, orbicular to elliptic, convex or concave, green to red, more or less stipitate; female flower pedicellate, perianth remnants constituting a 3-lobed calyx, styles 3, free to base or united partway down, bifid, recurved. Capsule smooth and sometimes pubescent, pendent or erect, 3(4--6)-locular, green or red, orbicular to acutely 3(4--6)-angled, then more or less deeply lobed; seeds 3(4--6), cylindrical to obtusely 4-angled, testa smooth, carunculate.

Distribution and habitat. – Widespread and most diverse in Madagascar, also native in the Arabian Peninsula (Oman and Yemen, including Socotra) and Africa (Angola, Namibia, Somalia, and South Africa). *Euphorbia tirucalli* is widespread across Africa, but it is not clear where the species is native (probably Madagascar and southern Africa). It is widely cultivated in India and in other tropical countries.

Species included (*25*) *– E. alcicornis* Baker, *E. analalavensis* Leandri, ***E. arahaka*** Poiss., ***E. arbuscula*** Balf.f., *E. bariensis* S. Carter, *E. boinensis* Denis ex Humbert & Leandri, ***E. carunculifera*** L.C. Leach, ***E. congestiflora*** L.C. Leach, ***E. damarana*** L.C. Leach, ***E. decorsei*** Drake, ***E. enterophora*** Drake, ***E. fiherenensis*** Poiss., ***E. gregaria*** Marloth, ***E. gummifera*** Boiss., *E. imerina* Cremers, ***E. kamponii*** Rauh & Pétignat, *E. mainty* (Poiss.) Denis ex Leandri, ***E. neochamaeclada*** Bruyns, *E. ramofraga* Denis & Humbert ex Leandri, *E. spissa* M. Thulin, ***E. stenoclada*** Baill., ***E. tirucalli*** L., ***E. uzmuk*** S. Carter & J.R.I. Wood, ***E. xylophylloides*** Brongn. ex Lem.

16. *Euphorbia* sect. *Rubellae* Dorsey, **sect. nov**. – Type: *Euphorbia rubella* Pax.

Monoecious geophytes. Root a subglobose to cone-shaped tuber or fibrous. Stems reduced and underground, tuberculate. Leaves elliptic in a rosette at ground level, long petiolate (2--6 cm); stipules threadlike or absent. Synflorescences subapical, produced before leaves emerge or with them, peduncle 2--11 cm long, more or less branched; cyathophylls orbicular-ovate, fused dorsally in *E. cryptocaulis*; cyathia sessile, glands 4 + 2 due to one being divided, oblong-elliptic, red or yellow; ovary exserted, oblong, smooth. Capsule long-exserted, oblong-ovoid; seeds ovoid, tuberculate, carunculate.

Distribution and habitat – Northeastern Africa (Sudan, Ethiopia, Kenya, Uganda); open sites in limestone crevices on well-drained soil, or under bushes in evergreen or deciduous bushland.

Species included (3) - ***E. brunellii*** Chiov., *E. cryptocaulis* M.G. Gilbert, ***E. rubella*** Pax.

This group of geophtytes from northeast Africa is unique in its cyathial gland morphology having 4 equal-sized glands and 2 smaller ones produced by the division of a single gland (Gilbert, 1987). Geophytes are present in several sections of subg. *Euphorbia*, but this unusual gland structure makes this restricted and small group easily recognizable. Bally (1967) considered *E. brunellii* a variety of *E. rubella*, but Gilbert (1995) treated them as separate species based on distinct root morphology, leaf size, cyathophyll shape, and capsule shape. We follow Gilbert (1995) and note that both the *ndh*F and ITS sequences differ more between these two species than between many other accepted species pairs in subg. *Euphorbia*.

17. *Euphorbia* sect. *Goniostema* Baill. ex Boiss. in DC., Prodr. 15(2): 10, 77. 1862. – Lectotype (Wheeler, 1943: 485): *Euphorbia lophogona* Lam.

= *Lacanthis* Raf., Fl. Tell. 2: 94. 1837. ≡ *Euphorbia* subg. *Lacanthis* (Raf.) M.G. Gilbert, Kew Bull. 42: 238. 1987. – Type: *Lacanthis splendens* (Bojer) Raf. (= *Euphorbia milii* Des Moul.).

= *Euphorbia* sect. *Diacanthium* Boiss. in DC., Prodr. 15 (2): 10, 78. 1862. – Lectoype (Wheeler, 1943: 485): *Euphorbia splendens* Bojer (= *E. milii* Des Moul.).

= *Sterigmanthe* Klotzsch & Garcke, Monatsber. Königl. Preuss. Akad. Wiss. Berlin 1859: 252. 1859. – Lectotype (Wheeler, 1943: 472): *Euphorbia splendens* Bojer (= *E. milii* Des Moul.).

= *Euphorbia* sect. *Rhizanthopsis* Croizat, Webbia 27(1): 178. 1972. – Type: *Euphorbia francoisii* Leandri.

Monoecious, prostrate geophytes, shrubs, or trees. Stems more or less succulent, branching or not; bark smooth, gray. Leaves, alternate, persistent or deciduous; shape highly variable; succulent in some taxa; petiole cylindrical or winged; stipular structures from gland-like to spines, individual or gathered in vertical comb-like structures, some branching, glabrous to pubescent. Synflorescences lateral (subterminal), of a variable number of cyathia (in some taxa, axillary buds can further develop the inflorescence by producing new cyathia); cyathophylls 2 per cyathium, usually well-developed and usually brightly colored, usually distinct from synflorescence bracts, patent or tightly enclosing the cyathia; cyathia bisexual, protandrous or protogynous; glands (4-)5(-6), shape variable, spreading or enveloping the involucre. Capsule dehiscent, smooth, erect, 3-locular; seeds 3, verrucose, ecarunculate.

Distribution and habitat. – Widespread across the island of Madagascar; in a wide variety of habitats from sea level to upper mountains.

Species included (76) – ***E. alfredii*** Rauh, *E. ambarivatoensis* Rauh & Bard.-Vauc., ***E. ambovombensis*** Rauh & Razaf., ***E. analavelonensis*** Rauh & Mangelsdorff, ***E. ankarensis*** Boiteau, ***E. ankazobensis*** Rauh & Hofstätter, ***E. annamarieae*** Rauh, *E. aureoviridiflora* (Rauh) Rauh, *E. banae* Rauh, ***E. beharensis*** Leandri, *E. berevoensis* Lawant & Buddens., *E. berorohae* Rauh & Hofstätter, *E. biaculeata* Denis, ***E. boissieri*** Baill., *E. boiteaui* Leandri, ***E. brachyphylla*** Denis, ***E. bulbispina*** Rauh & Razaf., ***E. capmanambatoensis*** Rauh, ***E. capuronii*** Ursch & Leandri, *E. caput-aureum* Denis, ***E. cremersii*** Rauh & Razaf., ***E. croizatii*** Leandri, ***E. cylindrifolia*** Marn.-Lap. & Rauh, ***E. decaryi*** Guillaumin, *E. delphinensis* Ursch & Leandri, *E. denisiana* Guillaumin, ***E. didiereoides*** Denis ex Leandri, *E. duranii* Ursch & Leandri, *E.*

erythrocucullata Mangelsdorff, *E. fianarantsoae* Ursch & Leandri, ***E. francoisii*** Leandri, *E. genoudiana* Ursch & Leandri, ***E. geroldii*** Rauh, ***E. gottlebei*** Rauh, *E. guillauminiana* Boiteau, *E. hermanschwartzii* Rauh, *E. hexadenia* Denis, *E. hofstaetteri* Rauh, ***E. horombensis*** Ursch & Leandri, ***E. iharanae*** Rauh, *E. isalensis* Leandri, *E. itremensis* Kimnach & Lavranos, *E. kondoi* Rauh & Razaf., *E. labatii* Rauh & Bard.-Vauc., *E. leuconeura* Boiss., ***E. lophogona*** Lam., ***E. mahafalensis*** Denis, *E. mainiana* Poiss., *E. mangelsdorffii* Rauh, *E. mangokyensis* Denis, *E. maromokotrensis* Rebmann, ***E. milii*** Des Moul., ***E. millotii*** Ursch & Leandri, *E. moratii* Rauh, *E. neobosseri* (Rauh) Rauh, ***E. neohumbertii*** Boiteau, ***E. pachypodioides*** Boiteau, ***E. parvicyathophora*** Rauh, ***E. paulianii*** Ursch & Leandri, ***E. pedilanthoides*** Denis, ***E. perrieri*** Drake, ***E. primulifolia*** Baker, ***E. quartziticola*** Leandri, *E. razafindratsirae* Lavranos, *E. razafinjohanyi* Ursch & Leandri, ***E. retrospina*** Rauh & Gerold, ***E. robivelonae*** Rauh, ***E. rossii*** Rauh & Buchloh, *E. sakarahaensis* Rauh, *E. suzannae-marnierae* Rauh & Pétignat, *E. tardieuana* Leandri, *E. thuarsiana* Baill., *E. tsimbazazae* Leandri, ***E. tulearensis*** (Rauh) Rauh, ***E. viguieri*** Denis, *E. waringiae* Rauh & Gérold.

Bruyns & al. (2006) circumscribed this section to include the entire Old World Clade II, but here we limit it to a more restricted clade containing the type, *E. lophogona*¸ and other species generally characterized by colorful cyathophylls. Section *Goniostema* is the largest clade in subg. *Euphorbia* on Madagascar and occurs across the island in diverse habitats. It is also a morphologically diverse clade including understory trees and shrubs in moist habitats (e.g., *E. geroldii*), spiny xerophytic or succulent shrubs and dwarfs from more arid habitats (*E. milii* complex), and geophytes in fire-prone regions and unusual substrates (e.g., *E. primulifolia* and *E. quartzicola*).

18. *Euphorbia* sect. *Deuterocalli* Croizat, Webbia 27(1): 179. 1972. ≡ *Euphorbia* subg. *Euphorbia* subsect. *Deuterocalli* (Croizat) Bruyns, Taxon 55: 415. 2006. – Type: *Euphorbia oncoclada* Drake (=*Euphorbia alluaudii* subsp. *oncoclada* (Drake) F. Friedmann & Cremers).

Dioecious shrubs or small trees. Stems succulent, terete (sometimes jointed as in a string of sausages because of alternation between long shoot and brachyblast morphology), bark gray, smooth, much delayed. Leaves alternate, soon deciduous, reduced to scales or lanceolate and petiolate; stipules modified as a waxy or glandular ring around the leaf insertion. Synflorescence lateral (subterminal), male cyathia usually more numerous; cyathophylls inconspicuous, green (identical to dichasial bracts); cyathia pedunculate; glands 5, elliptical, green-yellow. Capsule dehiscent, erect, 3-locular, green, smooth, with ridges tinted with purple; seeds 3, smooth, ecarunculate.

Distribution and habitat. – Widespread in Madagascar, mostly in dry scrub or among rocks in moister habitats, sea level to ca. 500 m.

Species included (3). – ***E. alluaudii*** Drake, ***E. cedrorum*** Rauh & Hebding, ***E. famatamboay*** F. Friedmann & Cremers.

This is a well-supported clade containing three species of trees that were previously grouped with *E. tirucalli* because of their leafless, pencil-stem growth form and dioecy. However, they differ from sect. *Tirucalli* in their thicker, more jointed stems, stipules that form glandular or waxy rings around the leaf scars, and ecarunculate seeds. *Euphorbia alluaudii* is treated here to include *E. leucodendron* Drake as well as *E. oncoclada* Drake.

19. *Euphorbia* sect. *Denisophorbia* (Leandri) Croizat, Webbia 27(1): 165. 1972. ≡ *Euphorbia* sect. *Euphorbium* subsect. *Denisophorbia* Leandri, Bull. Soc. Bot. France 104: 500. 1957. − Type: *Euphorbia pyrifolia* Lam.

Monoecious or dioecious shrubs or trees, without spines, sometimes with a basal caudex, the upper branching chandelier-like, with plagiotropic branches composed of one or more series of hypopodia (long, leafless internodes) and apical brachyblasts (very short branches with a rosette of leaves), the stems succulent or not, bark smooth, green or reddish-brown. Leaves spirally arranged, shape variable, present only at nodes or extremities of branches, deciduous to persistent, membranaceous to coriaceous (sometimes slightly succulent), petiolate or subsessile; stipules reduced to glandular dots. Synflorescences subterminal on brachyblasts, bearing few to many cyathia; cyathophylls 2, scarious and inconspicuous to well-developed and showy, green to yellow; glands (4)5(6), oval to kidney-shaped, green-yellow or pink. Capsule dehiscent, 3-locular, erect, smooth, round or strongly 3-angled in cross-section; seeds smooth, spherical, ecarunculate.

Distribution and habitat. – Madagascar, Mayotte and Comoro Islands, Seychelles, Mauritius.

Species included (13). –E. aprica Baill., *E. betacea* Baill., ***E. boivinii*** Boiss., *E. bongolavensis* Rauh, ***E. elliotii*** Leandri, ***E. hedyotoides*** N.E. Br., ***E. mahabobokensis*** Rauh, *E. mangorensis* Leandri, *E. martinae* Rauh, *E. physoclada* Boiss., *E. pyrifolia* Lam., *E. rangovalensis* Leandri, *E. zakamenae* Leandri.

This section is treated here in a more limited sense than Leandri's original circumscription of subsect. *Denisophorbia*. It includes a group of mostly dioecious species from Madagascar and other Indian Ocean islands that have a *Terminalia*-type of sympodial branching, with long arching branches ending in short brachyblasts that bear the leaves and cyathia. The group is currently under revision, and there appear to be at least five new species awaiting description (Haevermans et al., in prep.).

20. *Euphorbia* sect. *Monadenium* (Pax) Bruyns, Taxon 55: 411. 2006. ≡ *Monadenium* Pax, Bot. Jahrb. Syst. 19: 12 (1894). – Type: *Monadenium coccineum* Pax. (= *Euphorbia neococcinea* Bruyns).

= *Synadenium* Boiss. in DC., Prodr. 15 (2): 187. 1862. – Type: *Synadenium arborescens* Boiss. (= *Euphorbia cupularis* Boiss.).

= *Stenadenium* Pax, Bot. Jahrb. Syst. 30: 343. 1901. – Type: *Stenadenium spinescens* Pax (= *Euphorbia spinescens* (Pax) Bruyns).

= *Endadenium* L.C. Leach, Garcia de Orta 1: 31. 1973. – Type: *Endadenium gossweileri* (N.E. Br.) L.C. Leach (= *Euphorbia neogossweileri* Bruyns).

Monoecious geophytes, herbs, shrubs, or trees. Stems terete to angled, +/- fleshy to succulent. Leaves fleshy, stipules apparently absent or modified as spines or glands. Synflorescences axillary cymes to single cyathia; cyathophylls conspicuous, persistent, partly united along one edge then partly surrounding the involucre, or free; cyathia sessile; glands united into a horseshoe or ring that surrounds 5 involucral lobes. Capsule 3-lobed, dehiscent, exerted (through notch in gland ring if present); seeds oblong, carunculate.

Distribution and habitat. – Eastern, central, and southeastern tropical Africa; in a variety of mostly arid habitats: thickets, rock outcrops, and other open areas.

Species included (*90*). – *E. bianoensis* (Malaisse & Lecron) Bruyns, ***E. bicompacta*** Bruyns, ***E. biselegans*** Bruyns, ***E. bisellenbeckii*** Bruyns, *E. bisglobosa* Bruyns, *E. bodenghieniae*

(Malaisse & Lecron) Bruyns, *E. catenata* Bruyns, *E. chevalieri* Bruyns, *E. clarae* (Malaisse & Lecron) Bruyns, *E. crenata* (N.E. Br.) Bruyns, *E. cupricola* (Malaisse & Lecron) Bruyns, ***E. cupularis*** Boiss, E. *descampsii* (Pax) Bruyns, *E. dilunguensis* (Malaisse & Lecron) Bruyns, ***E. discoidea*** (P.R.O. Bally) Bruyns, *E. echinulata* (Stapf) Bruyns, *E. filiformis* (P.R.O. Bally) Bruyns, *E. friesii* (N.E. Br.) Bruyns, *E. fwambensis* (N.E. Br.) Bruyns, *E. gammaranoi* G. Will., *E. gladiata* (P.R.O. Bally) Bruyns, *E. guentheri* (Pax) Bruyns, *E. hedigeriana* (Malaisse & Lecron) Bruyns, *E. herbacea* (Pax) Bruyns, ***E. heteropoda*** Pax, *E. iancannellii* Bruyns, ***E. invenusta*** (N.E. Br.) Bruyns, ***E. kimberleyana*** (G. Will.) Bruyns, *E. kirkii* (N.E. Br.) Bruyns, *E. kundelunguensis* (Malaisse) Bruyns, *E. letestuana* (Denis) Bruyns, *E. letouzeyana* (Malaisse) Bruyns, ***E. lindenii*** (S. Carter) Bruyns, ***E. lugardiae*** (N.E. Br.) Bruyns, *E. mafingensis* (Hargreaves) Bruyns, ***E. magnifica*** (E.A. Bruce) Bruyns, *E. major* (Pax) Bruyns, *E. mamfwensis* (Malaisse & Lecron) Bruyns, *E. maryrichardsiae* G. Will., *E. neoangolensis* Bruyns, ***E. neoarborescens*** Bruyns, *E. neocapitata* Bruyns, ***E. neococcinea*** Bruyns, *E. neocrispa* Bruyns, *E. neocymosa* Bruyns, *E. neogillettii* Bruyns, *E. neoglabrata* Bruyns, *E. neoglaucescens* Bruyns, *E. neogoetzei* Bruyns, ***E. neogossweileri*** Bruyns, ***E. neogracilis*** Bruyns, *E. neohalipedicola* Bruyns, *E. neokaessneri* Bruyns, ***E. neomontana*** Bruyns, *E. neoparviflora* Bruyns, ***E. neopedunculata*** Bruyns, ***E. neoreflexa*** Bruyns, ***E. neorubella*** Bruyns, *E. neorugosa* Bruyns, ***E. neospinescens*** Bruyns, ***E. neostolonifera*** Bruyns, ***E. neovirgata*** Bruyns, *E. orobanchoides* (P.R.O. Bally) Bruyns, *E. pereskiifolia* Houllet ex Baill., *E. pseudohirsuta* Bruyns, ***E. pseudolaevis*** Bruyns, ***E. pseudomollis*** Bruyns, *E. pseudonudicaulis* Bruyns, *E. pseudopetiolata* Bruyns, *E. pseudoracemosa* (P.R.O. Bally) Bruyns, *E. pseudosimplex* Bruyns, ***E. pseudostellata*** Bruyns, ***E. pseudotrinervis*** Bruyns, *E. pseudovolkensii* Bruyns, *E. pudibunda* (P.R.O. Bally) Bruyns, *E. renneyi* (S. Carter) Bruyns, ***E. rhizophora*** (P.R.O. Bally) Bruyns, *E. ritchiei* (P.R.O.

Bally) Bruyns, *E. schaijesii* (Malaisse) Bruyns, ***E. schubei*** Pax, *E. shebeliensis* (M.G. Gilbert) Bruyns, ***E. spectabilis*** (S. Carter) Bruyns, *E. spinulosa* (S. Carter) Bruyns, ***E. succulenta*** (Schweickerdt) Bruyns, *E. syncalycina* Bruyns, *E. syncameronii* Bruyns, ***E. torrei*** (L.C. Leach) Bruyns, *E. triangolensis* Bruyns, ***E. umbellata*** (Pax) Bruyns, *E. yattana* (P.R.O. Bally) Bruyns.

Section *Monadenium* corresponds to the formerly recognized genera *Monadenium, Synadenium,* and *Endadenium*. These were previously segregated from *Euphorbia* based on modifications of the cyathial glands – fused into a ring in *Endadenium* and *Synadenium,* or into a horseshoe shape in *Monadenium*. These modified gland structures continue to be phylogenetically informative, as the former *Synadenium* and *Endadenium* species group closely together within sect. *Monadenium*. These species can also be distinguished by their reduced caruncle relative to the distinctive one of most other species in the section. Section *Monadenium* is another example of the lability of growth form evolution in subg. *Euphorbia,* since it contains nearly every form present in the subgenus. Many species also bear spine-like structures of various forms that support their close relationship to both sect. *Goniostema* and sect. *Euphorbia,* although the homology of the "spines" in these three sections is not yet established (Fig. 2.1N--P).

21. *Euphorbia* sect. *Euphorbia* – Lectotype (Millspaugh 1909: 306): *Euphorbia antiquorum* L.

= *Elaeophorbia* Stapf in Johnst., Liberia 2: 646. 1906. – Type: *Elaeophorbia drupifera* (Thonn.) Stapf (= *Euphorbia drupifera* Thonn.).

= *Euphorbia* subg. *Tithymalus* Pers., Syn. Pl. 2: 10. 1806, non Gaertn. (1790). – Lectotype (Wheeler, 1943: 484): *Euphorbia antiquorum* L.

= *Euphorbia* sect. *Aculeatae* Haw., Philos. Mag. & Ann. Philos., n.s., 1: 275. 1827. – Type: *Euphorbia caerulescens* Haw.

= *Euphorbia* sect. *Cereis* Webb & Berthelot, Hist. Nat. Iles Canaries 2(3): 255. 1847. – Type: *Euphorbia canariensis* L.

= *Euphorbia* sect. *Tekeanae* Croizat, Bull. Jard. Bot. Brux. 15: 119. 1938. – Type: *Euphorbia teke* Schweinf.

= *Euphorbia* sect. *Ballyeuphorbia* Croizat, Webbia 27(1): 181. 1972. – Type: *Euphorbia decidua* P.R.O. Bally & L.C. Leach.

Monoecious geophytes, shrubs or trees. Stems succulent, green, photosynthetic (some species forming bark in older stems), winged, angled or tuberculate. Leaves opposite, usually strongly reduced and caducous, but large and persistent in some tree or shrub species; stipules apparently modified as spines (rarely fused) and borne on a spine-shield subtending or surrounding the leaf that can also bear a pair of prickles. Synflorescences axillary, simple (compound), dichasia usually of three cyathia; cyathophylls inconspicuous; peduncles usually short; cyathia bisexual; glands 5, entire, typically elliptical, yellow to red; female flower pedicellate. Capsule 3-locular, globose to deeply acutely lobed, in a few species fleshy and indehiscent; seeds subglobose to ovoid, smooth to minutely tuberculate, ecarunculate.

Distribution and habitat. – Widespread across most of Africa, the Arabian Peninsula, and in southern Asia from Pakistan to Malaysia, Indonesia and Papua New Guinea; in a wide variety of habitats, but especially in arid landscapes, open areas, dry forests, scrub, rock outcrops, with some species occurring in moist forests.

Species included (344). – ***E. abdelkuri*** Balf.f., ***E. abyssinica*** J.F. Gmel., *E. acervata* S. Carter, *E. actinoclada* S. Carter, *E. adjurana* P.R.O. Bally & S. Carter, ***E. aeruginosa***

Schweick., *E. ambacensis* N.E. Br., ***E. ambroseae*** L.C. Leach, *E. amicorum* S. Carter, ***E. ammak*** Schweinf., *E. ammophila* S. Carter & Dioli, ***E. amplophylla*** Pax, *E. angularis* Klotzsch, *E. angustiflora* Pax, ***E. antiquorum*** L., *E. asthenacantha* S. Carter, *E. atrocarmesina* L.C. Leach, *E. atroflora* S. Carter, *E. atrox* F.K. Horw. ex S. Carter, ***E. avasmontana*** Dinter, *E. awashensis* M.G. Gilbert, *E. baga* A.Chev., *E. baioensis* S. Carter, *E. baleensis* M.G. Gilbert, *E. ballyana* Rauh, *E. ballyi* S. Carter, *E. baradii* S. Carter, *E. barnardii* A.C. White, R.A. Dyer & B. Sloane, *E. baylissii* L.C. Leach, *E. beillei* A. Chev., *E. bertemariae* Bisseret & Dioli, *E. biharamulensis* S. Carter, *E. bitataensis* M.G. Gilbert, *E. borenensis* M.G. Gilbert, ***E. bougheyi*** L.C. Leach, ***E. breviarticulata*** Pax, *E. brevis* N.E. Br., ***E. brevitorta*** P.R.O. Bally, *E. burgeri* M.G. Gilbert, *E. buruana* Pax, ***E. bussei*** Pax, *E. bwambensis* S. Carter, ***E. cactus*** Ehrenb. ex Boiss., ***E. caducifolia*** Haines, ***E. caerulescens*** Haw., *E. caloderma* S. Carter, ***E. canariensis*** L., *E. candelabrum* Trémaux ex Kotschy, *E. cannellii* L.C. Leach, *E. carteriana* P.R.O. Bally, *E. cataractarum* S. Carter, ***E. cattimandoo*** Elliot ex Wight, *E. classenii* P.R.O. Bally & S. Carter, ***E. clavigera*** N.E. Br., ***E. clivicola*** R.A. Dyer, *E. coerulans* Pax, *E. collenetteae* D. Al-Zahrani & El-Karemy, *E. colubrina* P.R.O. Bally & S. Carter, *E. columnaris* P.R.O. Bally, *E. complanata* Warb., *E. complexa* R.A. Dyer, ***E. confinalis*** R.A. Dyer, *E. conspicua* N.E. Br., ***E. contorta*** L.C. Leach, ***E. cooperi*** N.E. Br. ex A. Berger, ***E. corniculata*** R.A. Dyer, *E. cryptospinosa* P.R.O. Bally, *E. cuneneana* L.C. Leach, ***E. cuprispina*** S. Carter, *E. cupularis* Boiss., ***E. curvirama*** R.A. Dyer, *E. cussonioides* P.R.O. Bally, *E. dalettiensis* M.G. Gilbert, *E. darbandensis* N.E. Br., *E. dasyacantha* S. Carter, *E. dauana* S. Carter, *E. dawei* N.E. Br., *E. debilispina* L.C. Leach, ***E. decidua*** P.R.O. Bally & L.C. Leach, *E. decliviticola* L.C. Leach, *E. dedzana* L.C. Leach, *E. deightonii* Croizat, *E. dekindtii* Pax, *E. demissa* L.C. Leach, *E. densispina* S. Carter, ***E. desmondii*** Keay & Milne-Redh., *E. dichroa* S. Carter, *E. discrepans* S. Carter, *E. dispersa* L.C.

Leach, *E. dissitispina* L.C. Leach, *E. distinctissima* L.C. Leach, ***E. drupifera*** Thonn., *E. dumeticola* P.R.O. Bally & S. Carter, ***E. eduardoi*** L.C. Leach, *E. eilensis* S. Carter, ***E. elegantissima*** P.R.O. Bally & S. Carter, *E. ellenbeckii* Pax, ***E. enormis*** N.E. Br., ***E. epiphylloides*** Kurz, *E. erigavensis* S. Carter, *E. erlangeri* Pax, ***E. evansii*** Pax, ***E. excelsa*** A.C. White, R.A. Dyer & B. Sloane, *E. exilispina* S. Carter, *E. eyassiana* P.R.O. Bally & S. Carter, ***E. fanshawei*** L.C. Leach, *E. fascicaulis* S. Carter, *E. faucicola* L.C. Leach, *E. fissispina* P.R.O. Bally & S. Carter, *E. fluminis* S. Carter, *E. forolensis* L.E. Newton, *E. fortissima* L.C. Leach, ***E. fractiflexa*** S. Carter & J.R.I. Wood, *E. franckiana* A. Berger, *E. frankii* Lavranos, ***E. fruticosa*** Forssk., *E. furcata* N.E. Br., ***E. fusiformis*** Buch.-Ham. ex D. Don, *E. galgalana* S. Carter, *E. garuana* N.E. Br., *E. geldorensis* S. Carter, *E. gemmea* P.R.O. Bally & S. Carter, *E. gillettii* P.R.O. Bally & S. Carter, *E. glochidiata* Pax, *E. godana* Buddens., Lawant & Lavranos, *E. gracilicaulis* L.C. Leach, *E. graciliramea* Pax, *E. grandialata* R.A. Dyer, ***E. grandicornis*** Goebel ex N.E. Br., ***E. grandidens*** Haw., *E. grandifolia* Haw., ***E. graniticola*** L.C. Leach, *E. greenwayi* P.R.O. Bally & S. Carter, *E. greuteri* N. Kilian, Kürschner & P. Hein, ***E. griseola*** Pax, ***E. groenewaldii*** R.A. Dyer, ***E. gymnocalycioides*** M.G. Gilbert & S. Carter, *E. halipedicola* L.C. Leach, ***E. handiensis*** Burchard, ***E. heterochroma*** Pax, ***E. heterospina*** S. Carter, *E. hiernii* (Croizat) Oudejans, *E. holmesiae* Lavranos, *E. horwoodii* S. Carter & Lavranos, *E. hottentota* Marloth, *E. hubertii* Pax, *E. imitata* N.E. Br., *E. immersa* P.R.O. Bally & S. Carter, *E. imparispina* S. Carter, *E. inaequispina* N.E. Br., ***E. inarticulata*** Schweinf., *E. inculta* P.R.O. Bally, ***E. ingens*** E. Mey. ex Boiss., *E. ingenticapsa* L.C. Leach, *E. inundaticola* L.C. Leach, *E. isacantha* Pax, *E. johannis* S. Carter, *E. jubata* L.C. Leach, *E. kalisana* S. Carter, *E. kamerunica* Pax, *E. kaokoensis* (A.C. White, R.A. Dyer, & B. Sloane) L.C. Leach, ***E. keithii*** R.A. Dyer, *E. khandallensis* Blatt. & Hallb., *E. knobelii* Letty, ***E. knuthii*** Pax, ***E. lacei*** Craib, ***E. lactea*** Haw., *E. laikipiensis* S. Carter, *E. ledienii* A. Berger, ***E. lenewtonii*** S. Carter, *E. leontopoda* S. Carter, *E. letestui* J. Raynal, ***E. limpopoana*** L.C. Leach ex S. Carter, ***E. lividiflora*** L.C. Leach, ***E. longispina*** Chiov., *E. louwii* L.C. Leach, *E. luapulana* L.C. Leach, *E. lukoseana* S. Carter, ***E. lydenburgensis*** Schweick. & Letty, *E. madinahensis* Fayed & D. Al-Zahrani, ***E. magnicapsula*** S. Carter, *E. makallensis* S. Carter, ***E. malevola*** L.C. Leach, *E. margaretae* S. Carter, *E. marrupana* Bruyns, *E. marsabitensis* S. Carter, *E. mayuranathanii* Croizat~~,~~ ***E. meenae*** S. Carter, *E. memoralis* R.A. Dyer, *E. meridionalis* P.R.O. Bally & S. Carter, *E. michaelii* Thulin, ***E. micracantha*** Boiss., *E. migiurtinorum* Chiov., *E. mitriformis* P.R.O. Bally & S. Carter, ***E. mlanjeana*** L.C. Leach, *E. momccoyae* Lavranos, *E. monacantha* Pax, ***E. monadenioides*** M.G. Gilbert, *E. mosaica* P.R.O. Bally & S. Carter, *E. multiclava* P.R.O. Bally & S. Carter, *E. mwinilungensis* L.C. Leach, *E. myrioclada* S. Carter, ***E. namuliensis*** Bruyns, ***E. nana*** Royle, ***E. neriifolia*** L., *E. nicholasii* Oudejans, *E. nigrispina* N.E. Br., *E. nigrispinoides* M.G. Gilbert, ***E. nivulia*** Buch.-Ham., *E. nubigena* L.C. Leach, *E. nyassae* Pax, *E. nyikae* Pax ex Engl., *E. obconica* Bojer ex N.E. Br., *E. obovalifolia* A. Rich., *E. odontophora* S. Carter, ***E. officinarum*** L., *E. oligoclada* L.C. Leach, *E. opuntioides* Welw. ex Hiern, ***E. otjingandu*** Swanepoel, *E. otjipembana* L.C. Leach, *E. paganorum* A. Chev., ***E. parciramulosa*** Schweinf., *E. parviceps* L.C. Leach, *E. patentispina* S. Carter, ***E. perangusta*** R.A. Dyer, *E. perarmata* S. Carter, *E. perplexa* L.C. Leach, ***E. persistentifolia*** L.C. Leach, *E. pervittata* S. Carter, *E. petraea* S. Carter, *E. petricola* P.R.O. Bally & S. Carter, *E. phillipsiae* N.E. Br., *E. phillipsioides* S. Carter, *E. piceoides* Thulin, ***E. piscidermis*** M.G. Gilbert, *E. platyrrhiza* L.C. Leach, *E. plenispina* S. Carter, ***E. poissonii*** Pax, ***E. polyacantha*** Boiss., *E. ponderosa* S. Carter, ***E. proballyana*** L.C. Leach, *E. prona* S. Carter, *E. pseudoburuana* P.R.O. Bally & S. Carter, ***E. pseudocactus*** A. Berger, *E. pteroclada* L.C. Leach, ***E. qarad*** Deflers, *E. quadrangularis* Pax, ***E. quadrialata*** Pax, *E. quadrilatera* L.C. Leach, *E. quadrispina* S. Carter, *E. quinquecostata* Volkens, ***E. ramipressa*** Croizat, ***E. ramulosa*** L.C. Leach, *E. reclinata* P.R.O. Bally & S. Carter, *E. reptans* P.R.O. Bally & S. Carter, ***E. resinifera*** O. Berg, *E. restricta* R.A. Dyer, *E. richardsiae* L.C. Leach, *E. ridleyi* Croizat, ***E. robecchii*** Pax, *E. rowlandii* R.A. Dyer, ***E. royleana*** Boiss., *E. rubrispinosa* S. Carter, *E. rubromarginata* L.E. Newton, *E. rugosiflora* L.C. Leach, *E. samburuensis* P.R.O. Bally & S. Carter, *E. santapaui* A.N. Henry, ***E. sapinii*** De Wild., *E. saudiarabica* Fayed & D. Al-Zahrani, *E. saxorum* P.R.O. Bally & S. Carter, *E. scarlatina* S. Carter, *E. schinzii* Pax, *E. schizacantha* Pax, *E. schmitzii* L.C. Leach, *E. scitula* L.C. Leach, ***E. sebsebei*** M.G. Gilbert, ***E. seibanica*** Lavranos & Gifri, ***E. sekukuniensis*** R.A. Dyer, *E. semperflorens* L.C. Leach, *E. septentrionalis* P.R.O. Bally & S. Carter, *E. sepulta* P.R.O. Bally & S. Carter, *E. serendipita* L.E. Newton, *E. seretii* De Wild., *E. sessiliflora* Roxb., *E. setispina* S. Carter, *E. similiramea* S. Carter, *E. songweana* S. Carter, *E. speciosa* L.C. Leach, ***E. spiralis*** Balf.f., *E. stapfii* A. Berger, ***E. stellata*** Willd., *E. stenocaulis* Bruyns, *E. strangulata* N.E. Br., ***E. subsalsa*** Hiern, *E. subscandens* P.R.O. Bally & S. Carter, ***E. sudanica*** A. Chev., *E. sumati* S. Carter, *E. susan-holmesiae* Binojkumar & Gopalan, *E. taifensis* Fayed & D. Al-Zahrani, ***E. tanaensis*** P.R.O. Bally & S. Carter, *E. taruensis* S. Carter, *E. teixeirae* L.C. Leach, ***E. teke*** Schweinf. ex Pax, *E. tellieri* A. Chev., *E. tenuirama* Schweinf. ex A. Berger, *E. tenuispinosa* Gilli, *E. tescorum* S. Carter, *E. tetracantha* Rendle, *E. tetracanthoides* Pax, ***E. tetragona*** Haw., *E. tholicola* L.C. Leach, *E. tisserantii* A.Chev. & Sillans, *E. torta* Pax & K. Hoffm., ***E. tortilis*** Rottler ex Ainslie, ***E. tortirama*** R.A. Dyer, *E. tortistyla* N.E. Br., ***E. triaculeata*** Forssk., ***E. triangularis*** Desf. ex A. Berger, *E. trigona* Mill., ***E. turbiniformis*** Chiov., *E. turkanensis* S. Carter, *E. uhligiana* Pax, *E. umbonata* S. Carter, *E. umfoloziensis* Peckover, *E. undulatifolia* Janse, ***E. unicornis*** R.A. Dyer, ***E. unispina*** N.E. Br., ***E. vajravelui*** Binojk. & N.P. Balakr., ***E. vallaris*** L.C. Leach, *E. vandermerwei* R.A. Dyer, *E.*

venenata Marloth, ***E. venenifica*** Tremaux ex Kotschy, *E. venteri* L.C. Leach ex R.H. Archer & S. Carter, *E. viduiflora* L.C. Leach, ***E. virosa*** Willd., *E. vittata* S. Carter, *E. volkmanniae* Dinter, *E. vulcanorum* S. Carter, *E. wakefieldii* N.E. Br., ***E. waterbergensis*** R.A. Dyer, *E. whellanii* L.C. Leach, *E. williamsonii* L.C. Leach, *E. xylacantha* Pax, ***E. zoutpansbergensis*** R.A. Dyer.

Sect. *Euphorbia* is the largest and one of the most distinctive groups in subg. *Euphorbia*. The defining character for this clade is the spine-shield – a horny pad of tissue subtending or surrounding each leaf base that typically bears two or four spiny outgrowths. These outgrowths are usually interpreted as a pair of stipular spines and a pair of prickles (Carter, 1994). The vast majority of species in sect. *Euphorbia* have spine-shields, but in a few species they have been lost (e.g. *E. piscidermis* and geophytes like *E. meenae*).

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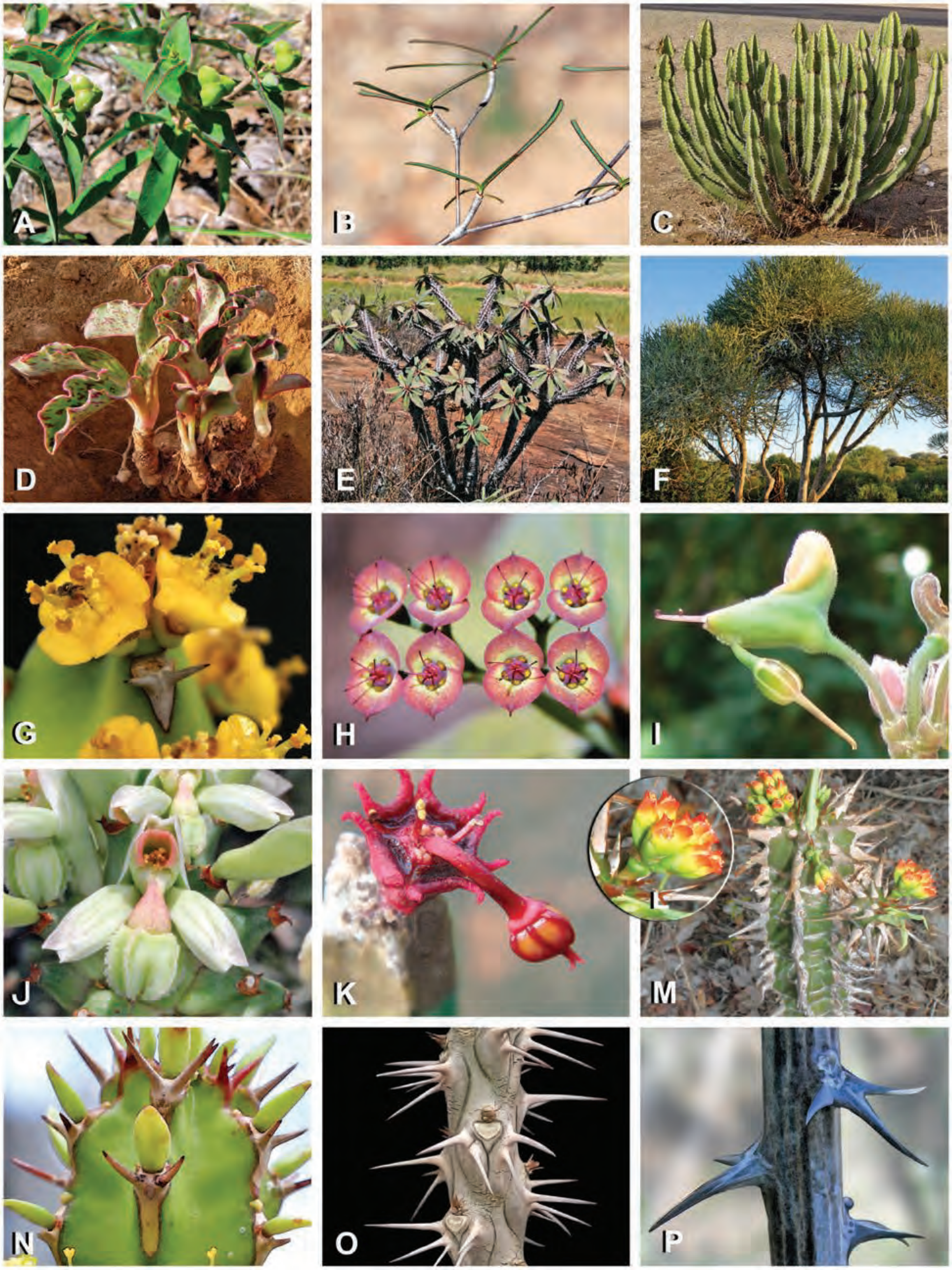
Table 2.1. Summary statistics of data sets used for phylogenetic inference. cpDNA data set consisted of concatenated *matK* and *ndhF* sequences and indel data. 3-gene data set consisted of concatenated cpDNA and ITS sequences.

	matK	*ndhF*	ITS	cpDNA	3-gene
Number of Accessions	177	162	281	232	314
Aligned Sequence Length	2115	1435	689	3550	4239
Variable DNA sites (%)	810 (39)	545 (38)	456 (66)	-	-
Indels Coded	99	38	-	137	137

Fig. 2.1. Examples of morphological diversity in subg. *Euphorbia*. (A-F) Growth forms: **A**, herb, *E. floridana*. **B**, Leafy shrub with long- and short-shoots, *E. hedyotoides*. **C**, cactiform succulent, *E. cooperi*. **D**, geophyte, *E. nana*. **E**, spiny, xerophytic shrub, *E. horombensis*. **F**, pencil-stem tree, *E. decorsei*. (G-L) Cyathial variations: **G**, simple dichasial form of sect. *Euphorbia*, *E. inarticulata*. **H**, spreading cyathophylls common in sect. *Goniostema, E. horombensis*. **I**, zygomorphic cyathium with nectar spur typical of sect. *Crepidaria, E. calcarata*. **J**, fused glands of sect. *Monadenium, E. heteropoda*. **K**, horned glands of sect. *Brasilienses, E. sipolisii*. **L**, cyathophylls forming a pseudofloral tube typical of several members of sect. *Goniostema, E. viguieri*. (M-P) Spine structures: **M**, comb-like stipular spines in sect. *Goniostema, E. viguieri*. **N**, spine-shields in sect. *Euphorbia*, *E. zoutpansbergensis*. **O**, stipular spines in section *Goniostema*, *E. milii*. **P**, spines of *E. neospinescens*, sect. *Monadenium*. (Photo credits - A: K. Gulledge, Atlas of Florida Vascular Plants; D: S. Dutta, I: P. Carillo-Reyes, L-M: B. van Ee, O: F. Vincentz, (www.euphorbia.de).


A
B
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D
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F
G
H
I
J
K
L
M
N
O
P

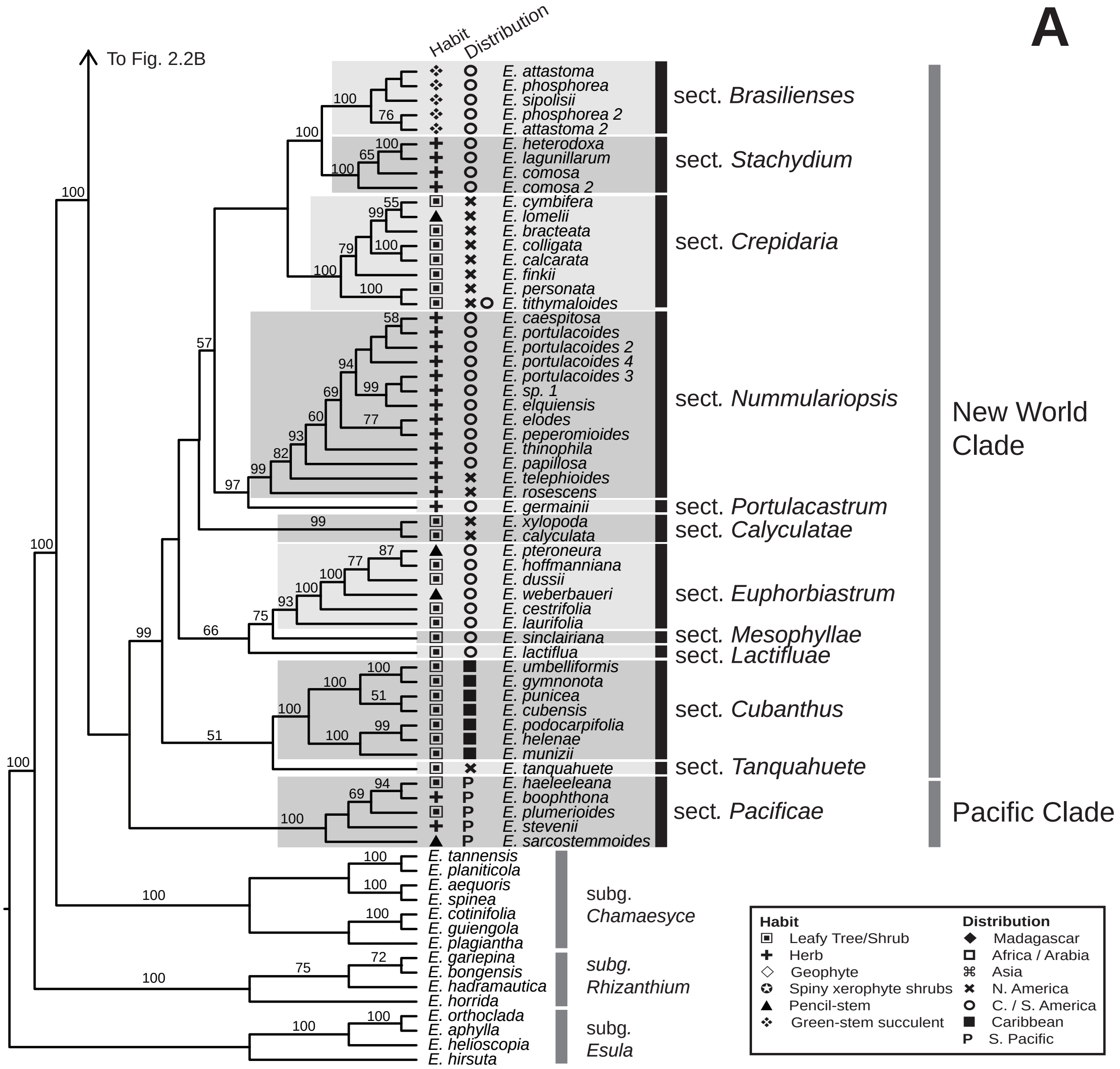
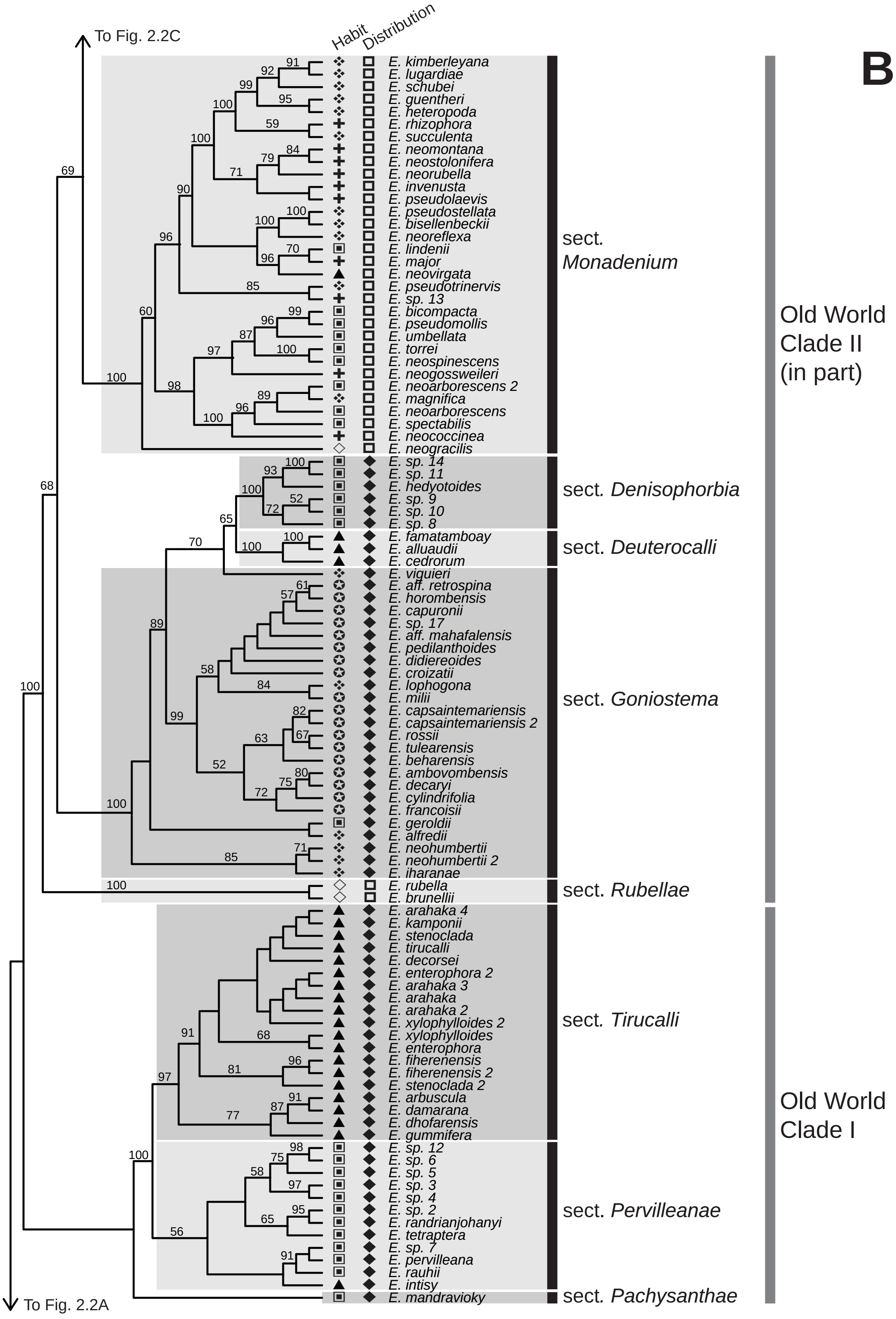


Fig. 2.2. Maximum likelihood estimate of the phylogenetic relationships of subg. *Euphorbia* based on the combined matrix of ITS, ndhF, and matK sequences. For clarity, multiple accessions from species shown to be exclusive lineages are excluded from the tree. Bootstrap support for clades > 50% is given above branches. Habit (left column) and distribution (right column) are indicated to the left of the taxa names – see inset for symbol definitions. Sections circumscribed in this study are indicated by shading and are named to the right of the taxa; the four major clades within subg. Euphorbia are indicated to the right of the sectional classification. A) Outgroups and New World Clade, B) Pacific Clade, Old World Clade I and Old World Clade II (in part), C) Old World Clade II (in part).


B
To Fig. 2.2C
Habit
Distribution
E. kimberleyana
E. lugardiae
E. schubei
E. guentheri
E. heteropoda
E. rhizophora
E. succulenta
E. neomontana
E. neostolonifera
E. neorubella
E. invenusta
E. pseudolaevis
E. pseudostellata
E. bisellenbeckii
E. neoreflexa
E. lindenii
E. major
E. neovirgata
E. pseudotrinervis
E. sp. 13
E. bicompacta
E. pseudomollis
E. umbellata
E. torrei
E. neospinescens
E. neogossweileri
E. neoarborescens 2
E. magnifica
E. neoarborescens
E. spectabilis
E. neococcinea
E. neogracilis
E. sp. 14
E. sp. 11
E. hedyotoides
E. sp. 9
E. sp. 10
E. sp. 8
E. famatamboay
E. alluaudii
E. cedrorum
E. viguieri
E. aff. retrospina
E. horombensis
E. capuronii
E. sp. 17
E. aff. mahafalensis
E. pedilanthoides
E. didiereoides
E. croizatii
E. lophogona
E. milii
E. capsaintemariensis
E. capsaintemariensis 2
E. rossii
E. tulearensis
E. beharensis
E. ambovombensis
E. decaryi
E. cylindrifolia
E. francoisii
E. geroldii
E. alfredii
E. neohumbertii
E. neohumbertii 2
E. iharanae
E. rubella
E. brunellii
E. arahaka 4
E. kamponii
E. stenoclada
E. tirucalli
E. decorsei
E. enterophora 2
E. arahaka 3
E. arahaka
E. arahaka 2
E. xylophylloides 2
E. xylophylloides
E. enterophora
E. fiherenensis
E. fiherenensis 2
E. stenoclada 2
E. arbuscula
E. damarana
E. dhofarensis
E. gummifera
E. sp. 12
E. sp. 6
E. sp. 5
E. sp. 3
E. sp. 4
E. sp. 2
E. randrianjohanyi
E. tetraptera
E. sp. 7
E. pervilleana
E. rauhii
E. intisy
E. mandravioky
sect. Monadenium
sect. Denisophorbia
sect. Deuterocalli
sect. Goniostema
sect. Rubellae
sect. Tirucalli
sect. Pervilleanae
sect. Pachysanthae
Old World Clade II (in part)
Old World Clade I
To Fig. 2.2A
91
92
99
95
100
59
100
84
79
71
69
90
100
100
96
70
96
85
60
99
96
87
97
100
100
98
89
96
100
100
93
68
100
52
72
65
70
100
100
61
57
89
58
84
100
82
99
67
63
52
80
75
72
100
71
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91
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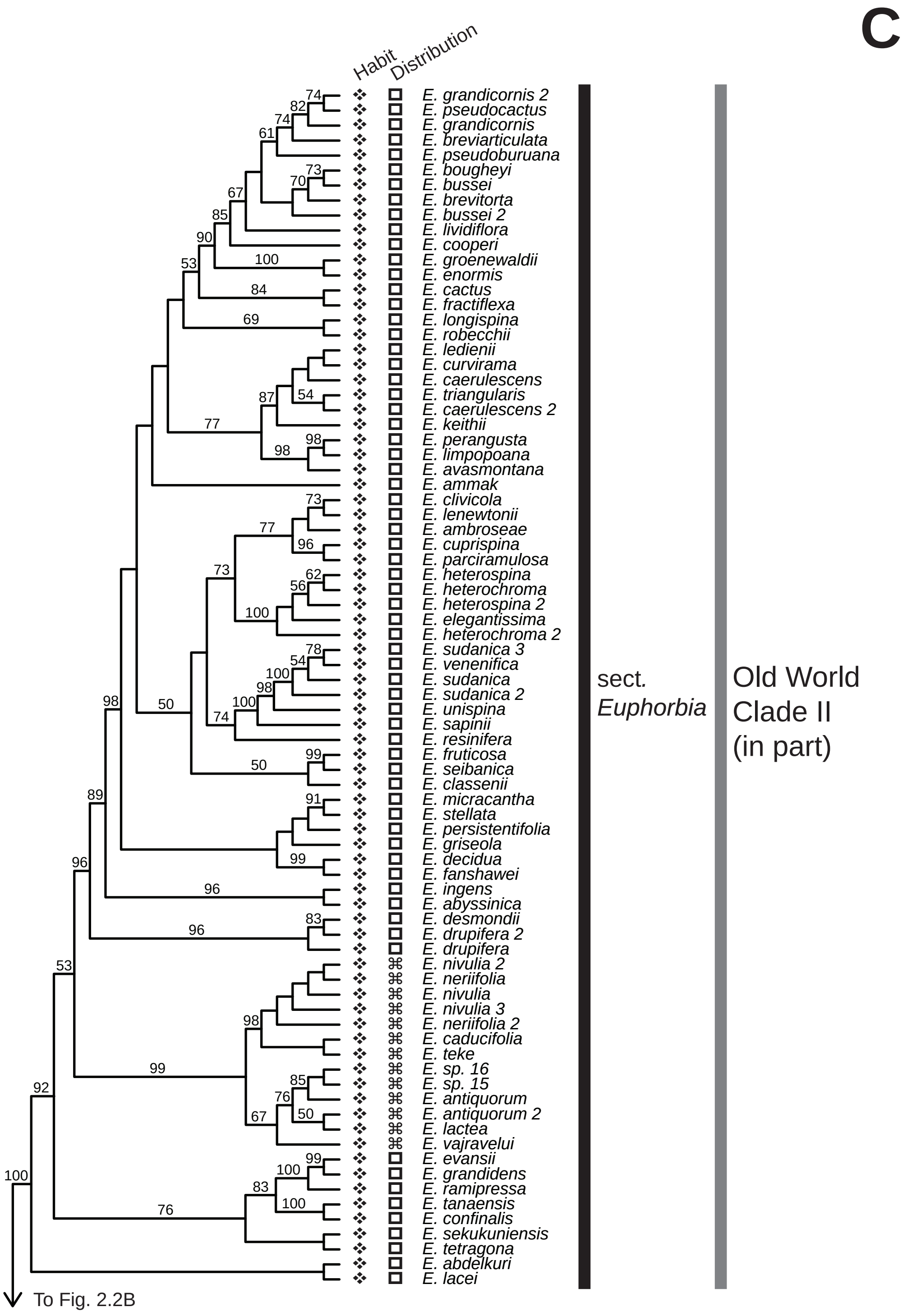
77
100
98
75
58
97
95
65
56
91


C
Habit
Distribution
E. grandicornis 2
E. pseudocactus
E. grandicornis
E. breviarticulata
E. pseudoburuana
E. bougheyi
E. bussei
E. brevitorta
E. bussei 2
E. lividiflora
E. cooperi
E. groenewaldii
E. enormis
E. cactus
E. fractiflexa
E. longispina
E. robecchii
E. ledienii
E. curvirama
E. caerulescens
E. triangularis
E. caerulescens 2
E. keithii
E. perangusta
E. limpopoana
E. avasmontana
E. ammak
E. clivicola
E. lenewtonii
E. ambroseae
E. cuprispina
E. parciramulosa
E. heterospina
E. heterochroma
E. heterospina 2
E. elegantissima
E. heterochroma 2
E. sudanica 3
E. venenifica
E. sudanica
E. sudanica 2
E. unispina
E. sapinii
E. resinifera
E. fruticosa
E. seibanica
E. classenii
E. micracantha
E. stellata
E. persistentifolia
E. griseola
E. decidua
E. fanshawei
E. ingens
E. abyssinica
E. desmondii
E. drupifera 2
E. drupifera
E. nivulia 2
E. neriifolia
E. nivulia
E. nivulia 3
E. neriifolia 2
E. caducifolia
E. teke
E. sp. 16
E. sp. 15
E. antiquorum
E. antiquorum 2
E. lactea
E. vajravelui
E. evansii
E. grandidens
E. ramipressa
E. tanaensis
E. confinalis
E. sekukuniensis
E. tetragona
E. abdelkuri
E. lacei
74
82
74
61
73
70
67
85
90
100
53
84
69
54
87
77
98
98
73
77
96
73
62
56
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78
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98
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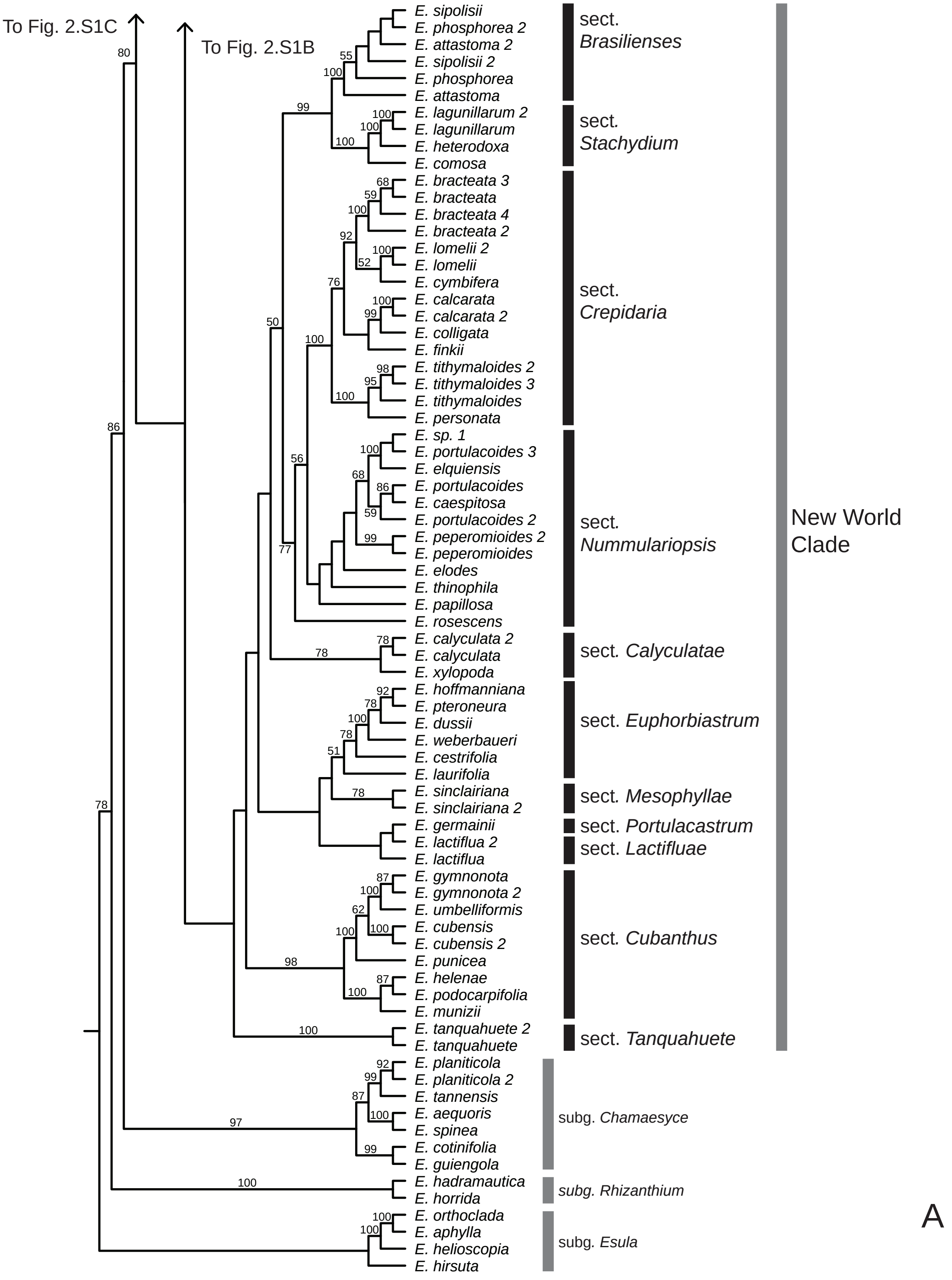
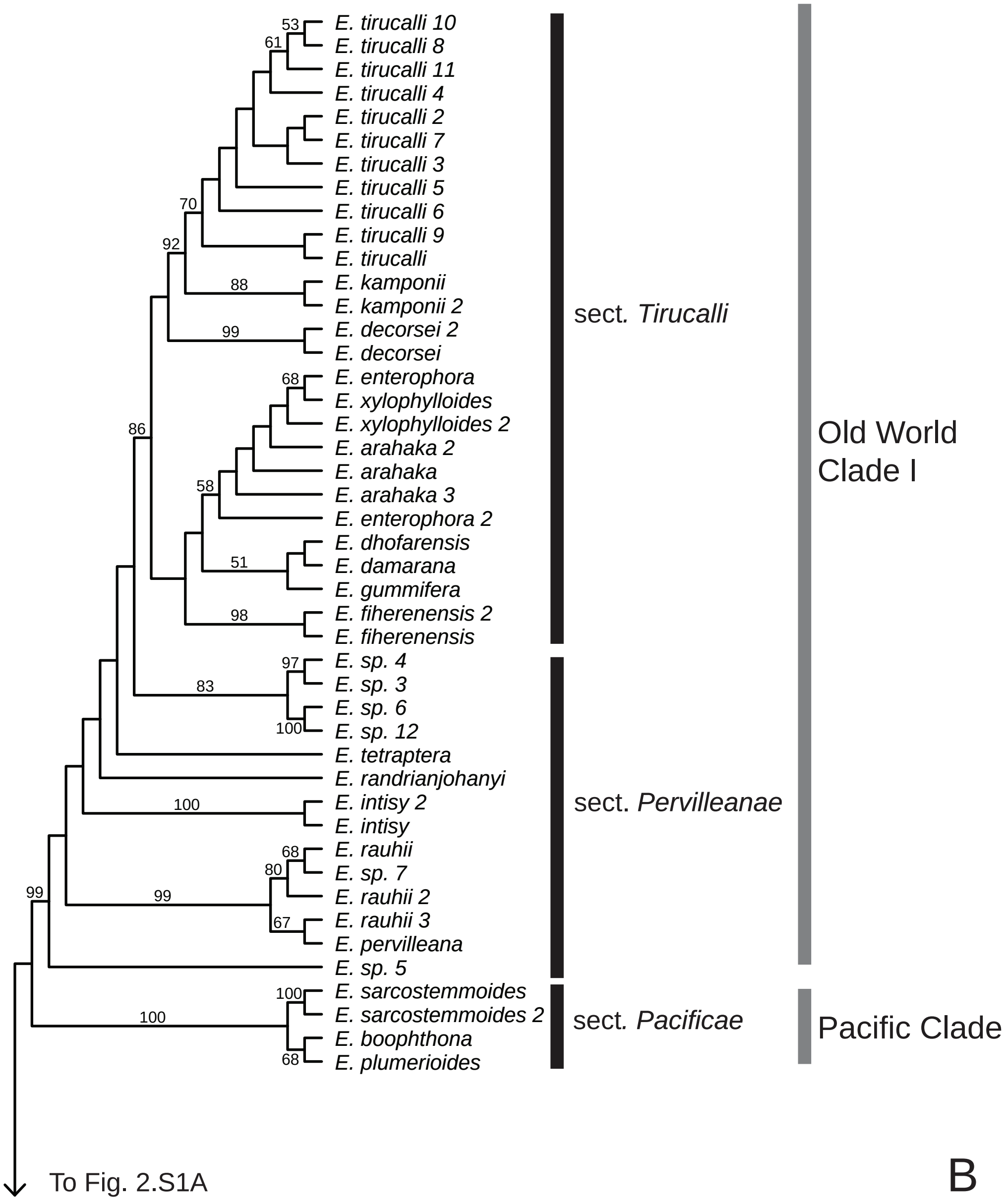
100
74
99
50
91
99
89
96
96
83
96
53
98
99
92
85
76
50
67
100
99
100
83
100
76
To Fig. 2.2B
sect. Euphorbia
Old World Clade II (in part)

Fig. 2.S1. Maximum likelihood estimation of the phylogenetic relationships of subg. *Euphorbia* based on ITS sequence data. Bootstrap support for clades > 50% is given above or below branches. Sections circumscribed in this study are indicated to the right of the taxa, and the four major lineages within subg. Euphorbia are indicated to the right of the sectional classification. Arrows indicate species or clades that are differently placed in the cpDNA tree.

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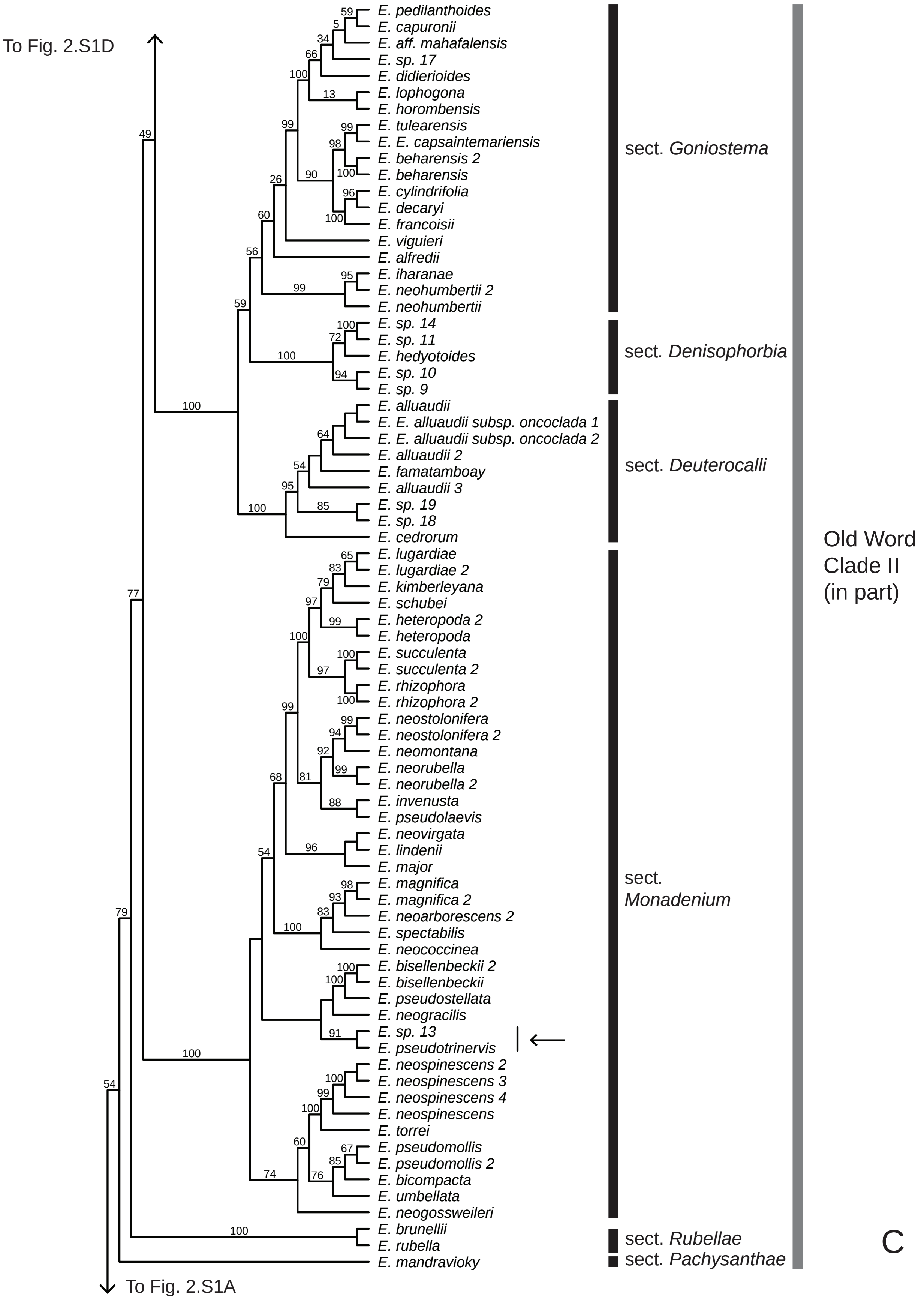
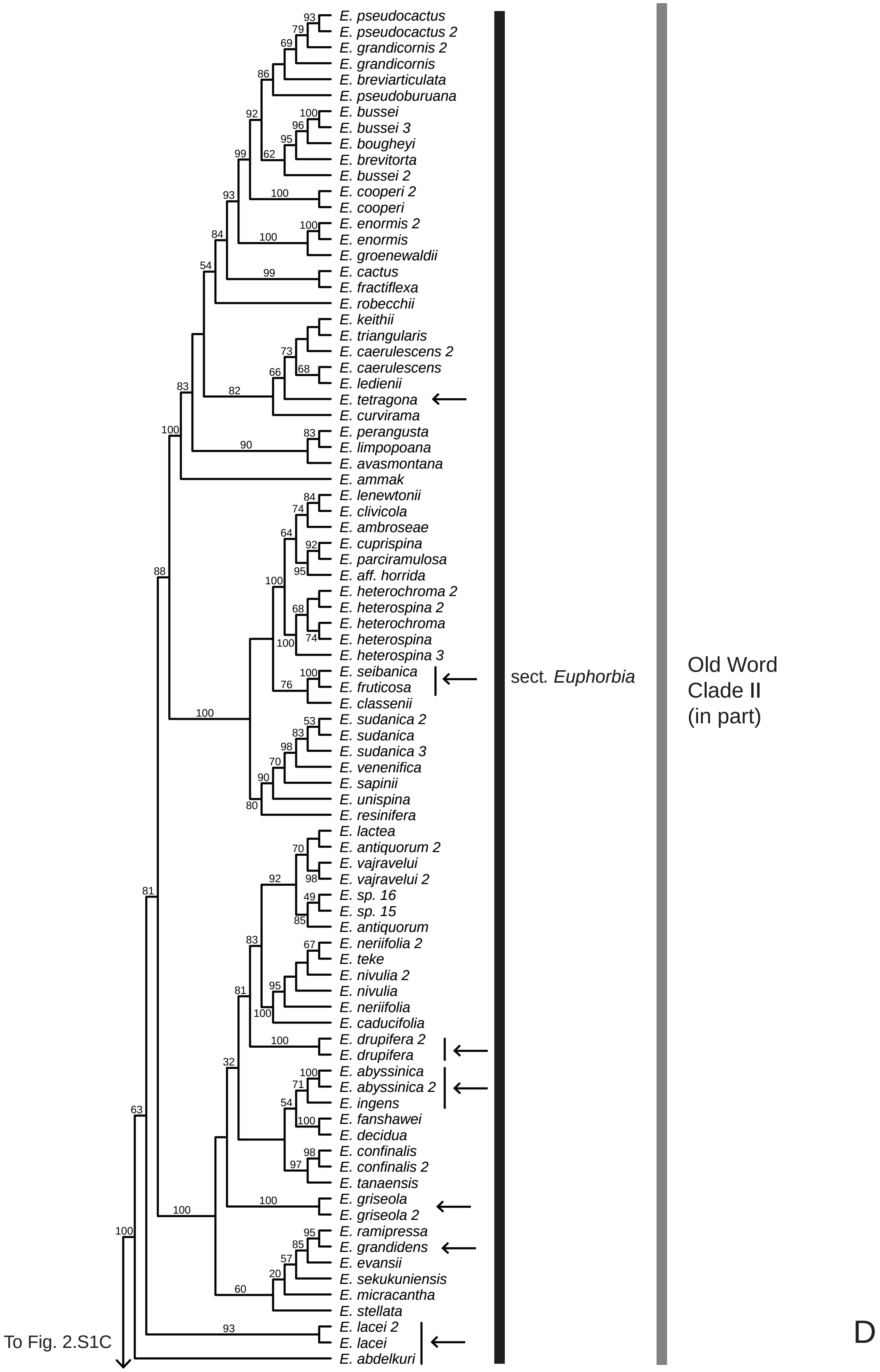
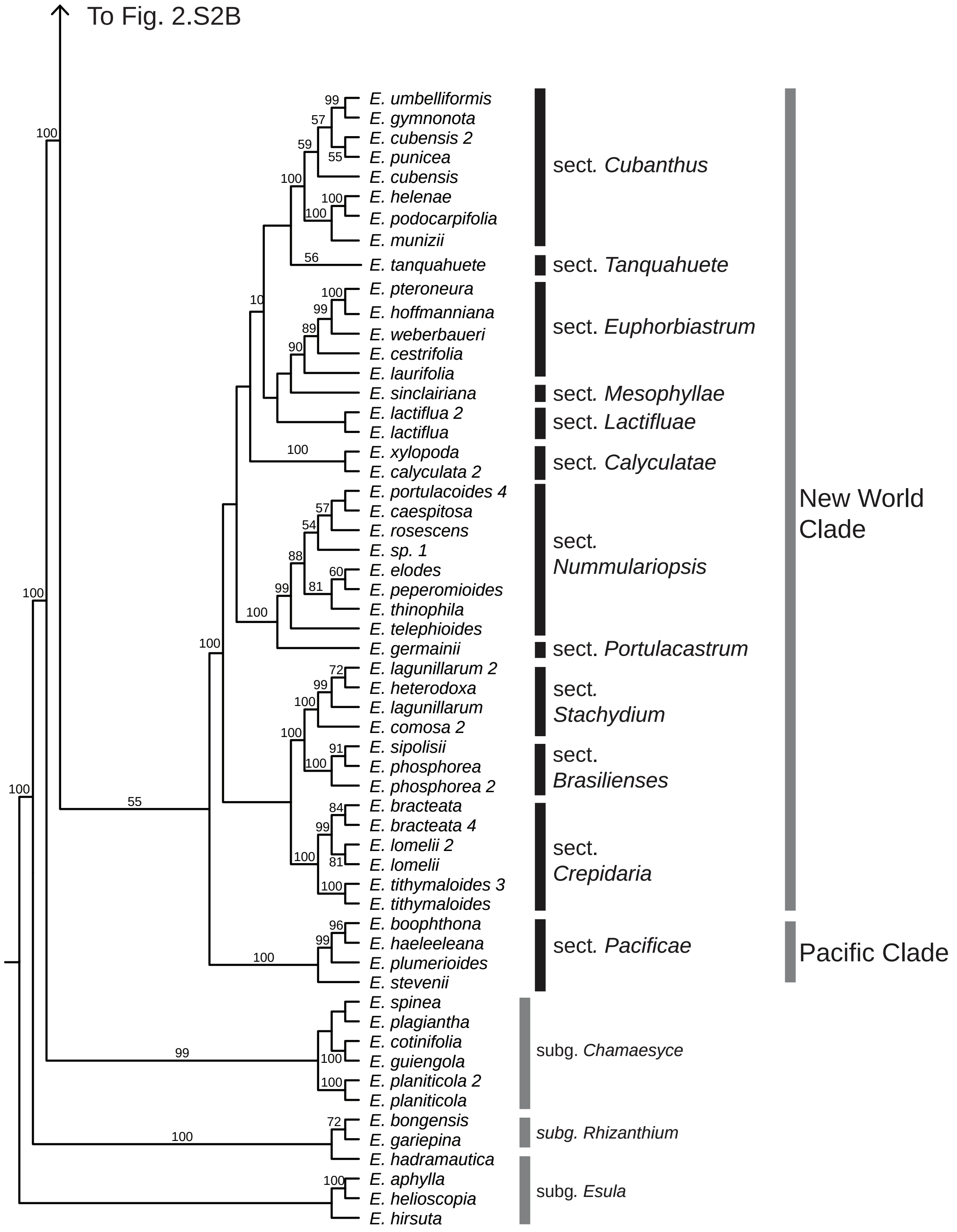
61
70
92
88
99
68
86
58
51
98
97
83
100
100
68
80
99
99
67
100
100
68
E. tirucalli 10
E. tirucalli 8
E. tirucalli 11
E. tirucalli 4
E. tirucalli 2
E. tirucalli 7
E. tirucalli 3
E. tirucalli 5
E. tirucalli 6
E. tirucalli 9
E. tirucalli
E. kamponii
E. kamponii 2
E. decorsei 2
E. decorsei
E. enterophora
E. xylophylloides
E. xylophylloides 2
E. arahaka 2
E. arahaka
E. arahaka 3
E. enterophora 2
E. dhofarensis
E. damarana
E. gummifera
E. fiherenensis 2
E. fiherenensis
E. sp. 4
E. sp. 3
E. sp. 6
E. sp. 12
E. tetraptera
E. randrianjohanyi
E. intisy 2
E. intisy
E. rauhii
E. sp. 7
E. rauhii 2
E. rauhii 3
E. pervilleana
E. sp. 5
E. sarcostemmoides
E. sarcostemmoides 2
E. boophthona
E. plumerioides
sect. Tirucalli
sect. Pervilleanae
sect. Pacificae
Old World Clade I
Pacific Clade
To Fig. 2.S1A
B

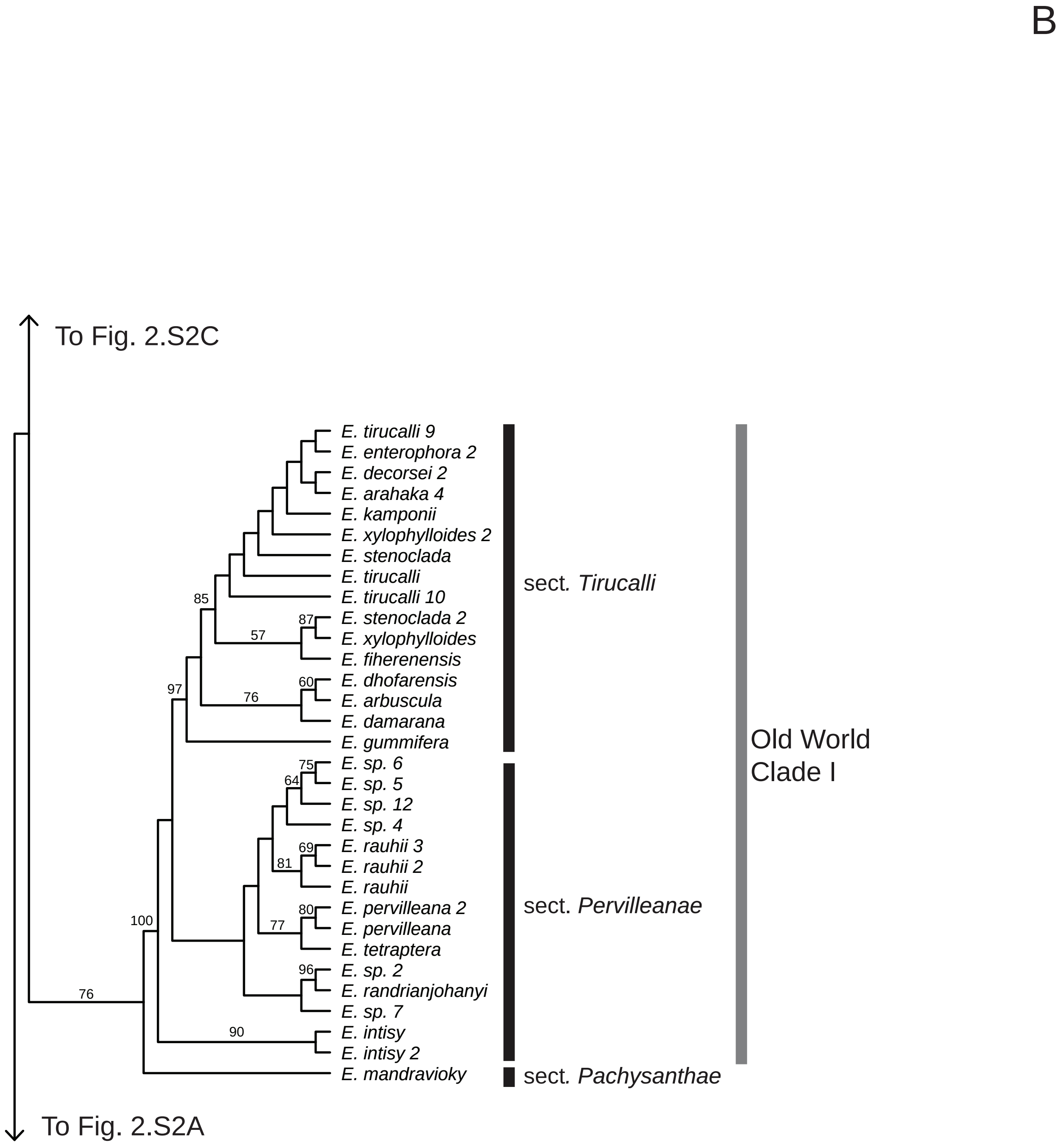

To Fig. 2.S1D
E. pedilanthoides
E. capuronii
E. aff. mahafalensis
E. sp. 17
E. didierioides
E. lophogona
E. horombensis
E. tulearensis
E. E. capsaintemariensis
E. beharensis 2
E. beharensis
E. cylindrifolia
E. decaryi
E. francoisii
E. viguieri
E. alfredii
E. iharanae
E. neohumbertii 2
E. neohumbertii
sect. Goniostema
E. sp. 14
E. sp. 11
E. hedyotoides
E. sp. 10
E. sp. 9
sect. Denisophorbia
E. alluaudii
E. E. alluaudii subsp. oncoclada 1
E. E. alluaudii subsp. oncoclada 2
E. alluaudii 2
E. famatamboay
E. alluaudii 3
E. sp. 19
E. sp. 18
E. cedrorum
sect. Deuterocalli
E. lugardiae
E. lugardiae 2
E. kimberleyana
E. schubei
E. heteropoda 2
E. heteropoda
E. succulenta
E. succulenta 2
E. rhizophora
E. rhizophora 2
E. neostolonifera
E. neostolonifera 2
E. neomontana
E. neorubella
E. neorubella 2
E. invenusta
E. pseudolaevis
E. neovirgata
E. lindenii
E. major
E. magnifica
E. magnifica 2
E. neoarborescens 2
E. spectabilis
E. neococcinea
E. bisellenbeckii 2
E. bisellenbeckii
E. pseudostellata
E. neogracilis
E. sp. 13
E. pseudotrinervis
E. neospinescens 2
E. neospinescens 3
E. neospinescens 4
E. neospinescens
E. torrei
E. pseudomollis
E. pseudomollis 2
E. bicompacta
E. umbellata
E. neogossweileri
sect. Monadenium
E. brunellii
E. rubella
sect. Rubellae
E. mandravioky
sect. Pachysanthae
Old Word Clade II (in part)
C
To Fig. 2.S1A

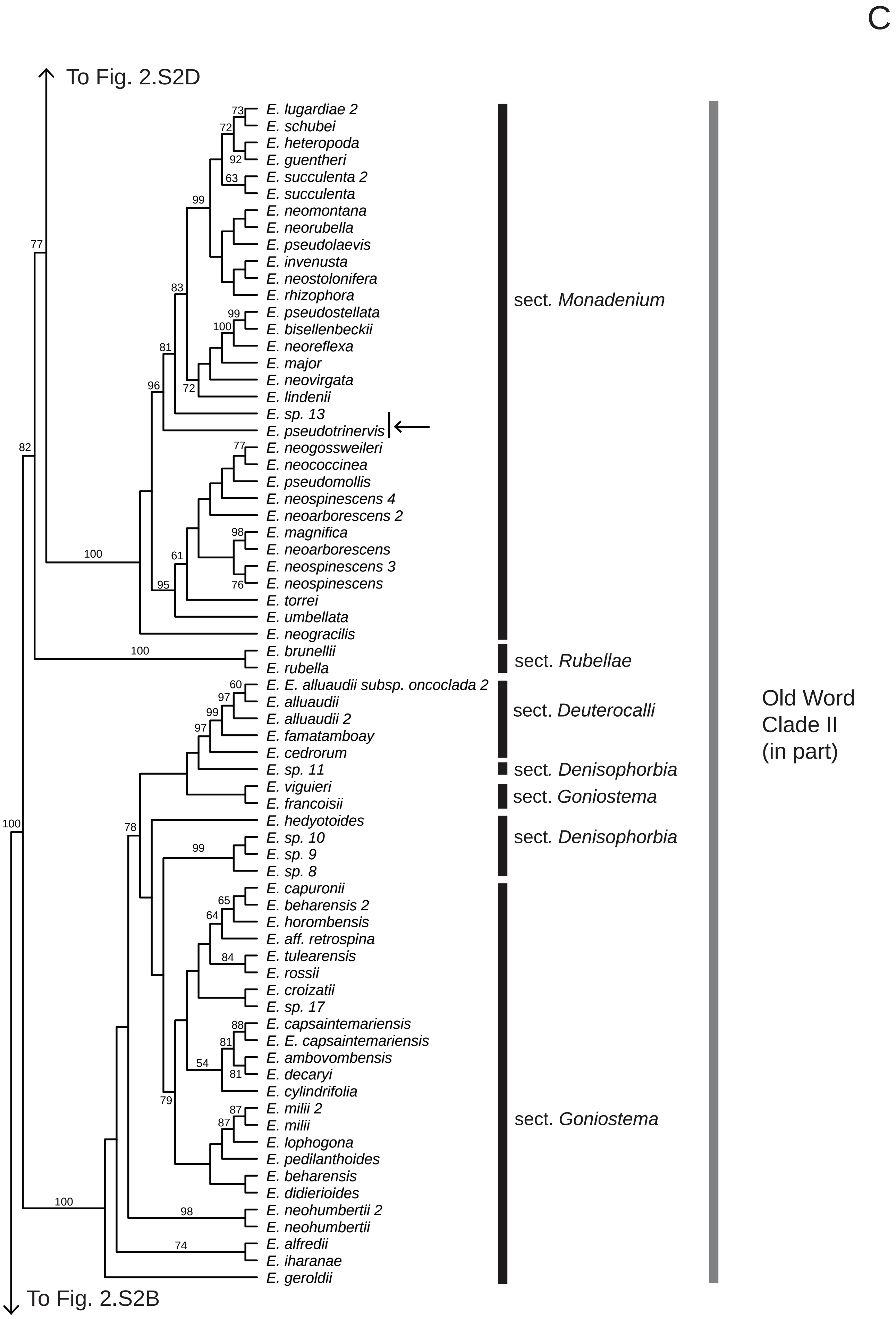

E. pseudocactus
E. pseudocactus 2
E. grandicornis 2
E. grandicornis
E. breviarticulata
E. pseudoburuana
E. bussei
E. bussei 3
E. bougheyi
E. brevitorta
E. bussei 2
E. cooperi 2
E. cooperi
E. enormis 2
E. enormis
E. groenewaldii
E. cactus
E. fractiflexa
E. robecchii
E. keithii
E. triangularis
E. caerulescens 2
E. caerulescens
E. ledienii
E. tetragona
E. curvirama
E. perangusta
E. limpopoana
E. avasmontana
E. ammak
E. lenewtonii
E. clivicola
E. ambroseae
E. cuprispina
E. parciramulosa
E. aff. horrida
E. heterochroma 2
E. heterospina 2
E. heterochroma
E. heterospina
E. heterospina 3
E. seibanica
E. fruticosa
E. classenii
E. sudanica 2
E. sudanica
E. sudanica 3
E. venenifica
E. sapinii
E. unispina
E. resinifera
E. lactea
E. antiquorum 2
E. vajravelui
E. vajravelui 2
E. sp. 16
E. sp. 15
E. antiquorum
E. neriifolia 2
E. teke
E. nivulia 2
E. nivulia
E. neriifolia
E. caducifolia
E. drupifera 2
E. drupifera
E. abyssinica
E. abyssinica 2
E. ingens
E. fanshawei
E. decidua
E. confinalis
E. confinalis 2
E. tanaensis
E. griseola
E. griseola 2
E. ramipressa
E. grandidens
E. evansii
E. sekukuniensis
E. micracantha
E. stellata
E. lacei 2
E. lacei
E. abdelkuri
sect. Euphorbia
Old Word
Clade II
(in part)
To Fig. 2.S1C
D

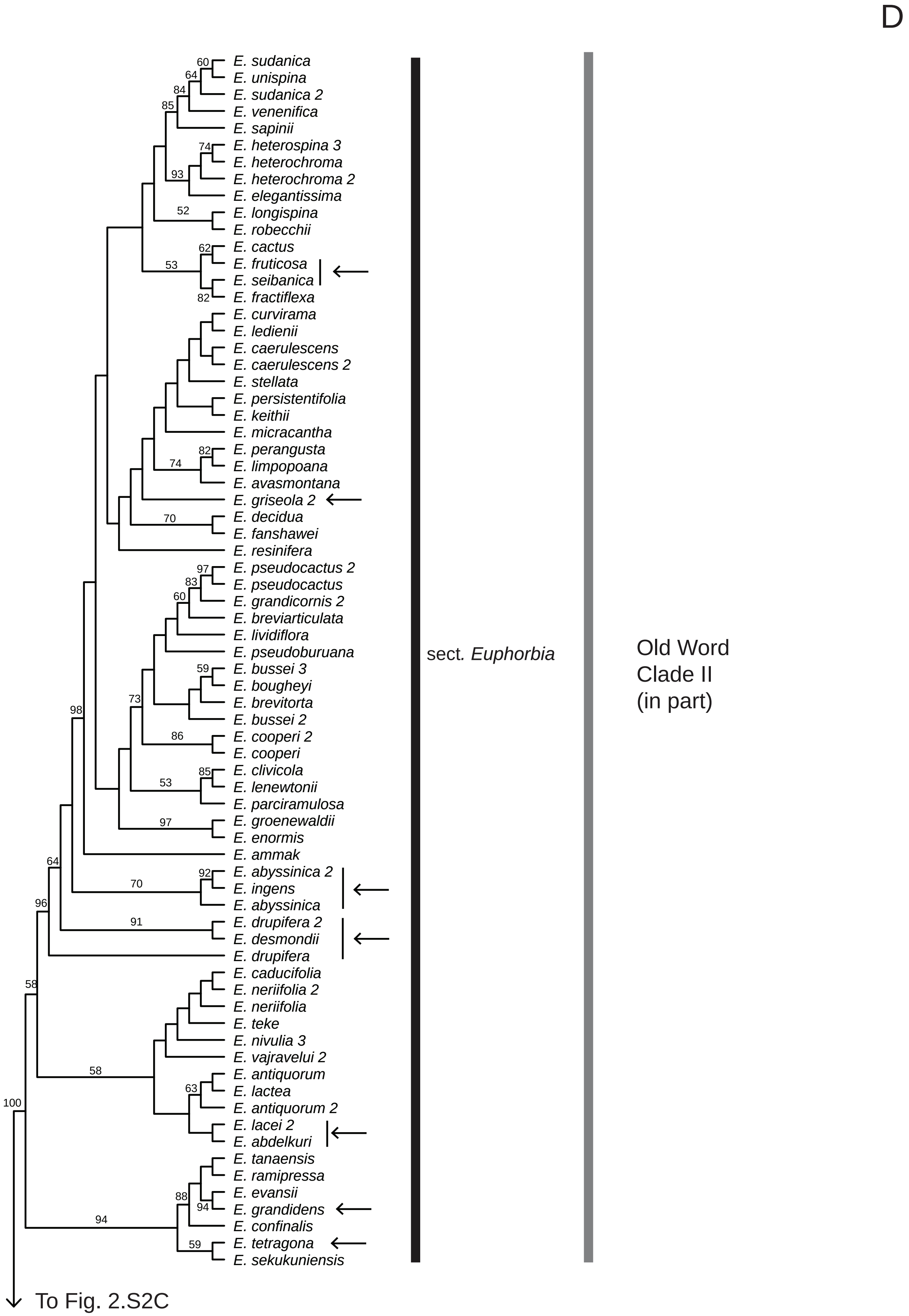
A

Fig. 2.S2. Maximum likelihood estimation of the phylogenetic relationships of subg. *Euphorbia* based on combined *ndh*F and *mat*K sequence and indel data (cpDNA data set). Bootstrap support for clades >50% is given above or below branches. Sections circumscribed in this study are indicated to the right of the taxa, and the four major lineages within subg. Euphorbia are indicated to the right of the sectional classification. Arrows indicate species or clades that are differently placed in the ITS tree.




B
To Fig. 2.S2C
E. tirucalli 9
E. enterophora 2
E. decorsei 2
E. arahaka 4
E. kamponii
E. xylophylloides 2
E. stenoclada
E. tirucalli
E. tirucalli 10
E. stenoclada 2
E. xylophylloides
E. fiherenensis
E. dhofarensis
E. arbuscula
E. damarana
E. gummifera
E. sp. 6
E. sp. 5
E. sp. 12
E. sp. 4
E. rauhii 3
E. rauhii 2
E. rauhii
E. pervilleana 2
E. pervilleana
E. tetraptera
E. sp. 2
E. randrianjohanyi
E. sp. 7
E. intisy
E. intisy 2
E. mandravioky
85
87
57
97
60
76
75
64
69
81
80
77
100
96
76
90
sect. Tirucalli
sect. Pervilleanae
sect. Pachysanthae
Old World Clade I
To Fig. 2.S2A

C
To Fig. 2.S2D
73
E. lugardiae 2
72
E. schubei
E. heteropoda
92
E. guentheri
63
E. succulenta 2
E. succulenta
99
E. neomontana
E. neorubella
E. pseudolaevis
77
E. invenusta
E. neostolonifera
83
E. rhizophora
99
E. pseudostellata
100
E. bisellenbeckii
81
E. neoreflexa
E. major
96
E. neovirgata
72
E. lindenii
E. sp. 13
E. pseudotrinervis
77
E. neogossweileri
82
E. neococcinea
E. pseudomollis
E. neospinescens 4
E. neoarborescens 2
98
E. magnifica
100
61
E. neoarborescens
E. neospinescens 3
95
76
E. neospinescens
E. torrei
E. umbellata
E. neogracilis
100
E. brunellii
E. rubella
60
E. E. alluaudii subsp. oncoclada 2
97
E. alluaudii
99
E. alluaudii 2
97
E. famatamboay
E. cedrorum
E. sp. 11
E. viguieri
E. francoisii
E. hedyotoides
100
78
99
E. sp. 10
E. sp. 9
E. sp. 8
E. capuronii
65
E. beharensis 2
64
E. horombensis
E. aff. retrospina
84
E. tulearensis
E. rossii
E. croizatii
E. sp. 17
88
E. capsaintemariensis
81
E. E. capsaintemariensis
54
E. ambovombensis
81
E. decaryi
E. cylindrifolia
79
87
E. milii 2
87
E. milii
E. lophogona
E. pedilanthoides
E. beharensis
E. didierioides
100
98
E. neohumbertii 2
E. neohumbertii
74
E. alfredii
E. iharanae
E. geroldii
To Fig. 2.S2B
sect. Monadenium
sect. Rubellae
sect. Deuterocalli
sect. Denisophorbia
sect. Goniostema
sect. Denisophorbia
sect. Goniostema
Old Word
Clade II
(in part)


D
E. sudanica
E. unispina
E. sudanica 2
E. venenifica
E. sapinii
E. heterospina 3
E. heterochroma
E. heterochroma 2
E. elegantissima
E. longispina
E. robecchii
E. cactus
E. fruticosa
E. seibanica
E. fractiflexa
E. curvirama
E. ledienii
E. caerulescens
E. caerulescens 2
E. stellata
E. persistentifolia
E. keithii
E. micracantha
E. perangusta
E. limpopoana
E. avasmontana
E. griseola 2
E. decidua
E. fanshawei
E. resinifera
E. pseudocactus 2
E. pseudocactus
E. grandicornis 2
E. breviarticulata
E. lividiflora
E. pseudoburuana
E. bussei 3
E. bougheyi
E. brevitorta
E. bussei 2
E. cooperi 2
E. cooperi
E. clivicola
E. lenewtonii
E. parciramulosa
E. groenewaldii
E. enormis
E. ammak
E. abyssinica 2
E. ingens
E. abyssinica
E. drupifera 2
E. desmondii
E. drupifera
E. caducifolia
E. neriifolia 2
E. neriifolia
E. teke
E. nivulia 3
E. vajravelui 2
E. antiquorum
E. lactea
E. antiquorum 2
E. lacei 2
E. abdelkuri
E. tanaensis
E. ramipressa
E. evansii
E. grandidens
E. confinalis
E. tetragona
E. sekukuniensis
60
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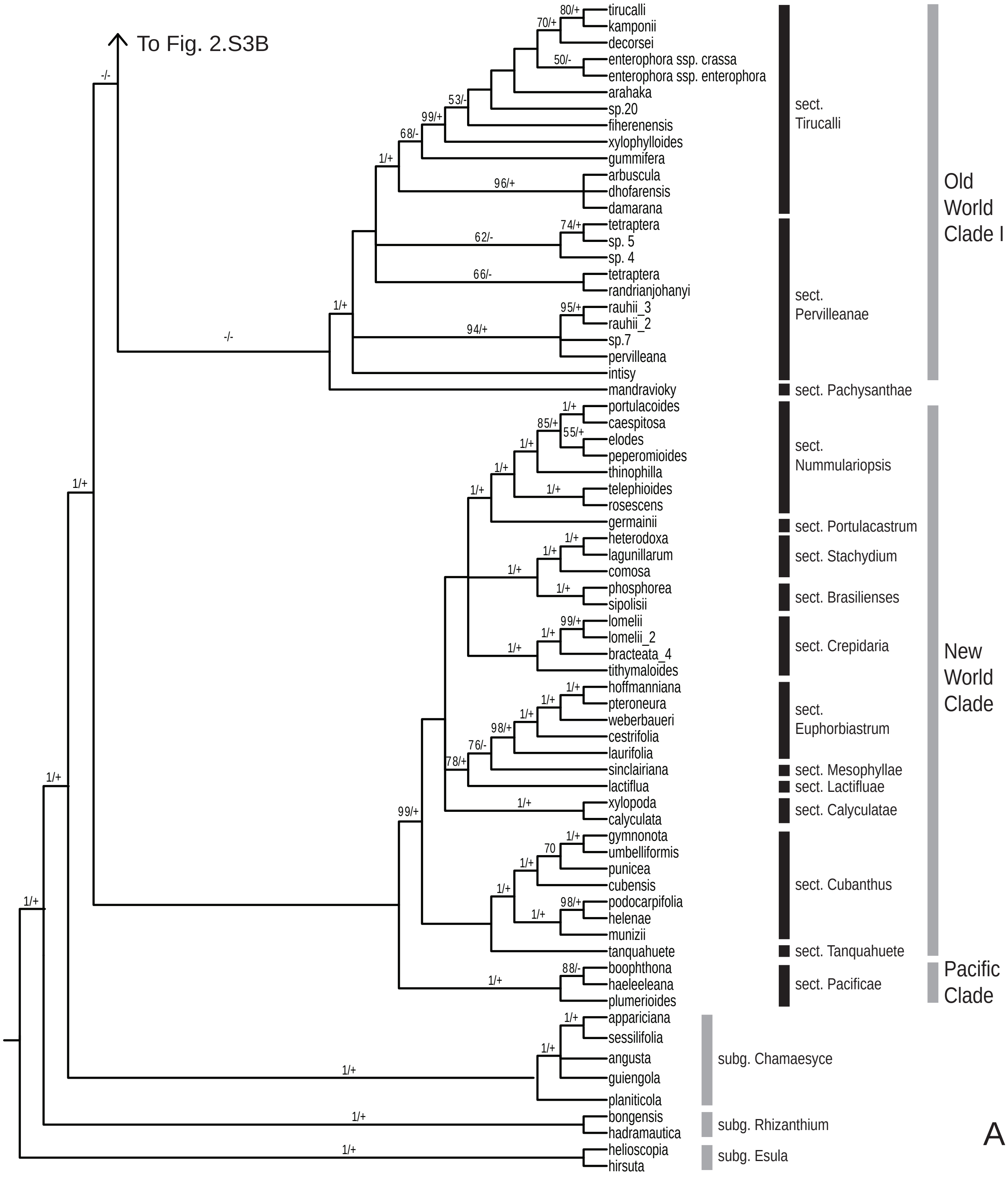
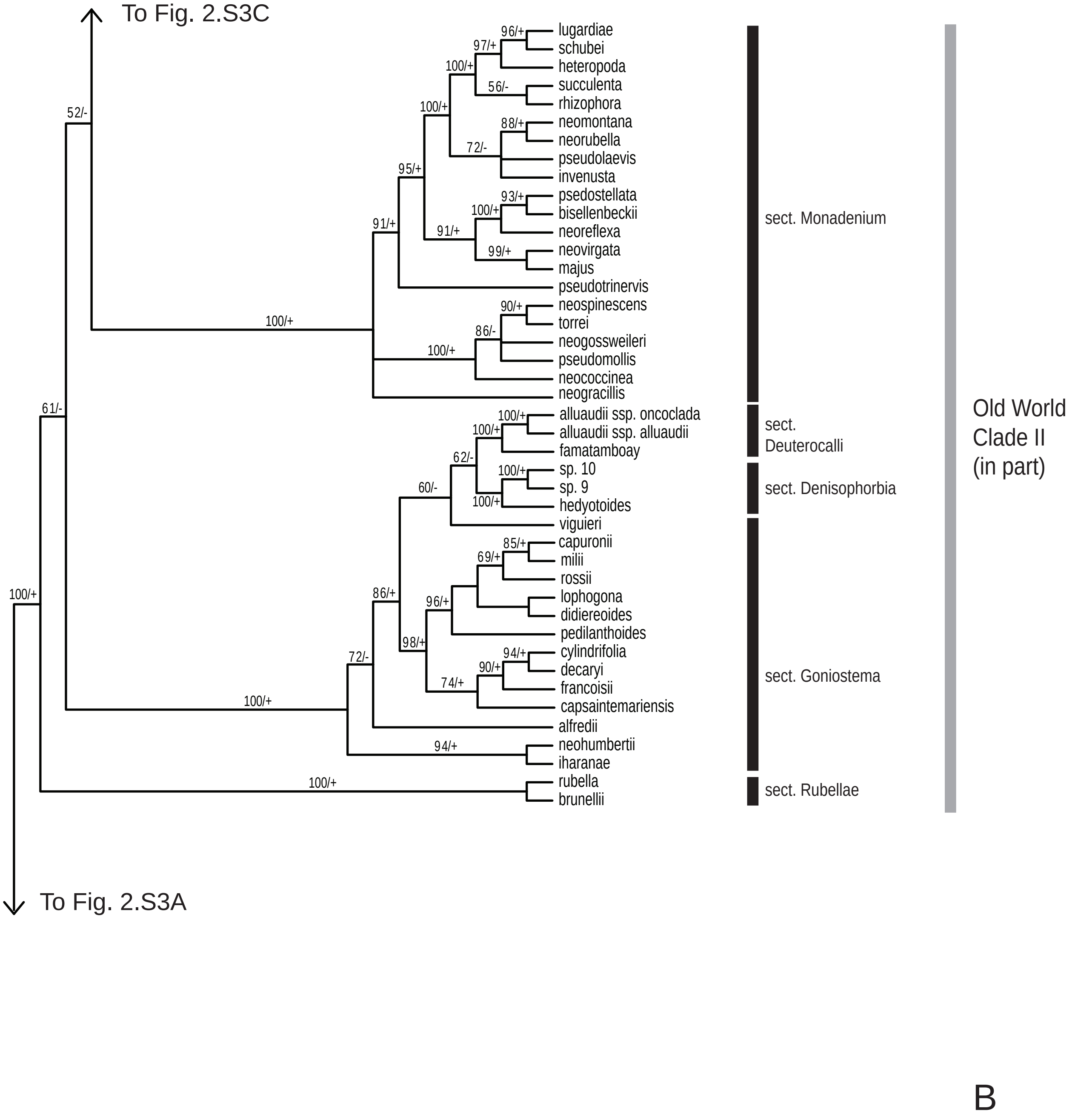
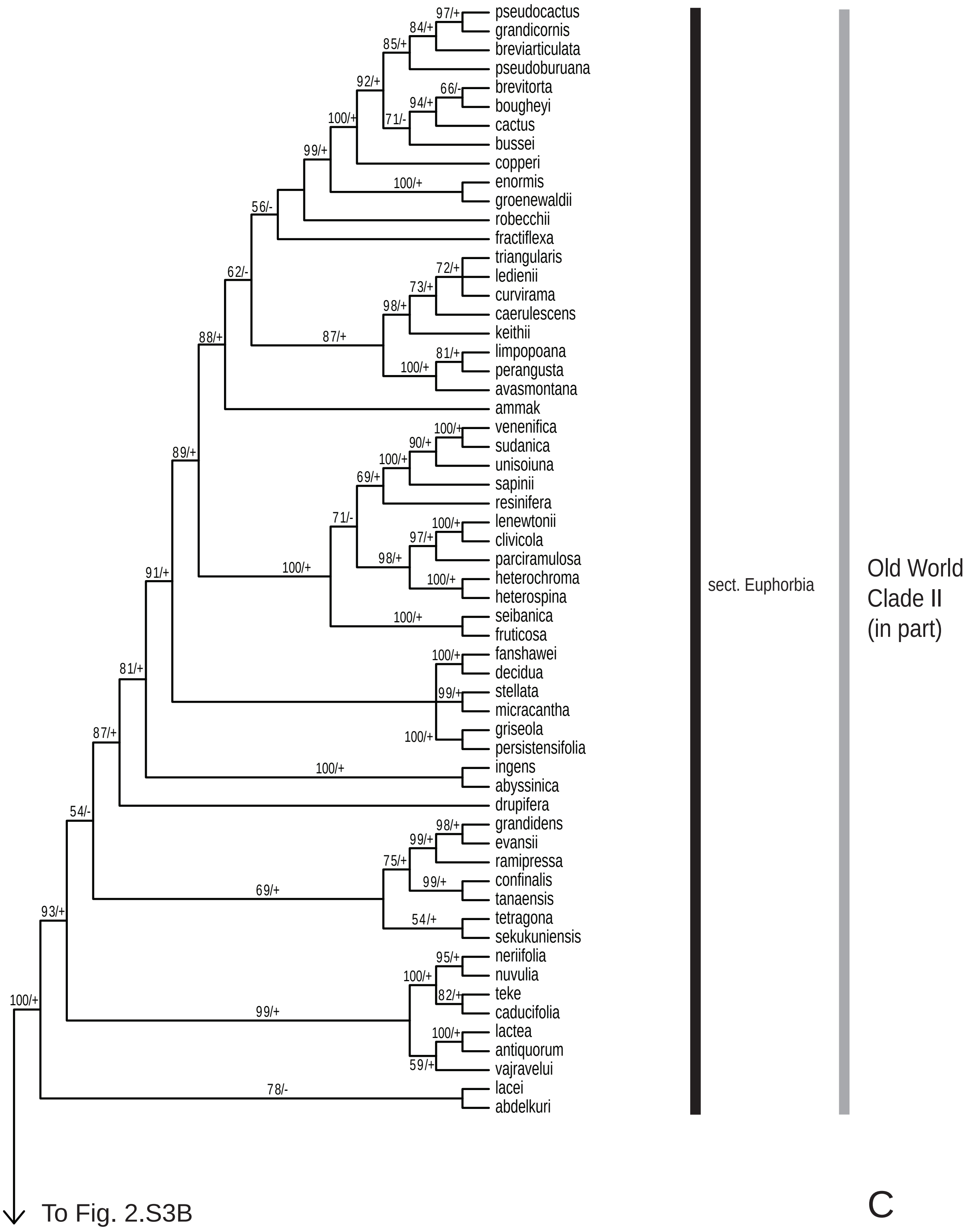
53
82
82
74
70
97
83
60
59
73
86
85
53
97
98
92
70
64
91
96
58
58
63
100
88
94
94
59
sect. *Euphorbia*
Old Word
Clade II
(in part)
To Fig. 2.S2C

Fig. 2.S3. Majority rule consensus tree of 50 maximum likelihood searches of the phylogenetic relationships of subg. *Euphorbia* based on a reduced taxa 3-gene (ITS, ndhF, matK) data set. Support for clades appear above branches. Numbers before the dash are bootstrap support > 50% based on 1000 pseudoreplicates and symbols after the dash indicate posterior probabilities (PP) for clades based on the posterior distribution of trees from the MrBayes analysis (plus sign indicates PP >95%, dash indicates PP < 95%). Sections circumscribed in this study are indicated to the right of the taxa, and the four major lineages within subg. *Euphorbia* are indicated to the right of the sectional classification.




To Fig. 2.S3C
96/+
lugardiae
97/+
schubei
100/+
heteropoda
56/-
succulenta
100/+
rhizophora
52/-
88/+
neomontana
neorubella
72/-
pseudolaevis
95/+
invenusta
93/+
psedostellata
100/+
bisellenbeckii
91/+
91/+
neoreflexa
99/+
neovirgata
majus
pseudotrinervis
90/+
neospinescens
100/+
86/-
torrei
neogossweileri
100/+
pseudomollis
neococcinea
neogracillis
61/-
100/+
alluaudii ssp. oncoclada
100/+
alluaudii ssp. alluaudii
famatamboay
62/-
100/+
sp. 10
60/-
sp. 9
100/+
hedyotoides
viguieri
85/+
capuronii
69/+
milii
rossii
86/+
96/+
lophogona
100/+
didiereoides
pedilanthoides
98/+
94/+
cylindrifolia
72/-
90/+
decaryi
74/+
francoisii
100/+
capsaintemariensis
alfredii
94/+
neohumbertii
iharanae
100/+
rubella
brunellii
sect. Monadenium
sect. Deuterocalli
sect. Denisophorbia
sect. Goniostema
sect. Rubellae
Old World Clade II (in part)
To Fig. 2.S3A
B


pseudocactus
grandicornis
breviarticulata
pseudoburuana
brevitorta
bougheyi
cactus
bussei
copperi
enormis
groenewaldii
robecchii
fractiflexa
triangularis
ledienii
curvirama
caerulescens
keithii
limpopoana
perangusta
avasmontana
ammak
venenifica
sudanica
unisoiuna
sapinii
resinifera
lenewtonii
clivicola
parciramulosa
heterochroma
heterospina
seibanica
fruticosa
fanshawei
decidua
stellata
micracantha
griseola
persistensifolia
ingens
abyssinica
drupifera
grandidens
evansii
ramipressa
confinalis
tanaensis
tetragona
sekukuniensis
neriifolia
nuvulia
teke
caducifolia
lactea
antiquorum
vajravelui
lacei
abdelkuri
97/+
84/+
85/+
92/+
66/-
94/+
100/+
71/-
99/+
100/+
56/-
62/-
72/+
73/+
98/+
88/+
87/+
81/+
100/+
100/+
90/+
89/+
100/+
69/+
71/-
100/+
97/+
98/+
100/+
91/+
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100/+
81/+
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87/+
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54/-
98/+
99/+
75/+
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69/+
54/+
93/+
95/+
100/+
82/+
100/+
99/+
100/+
59/+
78/-
sect. Euphorbia
Old World
Clade II
(in part)
To Fig. 2.S3B
C

Appendix. Taxon, collector and collection number (herbarium acronym), collection locality, and GenBank accession numbers for ITS, *mat*K, and *ndh*F sequences, respectively. Sequences without collection information were downloaded from GenBank.

Euphorbia abdelkuri Balf.f., *Berry, P.E. 7835* (MICH), USA, Living collection at UC-Davis, KC019681, KC019443, KC019836; ***Euphorbia abyssinica*** J.F. Gmel., *Morawetz, J. J. 372* (EA, K, MICH, MO), Kenya, Rift Valley Province, KC019616, KC019387, KC019793; ***Euphorbia abyssinica*** J.F. Gmel. **2**, *Berry, P.E. 7821* (MICH), USA, Living collection at UC-Davis, KC019670, KC019431, -; ***Euphorbia aequoris*** N.E. Br., *Riina, R. 1694* (MA), The Netherlands, From private living collection (RVV). Originally from South Africa (J&R 263), KC019720, -, -; ***Euphorbia alfredii*** Rauh, *Berry, P.E. 7824* (MICH), USA, Living collection at UC-Davis, KC019673, KC019434, KC019827; ***Euphorbia alluaudii*** Drake, *Dorsey, B.L. 104* (MICH), Madagascar, Toliara, KC019502, KC019320, KC019746; ***Euphorbia alluaudii* 2** Drake, *Dorsey, B.L. 133* (MICH), Madagascar, Toliara, KC019517, KC019328, -; ***Euphorbia alluaudii* 3** Drake, *Dorsey, B.L. 157* (MICH), Madagascar, Toliara, KC019531, -, -; ***Euphorbia alluaudii subsp. oncoclada*** (Drake) F. Friedmann & Cremers, *Dorsey, B.L. 125* (MICH), Madagascar, Toliara, KC019512, -, -; ***Euphorbia alluaudii subsp. oncoclada*** (Drake) F **2**. Friedmann & Cremers, *Dorsey, B.L. 111* (MICH), Madagascar, Toliara, KC019503, KC019321, -; ***Euphorbia ambovombensis*** Rauh & Razaf., *Dorsey, B.L. 144* (MICH), Madagascar, Toliara, -, KC019335, -; ***Euphorbia ambroseae*** L.C. Leach, *Dorsey, B.L. 258* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019576, -, -; ***Euphorbia ammak*** Schweinf., *Berry, P.E. 7813* (MICH), USA, Living collection at UC-Davis, KC019666, KC019427, KC019820; ***Euphorbia angusta*** Engelm., HQ645222, -, JQ750763; ***Euphorbia ankarensis*** Rauh, *Morawetz, J. J. 306* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (466/92), KC019611, KC019382, KC019878; ***Euphorbia antiquorum*** L., *Esser, H.-J. & M. van de Bult 08-15* (BKF), Thailand, Nokhon Sawon, KC019593, KC019369, KC019777; ***Euphorbia antiquorum*** L. **2**, *Yang, Y. 120* (MICH), U.S.A., Florida (Cultivated at Fairchild Bot. Garden), KC019741, KC019491, -; ***Euphorbia aphylla*** Brouss. ex Willd., *Dorsey, B.L. 4* (MICH), Spain, Las Palmas de Gran Canaria, KC019581, -, KC019773; ***Euphorbia appariciana*** Rizzini, JQ750877, -, JQ750765; ***Euphorbia arahaka*** Poiss., *Dorsey, B.L. 176* (MICH), Madagascar, Toliara, KC019541, -, -; ***Euphorbia arahaka*** Poiss. **2**, *Dorsey, B.L. 177* (MICH), Madagascar, Toliara, KC019542, -, -; ***Euphorbia arahaka*** Poiss. **3**, *Dorsey, B.L. 178* (MICH), Madagascar, Toliara, KC019543, -, -; ***Euphorbia arahaka***Poiss. **4**, *Dorsey, B.L. 135* (MICH), Madagascar, Toliara, -, KC019330, -; ***Euphorbia arbuscula*** Balf.f., *Berry, P.E. 7836* (MICH), USA, Living collection at UC-Davis, -, KC019444, KC019837; ***Euphorbia attastoma*** Rizzini, *Mello-Silva, R. 3195* (SPF), Brazil, Minas Gerais, KC019717, -, -; ***Euphorbia attastoma*** Rizzini **2**, AF537511, -, -; ***Euphorbia avasmontana*** Dinter, *Morawetz, J. J. 284* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (263/71), KC019602, KC019374, KC019783; ***Euphorbia beharensis*** Leandri, *Berry, P.E. 7829* (MICH), USA, Living collection at UC-Davis, KC019676, KC019439, KC019832; ***Euphorbia beharensis*** Leandri **2**, *Dorsey, B.L. 137* (MICH), Madagascar, Toliara, KC019520, KC019331, -; ***Euphorbia bicompacta*** Bruyns, *Berry, P.E. 7976* (MICH), U.S.A. (cultivated), Massachusetts, KC019696, -, -; ***Euphorbia bisellenbeckii*** Bruyns, *Berry, P.E. 7771* (MICH), USA, Living collection at UC-Davis, KC019649, KC019412, KC019810; ***Euphorbia bisellenbeckii*** Bruyns **2**, *Dorsey, B.L. 216* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019565, -, -;

Euphorbia bongensis Kotschy & Peyr. ex Boiss., *Riina, R. 1695* (MA), The Netherlands, From private living collection (RVV). Originally from South Africa (Vincent de Vries 288), -, KC019472, KC019860; ***Euphorbia boophthona*** C.A.Gardner, *Harris W.K. WKH2215* (BRI), Australia, Western Australia, KC019591, KC019368, KC019775; ***Euphorbia bougheyi*** L.C. Leach, *Berry, P.E. 7788* (MICH), USA, Living collection at UC-Davis, KC019658, KC019418, KC019814; ***Euphorbia bracteata*** Jacq., *Berry, P.E. 7870* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019691, KC019453, KC019845; ***Euphorbia bracteata*** Jacq. **2**, GU214909, -, -; ***Euphorbia bracteata*** Jacq **3**., *Dorsey, B.L. 292* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019578, -, -; ***Euphorbia bracteata*** Jacq **4**., *Berry, P.E. 7839* (MICH), USA, Living collection at UC-Davis, KC019683, KC019446, -; ***Euphorbia breviarticulata*** Pax, *Berry, P.E. 7819* (MICH), USA, Living collection at UC-Davis, KC019669, KC019429, KC019823; ***Euphorbia brevitorta*** P.R.O.Bally, *Riina, R. 1734* (MA), The Netherlands, From private living collection (JK). Originally from Kenya, KC019726, KC019478, -; ***Euphorbia brunellii*** Chiov., AF537486, -, AF538203; ***Euphorbia bussei*** Pax, *Dorsey, B.L. 256* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019575, -, -; ***Euphorbia bussei*** Pax **2**, *Morawetz, J. J. 393* (EA, MICH), Kenya, Rift Valley Province, KC019620, KC019388, KC019795; ***Euphorbia bussei*** Pax **3**, *Morawetz, J. J. 400* (EA, MICH), Kenya, K7. Coast Province, KC019621, -, KC019796; ***Euphorbia cactus*** Ehrenb. ex Boiss., *Morawetz, J. J. 327* (MICH, ON), Oman, Dhofar, KC019615, KC019386, -; ***Euphorbia caducifolia*** Haines, *Berry, P.E. 7759* (MICH), USA, Cultivated in the green house of UC-Davis. #B94.166, KC019648, KC019411, KC019809; ***Euphorbia caerulescens*** Haw., *Morawetz, J. J. 273* (MICH, NBG), South Africa, Eastern Cape, KC019601, -, KC019782; ***Euphorbia caerulescens*** Haw. **2**, *Berry, P.E. 7736* (MICH), USA, Cultivated in the green house of UC-Davis. #B81.233, KC019635, KC019397, -; ***Euphorbia caespitosa*** Lam., *Carrillo-Reyes, P. 5975* (SI), Argentina, Buenos Aires, KC019701, KC019459, -; ***Euphorbia calcarata*** (Schltdl.) V.W.Steinm., GU214912, GU214835, -; ***Euphorbia calcarata*** (Schltdl.) V.W.Steinm. **2**, GU214917, GU214857, -; ***Euphorbia calyculata*** Kunth, AF537524, -, AF538221; ***Euphorbia calyculata*** Kunth **2**, *Steinmann, V.W. 3472* (IEB), Mexico, Michoacan, KC019738, KC019486, -; ***Euphorbia capsaintemariensis*** (Rauh) Cremers, *Dorsey, B.L. 163* (MICH), Madagascar, Toliara, -, -, KC019760; ***Euphorbia capuronii*** Ursch & Leandri, *Berry, P.E. 7756* (MICH), USA, Cultivated in the green house of UC-Davis. #B68.269, KC019646, KC019409, -; ***Euphorbia cedrorum*** Rauh & Hebding, *Dorsey, B.L. 140* (MICH), Madagascar, Toliara, KC019522, KC019333, -; ***Euphorbia cestrifolia*** Kunth, AF537521, -, AF538213; ***Euphorbia classenii*** P.R.O.Bally & S.Carter, *Berry, P.E. 7811* (MICH), USA, Living collection at UC-Davis, KC019664, -, -; ***Euphorbia clivicola*** R.A.Dyer, *Becker, R. 976* (MICH, PRE, UNIN), South Africa, Limpopo Prov., KC019710, KC019465, KC019855; ***Euphorbia colligata*** V.W.Steinm., GU214920, GU214959, -; ***Euphorbia comosa*** Vell., AF537503, -, -; ***Euphorbia comosa*** Vell. **2**, -, -, AF538222; ***Euphorbia confinalis*** R.A.Dyer, *Becker, R. 1002* (MICH, PRE, UNIN), South Africa, Mpumalanga Prov., KC019703, KC019460, KC019849; ***Euphorbia confinalis*** R.A.Dyer **2**, *Dorsey, B.L. 225* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019570, -, -; ***Euphorbia cooperi*** N.E. Br. ex A. Berger, *Becker, R. 987* (MICH, PRE, UNIN), South Africa, Limpopo Prov., KC019714, KC019468, KC019858; ***Euphorbia cooperi*** N.E. Br. ex A. Berger **2**, *Berry, P.E. 7786* (MICH), USA, Living collection at UC-Davis, KC019656, KC019416, -; ***Euphorbia cotinifolia*** (L.) Millsp., *Dorsey, B.L. 174* (MICH), Madagascar, Toliara, KC019540, -, KC019764; ***Euphorbia croizatii*** Leandri, *Dorsey, B.L. 165* (MICH), Madagascar, Toliara, -, -, KC019761; ***Euphorbia cubensis*** Boiss., EF653254,

-, EF653259; ***Euphorbia cubensis*** Boiss. **2**, *W.O. 71* (P), Cultivated, KC019740, KC019489, -; ***Euphorbia cuprispina*** S.Carter, *Dorsey, B.L. 232* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019571, -, -; ***Euphorbia curvirama*** R.A. Dyer, *Morawetz, J. J. 309* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory, KC019612, KC019383, KC019791; ***Euphorbia cylindrifolia*** Marn.-Lap. & Rauh, *Berry, P.E. 7832* (MICH), USA, Living collection at UC-Davis, KC019678, KC019441, KC019834; ***Euphorbia cymbifera*** (Schltdl.) V.W.Steinm., GU214923, GU214834, -; ***Euphorbia damarana*** L.C. Leach, *Riina, R. 1729* (MA), The Netherlands, From private living collection (JK). Originally from Namibia, KC019725, KC019477, KC019866; ***Euphorbia decaryi*** Guillaumin, *Berry, P.E. 7828* (MICH), USA, Living collection at UC-Davis, KC019675, KC019438, KC019831; ***Euphorbia decidua*** P.R.O.Bally & L.C.Leach, *Riina, R. 1719* (MA), The Netherlands, From private living collection (JK) Origin Unkown, KC019723, KC019475, KC019864; ***Euphorbia decorsei*** Drake, *Dorsey, B.L. 161 (*MICH), Madagascar, Toliara, KC019534, -, -; ***Euphorbia decorsei*** Drake **2**, *Dorsey, B.L. 159* (MICH), Madagascar, Toliara, KC019533, KC019340, -; ***Euphorbia desmondii*** Keay & Milne-Redh., *Riina, R. 1755* (MA), The Netherlands, From private living collection (JK). Origin unknown, -, -, KC019871; ***Euphorbia dhofarensis*** S. Carter, *Morawetz, J. J. 324* (MICH, ON), Oman, Dhofar, KC019614, KC019385, -; ***Euphorbia didierioides*** Denis ex Leandri, *Yang, Y. 190* (MICH), U.S.A., Florida (Cultivated at Fairchild Bot. Garden), KC019742, KC019493, KC019877; ***Euphorbia drupifera*** Thonn., *Berry, P.E. 7774* (MICH), USA, Living collection at UC-Davis, KC019652, KC019413, -; ***Euphorbia drupifera*** Thonn. **2**, AF537480, -, AF538191; ***Euphorbia dussii*** Krug & Urb. ex Duss, *Graveson, R. 3064* (MICH), St. Lucia, Quarter of Castries, KC019715, -, -; ***Euphorbia elegantissima*** P.R.O.Bally & S.Carter, *Dorsey, B.L. 235* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, -, -, KC019771; ***Euphorbia elodes*** Boiss., *Cordeiro, I. 3053* (SP), Brazil, Sao Paulo, KC019596, KC019371, KC019779; ***Euphorbia elquiensis*** Phil., *Stoll, A. 90* (ULS), Chile, KC019497, -, -; ***Euphorbia enormis*** N.E. Br., *Berry, P.E. 7801* (MICH), USA, Living collection at UC-Davis, KC019661, KC019421, KC019816; ***Euphorbia enormis*** N.E. Br. **2**, *Becker, R. 979* (MICH, PRE, UNIN), South Africa, Limpopo Prov., KC019711, -, -; ***Euphorbia enterophora*** Drake, *Dorsey, B.L. 100* (MICH), Madagascar, Fianarantsoa, KC019500, -, -; ***Euphorbia enterophora*** Drake **2**, *Dorsey, B.L. 147* (MICH), Madagascar, Toliara, KC019525, KC019337, -; ***Euphorbia evansii*** Pax, *Morawetz, J. J. 293* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (206/97), KC019608, KC019379, KC019788; ***Euphorbia famatamboay*** F.Friedmann & Cremers, *Dorsey, B.L. 173* (MICH), Madagascar, Toliara, KC019539, KC019343, KC019763; ***Euphorbia fanshawei*** L.C. Leach, *Riina, R. 1723* (MA), The Netherlands, From private living collection (JK) Originally from Zambia, KC019724, KC019476, KC019865; ***Euphorbia fiherenensis*** Poiss., *Dorsey, B.L. 113* (MICH), Madagascar, Toliara, KC019505, KC019322, KC019749; ***Euphorbia fiherenensis*** Poiss. **2**, *Berry, P.E. 7833* (MICH), USA, Living collection at UC-Davis, KC019679, -, -; ***Euphorbia finkii*** (Boiss.) V.W.Steinm., GU214929, GU214852, -; ***Euphorbia fractiflexa*** S.Carter & J.R.I.Wood, *Riina, R. 1752* (MA), The Netherlands, From private living collection (JK). Origin unknown, KC019728, KC019481, KC019870; ***Euphorbia francoisii*** Leandri, *Berry, P.E. 7857* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019686, KC019447, KC019841; ***Euphorbia fruticosa*** Forssk., *Berry, P.E. 7780* (MICH), USA, Living collection at UC-Davis, KC019654, KC019415, KC019812; ***Euphorbia gariepina*** Boiss., *Becker, R. 918* (MICH, PRE, UNIN), South Africa, Northern Cape Prov., -, -, KC019853; ***Euphorbia germainii***

Phil., AF537499, -, AF538205; ***Euphorbia geroldii*** Rauh, *Dorsey, B.L. 145* (MICH), Madagascar, Toliara, -, KC019336, -; ***Euphorbia grandicornis*** Goebel ex N.E. Br., *Morawetz, J. J. 289* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (762/89), KC019605, -, -; ***Euphorbia grandicornis*** Goebel ex N.E **2**. Br., *Berry, P.E. 7787* (MICH), USA, Living collection at UC-Davis, KC019657, KC019417, KC019813; ***Euphorbia grandidens*** Haw., *Morawetz, J. J. 287* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (706/96), KC019604, KC019376, KC019785; ***Euphorbia griseola*** Pax, *Dorsey, B.L. 241* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019572, -, -; ***Euphorbia griseola*** Pax **2**, *Berry, P.E. 7812* (MICH), USA, Living collection at UC-Davis, KC019665, KC019426, KC019819; ***Euphorbia groenewaldii*** R.A. Dyer, *Becker, R. 972* (MICH, PRE, UNIN), South Africa, Limpopo Prov., KC019709, -, KC019854; ***Euphorbia guentheri*** (Pax) Bruyns, *Berry, P.E. 7743* (MICH), USA, Cultivated in the green house of UC-Davis. #B67.040, -, KC019399, KC019803; ***Euphorbia guiengola*** W.R. Buck & Huft, *Riina, R. 1699* (MA), The Netherlands, From private living collection (JK) Originally from Mexico, KC019721, KC019473, KC019861; ***Euphorbia gummifera*** Boiss., *Becker, R. 921* (MICH, PRE, UNIN), South Africa, Northern Cape Prov., KC019708, KC019464, -; ***Euphorbia gymnonota*** Urb., *Yang, Y. 185* (MICH), U.S.A., Florida (Cultivated at Fairchild Bot. Garden), JQ750886, KC019492, JQ750808; ***Euphorbia gymnonota*** Urb. **2**, AF537507, -, -; ***Euphorbia hadramautica*** Baker, *Morawetz, J. J. 320* (MICH, ON), Oman, Dhofar, KC019613, KC019384, KC019792; ***Euphorbia haeleeleana*** Herbst, *Wagner, W. L. 40455* (PTBG), U.S.A., Hawaii, -, KC019490, KC019876; ***Euphorbia hedyotoides*** N.E. Br., *Dorsey, B.L. 166* (MICH), Madagascar, Toliara, KC019535, KC019341, -; ***Euphorbia helenae*** Urb., EF653255, -, EF653261; ***Euphorbia helioscopia*** L., *Morawetz, J. J. 302* (MICH), South Africa, Western Cape, KC019610, KC019381, KC019790; ***Euphorbia heterochroma*** Pax, *Morawetz, J. J. 378* (EA, MICH, MO), Kenya, Rift Valley Province, KC019618, -, KC019794; ***Euphorbia heterochroma*** Pax **2**, *Morawetz, J. J. 402* (EA, MICH), Kenya, K7. Coast Province, KC019622, KC019389, KC019797; ***Euphorbia heterodoxa*** Müll.Arg., *Louzada, R. 128* (SP), Brazil, Bahia, KC019716, KC019469, KC019859; ***Euphorbia heteropoda*** Pax, *Berry, P.E. 7750* (MICH), USA, Cultivated in the green house of UC-Davis. #B67.031, KC019642, KC019405, KC019807; ***Euphorbia heteropoda*** Pax **2**, *Dorsey, B.L. 204* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019554, -, -; ***Euphorbia heterospina*** S.Carter, *Morawetz, J. J. 373* (EA, MICH), Kenya, Rift Valley Province, KC019617, -, -; ***Euphorbia heterospina*** S.Carter **2**, *Morawetz, J. J. 387* (EA, MICH), Kenya, Rift Valley Province, KC019619, -, -; ***Euphorbia heterospina*** S.Carter **3**, *Berry, P.E. 7875* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019693, KC019454, KC019847; ***Euphorbia hirsuta*** L., *Riina, R. 1769* (MICH), Spain, Madrid, KC019730, KC019483, KC019873; ***Euphorbia hoffmanniana*** (Klotzsch & Garcke) Boiss., AF537508, -, AF538211; ***Euphorbia horombensis*** Ursch & Leandri, *Dorsey, B.L. 191* (MICH), Madagascar, Toliara, KC019549, KC019348, KC019768; ***Euphorbia horrida*** Boiss., *Berry, P.E. 7783* (MICH), USA, Living collection at UC-Davis, KC019655, -, -; ***Euphorbia iharanae*** Rauh, *Berry, P.E. 7854* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019685, KC019479, KC019840; ***Euphorbia ingens*** E. Mey. ex Boiss., *Becker, R. 985* (MICH, PRE, UNIN), South Africa, Prov., KC019713, KC019467, KC019857; ***Euphorbia intisy*** Drake, *Dorsey, B.L. 112* (MICH), Madagascar, Toliara, KC019504, -, KC019748; ***Euphorbia intisy*** Drake **2**, *Dorsey, B.L. 127* (MICH), Madagascar, Toliara, KC019514, KC019325, KC019753; ***Euphorbia***

invenusta (N.E.Br.) Bruyns, *Berry, P.E. 7747* (MICH), USA, Cultivated in the green house of UC-Davis. #B67.042, KC019639, KC019402, KC019805; ***Euphorbia kamponii*** Rauh & Petignat, *Dorsey, B.L. 134* (MICH), Madagascar, Toliara, KC019518, KC019329, KC019755; ***Euphorbia kamponii*** Rauh & Petignat **2**, *Dorsey, B.L. 118* (MICH), Madagascar, Toliara, KC019507, -, -; ***Euphorbia keithii*** R.A. Dyer, *Morawetz, J. J. 290* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (80/97), KC019606, KC019377, KC019786; ***Euphorbia kimberleyana*** (G. Will.) Bruyns, *Downes, P.E. 9-85* (BRI), South Africa, Limpopo Prov., KC019698, -, -; ***Euphorbia lacei*** Craib, *Haevermans, T. 452* (P), Cultivated Heidelberg Botanic Gardens BGH142649 , KC019733, -, -; ***Euphorbia lacei*** Craib **2**, *Esser, H.-J. & M. van de Bult 08-10* (BKF), Thailand, Nokhon Sawon, KC019592, -, KC019776; ***Euphorbia lactea*** Haw., *Berry, P.E. 7816* (MICH), USA, Living collection at UC-Davis, KC019667, -, KC019821; ***Euphorbia lactiflua*** **2** Phil., AF537528, -, AF538219; ***Euphorbia lactiflua*** Phil., *Carrillo-Reyes, P. 5928* (IEB), Chile, Atacama, KC019700, KC019457, -; ***Euphorbia lagunillarum*** Croizat, *Riina, R. 1836* (VEN), Venezuela, Merida, KC019731, -, KC019874; ***Euphorbia lagunillarum*** Croizat **2**, *Riina, R. 1693* (VEN), The Netherlands, From private living collection (RVV) Originally from Venezuela, KC019719, KC019471, -; ***Euphorbia laurifolia*** Juss. ex Lam., *Riina, R. 1601* (VEN), Venezuela, Merida, KC019718, KC019470, -; ***Euphorbia ledienii*** A. Berger, *Becker, R. 684* (MICH, PRE, UNIN), South Africa, Eastern Cape Prov., KC019707, KC019463, KC019852; ***Euphorbia lenewtonii*** S.Carter, *Berry, P.E. 7861* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019687, KC019448, KC019842; ***Euphorbia limpopoana*** L.C. Leach ex S. Carter, *Becker, R. 633* (MICH, PRE, UNIN), South Africa, Limpopo Prov., KC019705, KC019461, KC019851; ***Euphorbia lindenii*** (S. Carter) Bruyns, *Dorsey, B.L. 217* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019566, KC019359, -; ***Euphorbia lividiflora*** L.C. Leach, *Riina, R. 1745* (MA), The Netherlands, From private living collection (JK). Origin unknown, -, -, KC019869; ***Euphorbia lomelii*** V.W.Steinm., *Van Devender, T.R. 2007-1105* (ASDM), Mexico, Sonora, KC019737, KC019485, KC019875; ***Euphorbia lomelii*** V.W.Steinm. **2**, *Berry, P.E. 7837* (MICH), USA, Living collection at UC-Davis, KC019682, KC019445, KC019838; ***Euphorbia longispina*** Chiov., *Riina, R. 1707* (MA), The Netherlands, From private living collection (JK). Origin unknown, -, -, KC019862; ***Euphorbia lophogona*** Lam., *Dorsey, B.L. 171* (MICH), Madagascar, Toliara, KC019538, KC019342, KC019762; ***Euphorbia lugardiae*** (N.E. Br.) Bruyns, *Cumming, D.M. 3217* (BRI), South Africa, Limpopo Prov., KC019588, -, -; ***Euphorbia lugardiae*** (N.E. Br.) Bruyns **2**, *Berry, P.E. 7751* (MICH), USA, Cultivated in the green house of UC-Davis. #B71.014, KC019644, KC019407, -; ***Euphorbia aff. mahafalensis*** , *Dorsey, B.L. 142* (MICH), Madagascar, Toliara, KC019523, -, -; ***Euphorbia magnifica*** (E.A.Bruce) Bruyns, *Berry, P.E. 7750a* (MICH), USA, In cultivation at the green house of UC-Davis. #B90.225, KC019643, KC019406, -; ***Euphorbia magnifica*** (E.A.Bruce) Bruyns **2**, *Dorsey, B.L. 202* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019552, -, -; ***Euphorbia major*** (Pax) Bruyns, *Dorsey, B.L. 210* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019559, KC019354, -; ***Euphorbia mandravioky*** Leandri, *Haevermans, T. 445* (P), Madagascar, Tsingy of Ankarana, KC019732, KC019484, -; ***Euphorbia micracantha*** Boiss., *Berry, P.E. 7802* (MICH), USA, Living collection at UC-Davis, KC019662, KC019422, KC019817; ***Euphorbia milii*** Des Moul., *Berry, P.E. 7826* (MICH), USA, Living collection at UC-Davis, -, KC019436, KC019829; ***Euphorbia milii*** Des Moul. **2**, *Dorsey, B.L. 101* (MICH), Madagascar, Fianarantsoa, -, -, KC019744; ***Euphorbia munizii*** Borhidi, EF653256, -, EF653262; ***Euphorbia neoarborescens***

Bruyns, *Dorsey, B.L. 219* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, -, KC019361, -; ***Euphorbia neoarborescens*** Bruyns **2**, *Berry, P.E. 7853* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019684, -, KC019839; ***Euphorbia neococcinea*** Bruyns, *Berry, P.E. 7749* (MICH), USA, Cultivated in the green house of UC-Davis. #B67.034, KC019641, KC019404, KC019806; ***Euphorbia neogossweileri*** Bruyns, *Dorsey, B.L. 213* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019562, KC019357, -; ***Euphorbia neogracilis*** Bruyns, *Dorsey, B.L. 211* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019560, KC019355, -; ***Euphorbia neohumbertii*** Boiteau, *Berry, P.E. 7830* (MICH), USA, Living collection at UC-Davis, KC019677, KC019440, KC019833; ***Euphorbia neohumbertii***Boiteau **2**, *Berry, P.E. 7874* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019692, -, KC019846; ***Euphorbia neomontana*** Bruyns, *Dorsey, B.L. 207* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019557, KC019352, -; ***Euphorbia neoreflexa*** Bruyns, *Berry, P.E. 7866* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, -, KC019449, KC019843; ***Euphorbia neorubella*** Bruyns, *Berry, P.E. 7746* (MICH), USA, Cultivated in the green house of UC-Davis. #BAA.167, KC019638, KC019401, KC019804; ***Euphorbia neorubella*** Bruyns **2**, *Dorsey, B.L. 214* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019563, -, -; ***Euphorbia neospinescens*** Bruyns, *Morawetz, J. J. 485* (DSM, K, MICH, MO, NHT), Tanzania, Morogoro Region, KC019627, KC019393, -; ***Euphorbia neospinescens*** Bruyns **2**, *Dorsey, B.L. 203* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019553, -, -; ***Euphorbia neospinescens*** Bruyns **3**, *Berry, P.E. 7748* (MICH), USA, Cultivated in the green house of UC-Davis. #B66.389, KC019640, KC019403, -; ***Euphorbia neospinescens*** Bruyns **4**, *Berry, P.E. 7773* (MICH), USA, Living collection at UC-Davis, KC019651, -, KC019811; ***Euphorbia neostolonifera*** Bruyns, *Berry, P.E. 7745* (MICH), USA, Cultivated in the green house of UC-Davis. #B84.108, KC019637, KC019400, -; ***Euphorbia neostolonifera*** Bruyns **2**, *Rauh, W. Ke179* (K), Kenya, Nairobi (Masai) District, KC019628, -, -; ***Euphorbia neovirgata*** Bruyns, *Dorsey, B.L. 215* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019564, KC019358, -; ***Euphorbia neriifolia*** L., *Dorsey, B.L. 243 (*MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019574, -, KC019772; ***Euphorbia neriifolia*** L. **2**, *Berry, P.E. 7776* (MICH), USA, Living collection at UC-Davis, KC019653, KC019414, -; ***Euphorbia nivulia*** Buch.-Ham., *Dorsey, B.L. 222* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019569, -, -; ***Euphorbia nivulia*** Buch.-Ham **2**., *Dorsey, B.L. 242* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019573, -, -; ***Euphorbia nivulia*** Buch.-Ham **3**., *Berry, P.E. 7809* (MICH), USA, Living collection at UC-Davis, -, KC019424, -; ***Euphorbia orthoclada*** Baker, *Dorsey, B.L. 136* (MICH), Madagascar, Toliara, KC019519, -, -; ***Euphorbia papillosa*** A.St.-Hil., *Barboza, G. 1677* (CORD), Argentina, KC019590, -, -; ***Euphorbia parciramulosa*** Schweinf., *Berry, P.E. 7878* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019694, KC019455, -; ***Euphorbia pedilanthoides*** Denis, *Berry, P.E. 7755* (MICH), USA, Cultivated in the green house of UC-Davis. #B68, KC019645, KC019408, KC019808; ***Euphorbia peperomioides*** Boiss., *Cordeiro, I. 3057* (SP), Brazil, Sao Paulo, KC019597, KC019372, KC019780; ***Euphorbia peperomioides*** Boiss. **2**, *Cordeiro, I. 3076* (SP), Brazil, Sao Paulo, KC019598, -, -; ***Euphorbia perangusta*** R.A. Dyer, *Becker, R. 664* (MICH, PRE, UNIN), South Africa, North West Prov., KC019706, KC019462, -; ***Euphorbia persistentifolia*** L.C. Leach, *Riina, R. 1743* (MA), The Netherlands, From private living collection (JK). Origin unknown, -, -, KC019868; ***Euphorbia personata*** (Croizat) V.W.Steinm., GU214940, GU214856, -; ***Euphorbia pervilleana*** Baill., *Dorsey, B.L. 188* (MICH), Madagascar, Toliara, JQ750888, -, JQ750835; ***Euphorbia pervilleana*** Baill. **2**, *Dorsey, B.L. 187* (MICH), Madagascar, Toliara, -, -, KC019767; ***Euphorbia phosphorea*** Mart., *Caruzo, M.B.R. 128* (SP), Brazil, Sao Paulo, KC019582, -, -; ***Euphorbia phosphorea*** Mart. **2**, *Caruzo, M.B.R. 145* (SP), Brazil, Bahia, JQ750889, KC019396, JQ750836; ***Euphorbia plagiantha*** Drake, *Dorsey, B.L. 164* (MICH), Madagascar, Toliara, -, -, JQ750838; ***Euphorbia planiticola*** D.C. Hassall, *Gillespie, L.J. 7324* (MICH), Australia, New South Wales, KC019629, KC019394, JQ750839; ***Euphorbia planiticola*** D.C. Hassall **2**, *Bean, A.R. 18393* (BRI), Australia, Queensland, KC019498, KC019318, -; ***Euphorbia plumerioides*** Teijsm. ex Hassk., *Berry, P.E. 7884* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019695, KC019456, KC019848; ***Euphorbia podocarpifolia*** Urb., EF653257, -, EF653263; ***Euphorbia portulacoides*** L., *Arroyo, M. 994664* (SGO), Chile, KC019630, -, -; ***Euphorbia portulacoides*** L. **2**, *Cocucci 3479* (CORD), Argentina, KC019585, -, -; ***Euphorbia portulacoides*** L. **3**, *Carrillo-Reyes, P. 5923* (IEB), Chile, Coquimbo, KC019699, -, -; ***Euphorbia portulacoides*** L. **4**, *Carrillo-Reyes, P. 5930* (IEB), Chile, Coquimbo, -, KC019458, -; ***Euphorbia pseudoburuana*** P.R.O. Bally & S. Carter, *Riina, R. 1742* (MA), The Netherlands, From private living collection (JK). Origin unknown, KC019727, KC019480, KC019867; ***Euphorbia pseudocactus*** A. Berger **2**, *Berry, P.E. 7735* (MICH), USA, Cultivated in the green house of UC-Davis. #B61.059, KC019634, -, KC019801; ***Euphorbia pseudocactus*** A. Berger, *Morawetz, J. J. 291* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (630/72), KC019607, KC019378, KC019787; ***Euphorbia pseudolaevis*** Bruyns, *Dorsey, B.L. 206* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019556, KC019351, -; ***Euphorbia pseudomollis*** Bruyns, *Morawetz, J. J. 440* (DSM, MICH, MO, NHT), Tanzania, Kilimanjaro Region, KC019625, KC019392, KC019799; ***Euphorbia pseudomollis*** Bruyns **2**, *Morawetz, J. J. 441* (MICH, NHT), Tanzania, Kilimanjaro Region, KC019626, -, -; ***Euphorbia pseudostellata*** Bruyns, *Berry, P.E. 7869* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019690, KC019452, -; ***Euphorbia pseudotrinervis*** Bruyns, *Berry, P.E. 7867* (MICH), USA, Living collection at The Huntington Botanical Gardens, San Marino, KC019688, KC019450, KC019844; ***Euphorbia pteroneura*** A. Berger, *Berry, P.E. 7792* (MICH), USA, Living collection at UC-Davis, KC019659, KC019419, KC019815; ***Euphorbia punicea*** Sw., *van Ee, B. 778* (MICH), Jamaica, Hanover, KC019584, KC019364, -; ***Euphorbia ramipressa*** Croizat, *Berry, P.E. 7820* (MICH), USA, Living collection at UC-Davis, KC019633, KC019430, KC019824; ***Euphorbia randrianjohanyi*** Haevermans & Labat, *Dorsey, B.L. 119* (MICH), Madagascar, Toliara, KC019508, KC019324, KC019751; ***Euphorbia rauhii*** Haevermans & Labat, *Dorsey, B.L. 181* (MICH), Madagascar, Toliara, KC019545, KC019345, KC019765; ***Euphorbia rauhii*** Haevermans & Labat **2**, *Dorsey, B.L. 182* (MICH), Madagascar, Toliara, KC019546, KC019346, -; ***Euphorbia rauhii*** Haevermans & Labat **3**, *Dorsey, B.L. 180* (MICH), Madagascar, Toliara, KC019544, KC019344, -; ***Euphorbia resinifera*** O.Berg, *Berry, P.E. 7817* (MICH), USA, Living collection at UC-Davis, KC019668, KC019428, KC019822; ***Euphorbia aff. retrospina*** , *Dorsey, B.L. 150* (MICH), Madagascar, Toliara, -, -, KC019757; ***Euphorbia rhizophora*** (P.R.O. Bally) Bruyns, *Berry, P.E. 7742* (MICH), USA, cultivated in the green house of UC-Davis. #B71.015, KC019636, KC019398, KC019802; ***Euphorbia rhizophora*** (P.R.O. Bally) Bruyns **2**, *Bally, P.R. E117* (BRI), Kenya, Mchakos District, KC019697, -, -; ***Euphorbia robecchii*** Pax, *Berry, P.E. 7822* (MICH), USA, Living collection at UC-Davis, KC019671, KC019432, KC019825; ***Euphorbia rosescens***

E.L.Bridges & Orzell, *Weekley, C. sn* (ARCH), U.S.A., Florida, KC019587, -, KC019774; ***Euphorbia rossii*** Rauh & Buchloh, *Berry, P.E. 7827* (MICH), USA, Living collection at UC-Davis, -, KC019437, KC019830; ***Euphorbia rubella*** Pax, AF537487, -, AF538204; ***Euphorbia sapinii*** De Wild., *Riina, R. 1756* (MA), The Netherlands, From private living collection (JK). Origin unknown, KC019729, KC019482, KC019872; ***Euphorbia sarcostemmoides*** J.H. Willis, *Forster, P.I. PIF35626* (BRI), Australia, Queensland, KC019589, -, -; ***Euphorbia sarcostemmoides*** J.H. Willis **2**, *Bean, A.R. 22376* (BRI), Australia, Queensland, KC019499, -, -; ***Euphorbia schubei*** Pax, *Dorsey, B.L. 200* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019551, KC019350, -; ***Euphorbia seibanica*** Lavranos & Gifri, *Riina, R. 1709* (MA), The Netherlands, From private living collection (JK). (Lavranos 30745), Originally from Yemen, KC019722, KC019474, KC019863; ***Euphorbia sekukuniensis*** R.A. Dyer, *Morawetz, J. J. 286* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (713/96), KC019603, KC019375, KC019784; ***Euphorbia sessilifolia*** Klotzsch ex Boiss., JQ750957, -, JQ750851; ***Euphorbia sinclairiana* 2** Benth., Reynel 4830 (MOL), Peru, KC019702, -, -; ***Euphorbia sinclairiana*** Benth., AF537495, -, AF538217; ***Euphorbia sipolisii*** N.E. Br. **2**, *Caruzo, M.B.R. 129* (SP), Brazil, Sao Paulo, KC019583, -, -; ***Euphorbia sipolisii*** N.E. Br., *Cordeiro, I. 3039* (SP), Brazil, Minas Gerais, KC019595, KC019370, KC019778; ***Euphorbia* sp**. **1**, *Taylor, C.M. & Richardson, P.M. 12412* (MO), Chile, Atacama, KC019586, KC019367, -; ***Euphorbia* sp. 2**, *Dorsey, B.L. 148* (MICH), Madagascar, Toliara, -, -, KC019756; ***Euphorbia* sp. 3**, *Dorsey, B.L. 186* (MICH), Madagascar, Toliara, KC019548, -, -; ***Euphorbia* sp. 4**, *Dorsey, B.L. 185* (MICH), Madagascar, Toliara, KC019547, KC019347, KC019766; ***Euphorbia* sp. 5**, *Dorsey, B.L. 128* (MICH), Madagascar, Toliara, KC019515, KC019326, KC019754; ***Euphorbia* sp. 6**, *Dorsey, B.L. 126* (MICH), Madagascar, Toliara, KC019513, -, KC019752; ***Euphorbia* sp. 7**, *Dorsey, B.L. 158* (MICH), Madagascar, Toliara, KC019532, KC019339, KC019759; ***Euphorbia* sp. 8**, *Dorsey, B.L. 105* (MICH), Madagascar, Toliara, -, -, KC019747; ***Euphorbia* sp. 9**, *Dorsey, B.L. 192* (MICH), Madagascar, Toliara, KC019550, KC019349, KC019769; ***Euphorbia* sp**. **10**, *Dorsey, B.L. 151* (MICH), Madagascar, Toliara, KC019526, KC019338, KC019758; ***Euphorbia* sp**. **11**, *McPherson & Rabenantoandro, J. 18330* (MO), Madagascar, Fianarantsoa, KC019632, KC019366, -; ***Euphorbia* sp**. **12**, *Dorsey, B.L. 129* (MICH), Madagascar, Toliara, KC019516, KC019327, -; ***Euphorbia* sp**. **13**, *Dorsey, B.L. 209* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019558, KC019353, -; ***Euphorbia* sp**. **14**, *Randrianasolo, A. 1129* (MO), Madagascar, Toamasina, KC019496, -, -; ***Euphorbia* sp. 15**, *Haevermans, T. 532* (P), Vietnam, KC019735, -, -; ***Euphorbia* sp. 16**, *Haevermans, T. 547* (P), Vietnam, KC019736, -, -; ***Euphorbia* sp. 17**, *Dorsey, B.L. 138* (MICH), Madagascar, Toliara, KC019521, KC019332, -; ***Euphorbia* sp. 18**, *Dorsey, B.L. 167* (MICH), Madagascar, Toliara, KC019536, -, -; ***Euphorbia* sp. 19**, *Dorsey, B.L. 168* (MICH), Madagascar, Toliara, KC019537, -, -; ***Euphorbia* sp. 20**, *Dorsey, B.L. 130* (MICH), Madagascar, Toliara, KC019743, KC019494, KC019879; ***Euphorbia spectabilis*** (S.Carter) Bruyns, *Dorsey, B.L. 205* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019555, -, -; ***Euphorbia spinea*** N.E. Br., *Becker, R. 967* (MICH, PRE, UNIN), South Africa, Northern Cape Prov., JQ750964, -, JQ750862; ***Euphorbia stellata*** Willd., *Morawetz, J. J. 297* (MICH), South Africa, Western Cape, In cultivation at the Kirstenbosch National Botanical Garden Conservatory (944/97), KC019609, KC019380, KC019789; ***Euphorbia stenoclada*** Baill., *Dorsey, B.L. 115* (MICH), Madagascar, Toliara, -, -, KC019750; ***Euphorbia stenoclada*** Baill. **2**, *Berry, P.E. 7804* (MICH), USA, Living collection at UC-Davis, -, KC019423, -; ***Euphorbia stevenii*** F.M.Bailey, *Walmsley PW33* (BRI), Australia, Queensland, -, KC019488, -; ***Euphorbia succulenta*** (Schweickerdt) Bruyns, *Dorsey, B.L. 218* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019567, KC019360, -; ***Euphorbia succulenta*** (Schweickerdt) Bruyns **2**, *Morawetz, J. J. 423* (EA, MICH), Kenya, Rift Valley Province, KC019624, KC019391, -; ***Euphorbia sudanica*** A.Chev., *Berry, P.E. 7757* (MICH), USA, Cultivated in the green house of UC-Davis. #B79.257, KC019647, KC019410, -; ***Euphorbia sudanica*** A.Chev. **2**, *Berry, P.E. 7810* (MICH), USA, Living collection at UC-Davis, KC019663, KC019425, KC019818; ***Euphorbia sudanica*** A.Chev. **3**, *Haevermans, T. 462* (P), Cultivated , KC019734, -, -; ***Euphorbia tanaensis*** P.R.O. Bally & S. Carter, *Morawetz, J. J. 415* (MICH), Kenya, Cultivated in the garden of A. Robertson, originally from Witu Forest, Kenya, KC019623, KC019390, KC019798; ***Euphorbia tannensis*** Spreng., *Halford Q9447* (BRI), Australia, Queensland, KC019594, -, -; ***Euphorbia tanquahuete*** Sessé & Moç., AF537525, -, AF538224; ***Euphorbia tanquahuete*** Sessé & Moç. **2**, *Martinez, M. 6205* (IEB), Mexico, Guanajuato, KC019631, -, -; ***Euphorbia teke*** Schweinf. ex Pax, *Berry, P.E. 7834* (MICH), USA, Living collection at UC-Davis, KC019680, KC019442, KC019835; ***Euphorbia telephioides*** Chapm., *Black, C. 1* (FSU), U.S.A., Florida, -, KC019365, -; ***Euphorbia tetragona*** Haw., *Morawetz, J. J. 260* (MICH, NBG), South Africa, Eastern Cape, KC019599, KC019373, KC019781; ***Euphorbia tetraptera*** Baker, *Randrianasolo, A. 1113* (MO), Madagascar, Toamasina, KC019495, KC019317, -; ***Euphorbia thinophila*** Phil., AF537530, -, AF538218; ***Euphorbia tirucalli*** L., *Becker, R. 982* (MICH, PRE, UNIN), South Africa, Prov., KC019712, KC019466, KC019856; ***Euphorbia tirucalli*** **2** L., *Dorsey, B.L. 121* (MICH), Madagascar, Toliara, KC019510, -, -; ***Euphorbia tirucalli*** **3** L., *Dorsey, B.L. 122* (MICH), Madagascar, Toliara, KC019511, -, -; ***Euphorbia tirucalli*** **4** L., *Dorsey, B.L. 120* (MICH), Madagascar, Toliara, KC019509, -, -; ***Euphorbia tirucalli*** **5** L., *Dorsey, B.L. 153* (MICH), Madagascar, Toliara, KC019527, -, -; ***Euphorbia tirucalli*** **6** L., *Dorsey, B.L. 154* (MICH), Madagascar, Toliara, KC019528, -, -; ***Euphorbia tirucalli*** **7** L., *Dorsey, B.L. 155* (MICH), Madagascar, Toliara, KC019529, -, -; ***Euphorbia tirucalli*** **8** L., *Dorsey, B.L. 156* (MICH), Madagascar, Toliara, KC019530, -, -; ***Euphorbia tirucalli*** **9** L., *Becker, R. 1065* (MICH, PRE, UNIN), South Africa, Limpopo Prov., KC019704, -, KC019850; ***Euphorbia tirucalli*** **10** L., *Dorsey, B.L. 117* (MICH), Madagascar, Toliara, KC019506, KC019323, -; ***Euphorbia tirucalli*** **11** L., *Berry, P.E. 7772* (MICH), USA, Living collection at UC-Davis, KC019650, -, -; ***Euphorbia tithymaloides*** L., *Dorsey, B.L. 294* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019580, KC019363, -; ***Euphorbia tithymaloides*** **2** L., *Dorsey, B.L. 293* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019579, -, -; ***Euphorbia tithymaloides*** **3** L., *Dorsey, B.L. 102* (MICH), Madagascar, Fianarantsoa, KC019501, KC019319, KC019745; ***Euphorbia torrei*** (L.C. Leach) Bruyns, *Dorsey, B.L. 212* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019561, KC019356, -; ***Euphorbia triangularis*** Desf. ex A.Berger, *Morawetz, J. J. 270* (MICH, NBG), South Africa, Eastern Cape, KC019600, -, -; ***Euphorbia tulearensis*** (Rauh) Rauh, *Dorsey, B.L. 143* (MICH), Madagascar, Toliara, KC019524, KC019334, -; ***Euphorbia umbellata*** (Pax) Bruyns, AF537469, -, JN249237; ***Euphorbia umbelliformis*** (Urb. & Ekman) V.W.Steinm. & P.E.Berry, EF653253, -, EF653258; ***Euphorbia unispina*** N.E.Br., *Berry, P.E. 7798* (MICH), USA, Living collection at UC-Davis, KC019660, KC019420, JN249239; ***Euphorbia vajravelui*** Binojk. & N.P.Balakr., *Dorsey, B.L. 289* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019577, -, -; ***Euphorbia vajravelui*** **2** Binojk. & N.P.Balakr., *Dorsey, B.L. 220* (MICH), USA, In cultivation at Arid Lands Greenhouses, Tucson, AZ, KC019568, KC019362, KC019770; ***Euphorbia venenifica*** Tremaux ex Kotschy, *Berry, P.E. 7868* (MICH), USA, Living collection at The

Huntington Botanical Gardens, San Marino, KC019689, KC019451, -; ***Euphorbia viguieri*** Denis, *Berry, P.E. 7825* (MICH), USA, Living collection at UC-Davis, KC019674, KC019435, KC019828; ***Euphorbia weberbaueri*** Mansf., AF537519, -, AF538212; ***Euphorbia xylophylloides*** Brongn. ex Lem., *Berry, P.E. 7823* (MICH), USA, Living collection at UC-Davis, KC019672, KC019433, KC019826; ***Euphorbia xylophylloides* 2** Brongn. ex Lem., AF537467, -, AF538214; ***Euphorbia xylopoda*** Greenm., *Steinmann, V.W. 6752* (IEB), Mexico, Mexico, KC019739, KC019487, -;

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Chapter III

Climatic niche, biogeography, and growth form evolution in *Euphorbia* sect. *Euphorbia*

INTRODUCTION

Understanding the causes of morphological evolution and the biogeographic history of clades are two fundamental goals of evolutionary biology. Because patterns of morphological evolution can be linked to historical biogeography in a number of ways, it is important to incorporate these two goals. For example, *in situ* selection by changing environmental factors (e.g. climatic parameters) may result in the morphological evolution of a lineage. Alternatively, filtering of species into novel habitats according to their historical ranges/habitats may better explain patterns of morphological traits than do hypotheses of in situ adaptation (Ackerly, 2004). For land plants, extremes in water availability and temperature have apparently selected for novel growth forms, as evidenced by the evolution of many succulent and xerophytic forms in hot, arid regions. Recent studies involving parameters of water availability and temperature at a site, or ‘climatic niche’ sensu Evans & al. (2009), have focused on the role of life history in the evolution of climatic tolerances (Smith & Beaulieu, 2009), estimated the extent of niche diversification (Evans & al., 2009), and investigated the influence of species’ niche on biogeographic patterns (Smith & Donoghue, 2010). However, the influence of climatic parameters on growth form evolution has, surprisingly, not been well studied. This may be because to explicitly do so in a macroevolutionary context requires data and methods that have only recently become available, namely, phylogenetic relationships (including branch lengths) for groups in which distinct growth forms have evolved multiple times; estimates of each species' climatic niche; and methods that incorporate phylogenetic non-independence while explicitly testing hypotheses of adaptation. These requirements have recently become less daunting due to the development of global climatic data sets (Hijmans & al., 2005), new comparative methods that model selection and drift explicitly (Butler & King, 2004), and improved phylogenetic histories of morphologically diverse clades. Among these morphologically diverse clades is the species rich and cosmopolitan angiosperm genus *Euphorbia* (Euphorbiaceae, Malpighiales). With its impressive morphological diversity and convergent evolution of multiple xerophytic growth forms, *Euphorbia* provides many putative examples of adaptive morphological evolution (Steinmann & Porter, 2002; Bruyns & al., 2011; Horn & al., 2012; Yang & al., 2012; Dorsey & al., in press). In fact, these patterns of divergent and convergent morphological evolution of growth form are found both at the level of the entire genus and within individual sections. Here we investigate the roles of biogeographic history and climatic niche in the evolution of growth form within *Euphorbia* sect. *Euphorbia*, a diverse clade of stem-succulent species within *Euphorbia*.

The spine-shield euphorbias (*Euphorbia* sect. *Euphorbia*), so-called because of the hard pad of tissue at each leaf axil that bears several spines, are perhaps the most recognizable of the xerophytic groups within *Euphorbia*, and the clade is an excellent group in which to investigate both historical biogeography and climatic niche, and their combined role in the evolution of growth form. Section *Euphorbia* includes roughly 360 species and is the largest section in *Euphorbia*. It is distributed throughout most of Africa but approximately twenty species occur in South or Southeast Asia. The greatest species diversity is in eastern and southern Africa, where the spine-shield euphorbias are often dominant members of the xerophytic communities. Section *Euphorbia* is almost exclusively made up of spiny stem-succulent species that range from dwarf shrubs less than 15 cm tall (e.g. cushion plants) to large trees up to 30 m high, but a few geophytic species have also evolved (Fig. 3.1). Because of this great range of sizes, the diversity of growth forms within the spine-shield euphorbias can best be characterized as variation on the stem-succulent form in terms of plant stature.

The spine-shield euphorbias are often compared to cacti as a classic example of convergent adaptation to xeric habitats in distantly related groups but convergence of growth form has also been an important process within the evolutionary history of *Euphorbia*. Horn & al. (2012) found "… at least five independent origins of the herbaceous habit, at least seven transitions from herbs to secondary woodiness, and 14 origins of strongly xeromorphic growth forms" within *Euphorbia.* In a recent phylogenetic study of *Euphorbia* in the Old World, Bruyns & al. (2011) discussed a pattern of convergent growth form evolution among the large succulent tree species found in sect. *Euphorbia*. Dorsey & al. (in press) provided a comprehensive revision of *Euphorbia* subg. *Euphorbia* and noted a pattern of convergent evolution of similar growth forms throughout the subgenus. These patterns of widespread convergence suggest that adaptation has been a major force in the evolution of growth form within sect. *Euphorbia* and among its congeners, but the specific selective filters that have resulted in this adaptation remain unknown.

Because growth form variation in sect. *Euphorbia* is mainly the result of changes in plant size, we expect factors that limit or promote plant growth to have been important in the evolution of growth form within this group. Game theory models (reviewed in Falster & Westoby, 2003) and allocation tradeoff models (Givnish, 1995) have been proposed to explain optimal plant height, and in general these predict that taller plants will be favored in highly productive habitats with high light competition. Conversely, the physiological requirement of water per unit carbon assimilated should limit plant growth in regions of low water availability. Competition for light is not likely to be a dominant force in the open, tropical habitats of most species in sect. *Euphorbia*. However, the relationship between plant stature and site productivity, which is partly determined by water availability and thus likely to be very important in such habitats, suggests that climatic parameters have been an important factor in the evolution of growth form within this clade. Consistent with this, and based on the assumption that a leafy, non- or semi-succulent tree was the ancestral form in sect. *Euphorbia,* Carter (1994) suggested that sect. *Euphorbia* evolved first in mesic forests of southern Asia and subsequently dispersed into Africa, where more xeric conditions favored smaller size, reduced or absent leaves, and an increase in succulent tissue. If Carter's hypothesis is correct and sect. *Euphorbia* originated in southern Asia and then expanded into Africa, concurrent with the adaptive evolution of smaller growth forms in increasingly xeric habitats, we would expect that 1) the ancestor of sect. *Euphorbia* occurred in southern Asia and subsequently dispersed into Africa, 2) the ancestral growth form of the section was a tree, and the most derived forms are dwarf shrubs, and 3) the evolution of smaller forms is associated with climatic parameters that reduce water-availability. In this study we test the predictions of Carter’s hypothesis in order to understand the adaptive significance of climatic niche to growth form evolution and the link between biogeography and morphology within sect. *Euphorbia*. To that end, we produced the most complete phylogeny of sect. *Euphorbia* to date and then used this phylogenetic framework to reconstruct both the ancestral growth forms and ancestral ranges of the group. We then estimated the climatic niche of each species and tested the relative fit of a model of selection for growth form by climatic parameters versus a pure drift model.

MATERIALS AND METHODS

Phylogenetic Analysis — We sampled 190 of the ca. 360 species of *Euphorbia* sect. *Euphorbia* and five members of the closely related *Euphorbia* sect. *Monadenium*, which were used as an outgroup following the relationships in Dorsey & al. (in press). We selected species to maximize the geographic coverage and morphological diversity of our sampling. We sequenced a total of five DNA regions: the internal transcribed spacer region of the nuclear ribosomal DNA (ITS), chloroplast (cpDNA) coding regions *mat*K (including the partial *trn*K intron) and *ndh*F, and the cpDNA intergenic spacers *psb*B-*psb*H and *trn*S-*trn*G-*trn*G. DNA extraction and sequencing, and sequence alignment followed Dorsey & al. (in press), with the addition of primers for the *psb*B-*psb*H and *trn*S-*trn*G-*trn*G spacers (Shaw & al., 2005; Shaw & al., 2007). We obtained Bayesian estimates of phylogeny and relative divergent times using the program BEAST (v. 1.7.2, Drummond & Rambaut, 2007) for the ITS and the concatenated chloroplast (cpDNA) data sets. We analyzed each of these two data sets separately under the GTR+gamma model of nucleotide evolution and an uncorrelated log-normal relaxed clock model (Drummond & al., 2006), with separate partitions defined for each region of the cpDNA matrix. We performed two runs for the ITS matrix and 4 for the cpDNA matrix, each of at least 10^8 generations, and evaluated convergence and stationarity using Tracer (v. 1.5, Rambaut & Drummond, 2007) and then compared the maximum clade credibility (MCC) trees from each matrix for topological congruence. Because of problems with convergence of the BEAST runs analyzing a concatenated matrix of ITS and cpDNA sequences, even after 2×10^8 generations, we performed a maximum likelihood (ML) analysis of this data set using RAxML (version 7.2.8, Stamatakis, 2006). We partitioned this set by gene region and assigned each a separate GTR+gamma model of nucleotide evolution. We also ran one thousand bootstrap replicates in RAxML under the same model of evolution to evaluate support for clades. We then transformed the ML phylogram into a relatively dated ultrametric tree using the penalized likelihood method of Sanderson (2002) implemented in the R package "ape" (Paradis E. & Strimmer, 2004; R Core Team, 2012). For subsequent analyses, we used the MCC tree from the ITS and the cpDNA data sets and the transformed ML tree from the concatenated data set.

***Ancestral State Reconstruction* —** To infer the growth form of ancestors within sect. *Euphorbia* we performed maximum likelihood reconstruction (Schluter & al., 1997; Pagel, 1999) of growth form under the Mk1 model of character evolution (Lewis, 2001) as implemented in the program Mesquite (Maddison & Maddison, 2006, 2011), using pruned phylogenies that excluded the outgroup. We coded species as trees, shrubs, or dwarfs (i.e., < 30cm tall; Fig. 3.1) according to original species descriptions or regional floras and checklists (White & al., 1941; Carter, 1988, 2002; Binojkumar & Balakrishnan, 2010). We designated the few species that were described as either a tree or shrub as trees because the method in Mesquite does not allow for ambiguous states. We assigned a given state to a node if its raw likelihood was >2 log units better than those of the other states and recorded relative probabilities of all assigned states at each node.

***Ancestral Area Reconstruction* —** We obtained maximum likelihood estimates of ancestral areas on the ingroup trees using the program Lagrange, which implements a dispersal-extinction-cladogenesis (DEC) model of range evolution (Ree & al., 2005; Ree & Smith, 2008). We designated nine areas based on the level two regions defined in the World Geographic Scheme for Recording Plant Distributions (Fig. 3.3; Brummitt & al., 2001) with the exception that we combined the Arabian Peninsula with Northeast Tropical Africa because the few species that occur in the former area did not justify a more complex model. These regions accommodated all the species within sect. *Euphorbia* without dividing the entire range unnecessarily finely; the majority of species occur in only one region and only six species occur in three regions. In Lagrange, the DEC model is set by defining the following: a matrix of allowed ranges (each range consists of one or more areas, which are equal to the level two regions mentioned above); a matrix describing the connectivity of the areas (i.e., rates of dispersal between each area); and a maximum number of areas allowed at each node. Because model selection could potentially affect our results, we tested the fit of five models that varied in two of the three parameters (Table 3.2). While the rate of dispersal between areas likely differs among the possible pairs of areas, initial runs with differing connectivity values did not differ in ancestral ranges. Furthermore, choosing which cells in the matrix should be down-weighted and assigning meaningful relative rates of dispersal among areas is somewhat subjective, so we left them at the default value of equal rates. We instead chose to vary the specific ranges considered and the maximum number of areas allowed in a given range. Restricting the maximum number of areas allowed in the reconstructed range at a node makes biological sense given that all extant species are restricted to less than three areas with most occurring in only one. Also, disallowing widely disjunct ranges of species follows from the fact that no species is currently distributed in widely disjunct areas. This results in models that vary in the total number of ranges that are considered possible and we refer to the models we tested according to the total number of unique ranges allowed in each. The most general model we tested (502-range model) allowed all possible ranges and the maximum number of areas per range, which is all nine areas we defined. We restricted this model by allowing only four areas in ancestral ranges for the 246-range model. We also tested three models that restricted the level of disjunction within ranges. The 81-range model allowed only ranges of contiguous areas or with areas disjunct by only one intervening area and only three areas per range. The 59-range and 31-range models allowed only ranges of contiguous areas with four or three areas per range, respectively. The log likelihood scores of each model were compared using a likelihood ratio test to determine which one best fit the data.

***Estimation of climatic niche* —**To estimate the climatic niche of species in sect. *Euphorbia*, we used locality data for collected species and the global data set of 19 bioclimatic variables available at www.worldclim.org (Hijmans & al., 2005). We downloaded locality coordinates for all herbarium-archived species of sect. *Euphorbia* available from the Global Biodiversity Information Facility (data.gbif.org, accessed 2012-04-28 through 2012-05-09) and from the Tolkin database of *Euphorbia* phylogenetic and taxonomic information (http://app.tolkin.org/projects/72, Riina & Berry, 2012). We then verified coordinates by mapping all points to confirm that they fell within published distributions of species, by comparing recorded collection locality descriptions to coordinates, and by comparing specimen descriptions to published species descriptions. There were no coordinates available for two narrowly restricted species, *E. abdelkuri* and *E. epiphylloides*, endemic to Abd al Kuri Island, Socotra, and North Andaman Island, India, respectively. For these two species we generated random coordinates within their known ranges. We obtained a total of 650 points for 80 species (mean = eight points per species). These species were broadly distributed across the phylogeny of sect. *Euphorbia,* representing almost all major clades and all three main growth form categories. We used the program DIVA-GIS (Hijmans & al., 2001) to extract values for each of the 19 bioclimatic variables at each set of coordinates. We then used Principle Components Analysis (PCA) on these data to account for covariation among the bioclimatic variables and to summarize the climatic niche of each species. We used the loadings of the original variables on the principal components to identify those variables that are most strongly correlated with a species' PC score (Smith & Donoghue, 2010).

Modeling Growth Form Evolution — We tested for an association between climatic niche and growth form in sect. *Euphorbia* by modeling adaptive evolution and random drift using the R package OUCH (Butler & King, 2004; King & Butler, 2009). This package estimates the strength of stabilizing selection, as well as random or unaccounted for factors such as drift, in the evolution of a character using an Ornstein-Uhlenbeck (OU) model of evolution (Hansen, 1997). OUCH takes as input a phylogeny, a set of character values at the tips, and one or more historical selection regime scenarios. We used species' mean scores from the first two PCA axes as estimates of each species' current climatic niche and a pruned ultrametric phylogeny that included those species for which we had estimated a climatic niche. The OUCH package can be used to compare alternative hypotheses of character evolution by specifying any number of historical selection regimes (optimal climatic niches in our case) hypothesized to have influenced the evolution of a character (growth form) and assigning these to specific lineages, clades and/or individual species across the phylogeny of the group. We hypothesized that there have been three distinct optimal climatic niches, corresponding to the three basic growth forms in sect. *Euphorbia*. We used our ML estimates of ancestral growth form throughout sect. *Euphorbia* to assign distinct selection regimes to lineages according to their reconstructed growth form (Fig. 3.5). Specifically, we assigned a regime to each extant species according to their growth form and a regime to each internal branch according to the reconstructed growth form at the node that it subtends. Our assumption was that when growth form did not change between ancestor and descendant, the selection regime (i.e., niche) was also constant, but when a descendant differed in form from its ancestor, a distinct selection regime had been encountered along the branch that connects them. As a null model, we also modeled evolution of growth form according to a pure drift Brownian motion process. Because selection and drift each contribute to the ML estimates of trait values throughout the phylogeny in the OU model, the OUCH package provides estimates of the strength of these two processes in the evolution of the character of interest. In fact, the OU model reduces to a Brownian motion model when the selection term equals zero (Hansen, 1997; Butler & King, 2004). This allowed for the use of likelihood ratio tests and the Akaike information criterion (AIC) to determine the best fit among models of selection and drift. We performed parametric bootstrapping (BS) to obtain confidence intervals (CI) for the parameters of the OU model.

RESULTS

***Phylogeny of sect. Euphorbia* —** Summary statistics for the DNA matrices are given in Table 3.1. In general, the backbone of the ML tree from the concatenated matrix (Fig. 3.2) is better supported than either of the individual data sets, and it resolves most of the same subclades that are common to both (Fig. 3.2, 3.S1—3.S2). As in the cpDNA tree (Fig. 3.S2), the base of the concatenated tree is a grade that contains the species forming clade A in the ITS tree (Fig. 3.S1), although the order of divergence among the constituent clades is different and the topology is better supported. The first two diverging clades consist of Asian species, which are split up as in the ITS tree. *Euphorbia abdelkuri*, from Abd al Kuri island in the Socotra archipelago, is only weakly supported as part of the first diverging clade (BS=58%), whereas it was well-supported as closely related to the other Asian species in the individual trees (PP=0.96-1.0, Fig. 3.S1—3.S2). Also resolved by the concatenated data are two clades that correspond to clades B and C in the ITS tree (Fig. 3.S1, Fig. 3.2, nodes 9 and 10) but which are here resolved sister to each other with better support (BS = 85%).

Individual analyses of the ITS and cpDNA data sets produced estimates of phylogenetic relationships (MCC trees) that resolved most of the same monophyletic groups, although the relationships among these clades are not consistent between the two trees (Figs. 3.S1—3.S2). The ITS tree is composed of three main clades (Fig. 3.S1, clades A-C), whereas the cpDNA tree includes a basal grade subtending two major clades (Fig. 3.S2). Despite the fact that many of the same clades are inferred between the two analyses, there are several cases of significant incongruence. The main conflict between the trees lies in the placement of several clades that form a monophyletic group in the ITS tree (clade A, Fig. 3.S1, PP=1.0), but that form a grade at the base of the cpDNA tree (Fig. 3.S2). Importantly, the Asian species form the second diverging clade in the basal grade of the cpDNA tree (Fig. 3.S2, black bar), whereas they are split into two separate clades in the ITS tree, one of which (PP=0.96) is outside clade A.1 and the other nested within it (Fig. 3.S1, black bars, PP=1.0). Another conflicting result involves the clade containing *E. multiclava* and *E. eilensis* in the cpDNA tree (Fig. 3.S2, striped bar), the species of which are nested within clade C.1 in the ITS tree (Fig. 3.S1, PP=0.98-1.0). Similarly, three species from clade A.2 in the ITS tree (Fig. 3.S1, grey bar), *E. stellata, E. micracantha*, and *E. squarrosa*, are in a different position in the cpDNA tree, nested within species that are part of the ITS clade B.1 (Figs 3.S1—3.S2, grey bars). Several other individual species are also placed in distinct positions between the two trees, some with high support.

***Reconstruction of Ancestral Growth Form* —** Tip values of growth form on all phylogenies indicate that trees, shrubs, and dwarf shrubs are scattered across sect. *Euphorbia* and almost every major clade contains multiple growth forms (Figs. 3.2, 3.S1—3.S2). In the better-supported concatenated tree, the ML reconstructions of growth form are unambiguous at nearly all nodes with the exception of a few distal clades (Fig. 3.2), while the ancestral states inferred at the basal nodes of the two individual trees are less certain (Fig. 3.S1—3.S2). Outside the basal nodes, the growth form reconstructions for the concatenated (Fig. 3.2) and individual phylogenies (Figs. 3.S1—3.S2) are largely congruent and the results here focus primarily on the concatenated tree (Fig. 3.2), although the same general patterns are found on the two individual topologies. The most recent common ancestor (MRCA) of the section is inferred to be a tree, as are all nodes in the basal grade (nodes 1-7 and their descendants) except the MRCA of the dwarf shrubs *E. stellata, E. micracantha*, and *E. squarrosa*. The shrub form evolved from this tree ancestor at node 8 and possibly one node prior. According to the concatenated ML tree, dwarf shrubs evolved at least five times in the basal grade of the section and 24 separate times within clade 8. Also, the geophyte form evolved in at least two separate clades both in Asia and Africa. In addition to these instances of repeated evolution, multiple reversals to ancestral forms are found across the tree.

***Ancestral Area Reconstruction* —** The log likelihood scores of each of the 5 DEC models are presented in Table 3.2. Across all phylogenies the likelihood was highest for the 59-range model. Even though these models differ in the number of ranges allowed, they estimate the same two free parameters of dispersal and extinction rates and differ only in additional parameters fixed at zero for rates involving disallowed areas (Ree & Smith, 2008). Consequently, they cannot be compared with a likelihood ratio test. However, the best score is more than the standard 2 log units better than the next best model, suggesting a uniquely best fit to the data. Current ranges of species and ancestral area reconstructions from the concatenated data set are shown in Fig. 3.2. Differences in topology produced somewhat different historical biogeographic scenarios among the three trees (Fig. 3.2, 3.S3—3.S4), which are nonetheless compatible given the uncertainty among the deeper nodes of both the ITS and cpDNA phylogenies. Because the concatenated tree is better supported and the reconstruction of ancestral areas is unambiguous at nearly all nodes on this tree, we report results from that phylogeny but refer the reader to the individual tree figures (Fig. 3.S3—3.S4) for alternative reconstructions.

The ancestor of sect. *Euphorbia* was most likely widespread across South Tropical, East, and Northeast Tropical Africa and South Asia. Two early vicariance events between Asia and Africa (Fig. 3.2, nodes 1, 2) split this ancestral range and the resulting two lineages gave rise to all the extant members of the section that occur in South and Southeast Asia. This was followed by two divergences within South Tropical Africa (Fig. 3.2, node 3) and East Tropical Africa (Fig. 3.2, node 4), while the ancestral species retained the widespread range. Several species from West-Central Tropical and West Tropical Africa descend from nodes 4 and 5, but the topology is not well supported between nodes 4 and 7, so the exact order of biogeographic events is unclear. However, it appears that there were two expansions into the western half of Africa (Fig. 3.2, nodes 5 and 6). The ancestral range was expanded at node 7 to include Southern Africa, and this was retained throughout clade 9 with multiple vicariance events within the individual areas giving rise to generally more restricted subclades. Clade 10 is the result of cladogenesis within Northeast Tropical Africa at node 8. This clade eventually expanded into East Tropical Africa only to be split between the two areas by a vicariance event at node 11. The resulting clades are generally restricted to these two areas, but a few lineages eventually expanded in to South Tropical and Southern Africa.

***Climatic Niche Estimation* —** The first two principal components (PC) from the PCA analysis of 19 bioclimatic variables explained 61% of the variation present in the data set. The original variables that loaded most strongly on PC1 were annual mean temperature, minimum temperature of the coldest month, mean temperature of both the coldest and driest quarters, and temperature seasonality. PC2 was most strongly associated with precipitation seasonality, annual precipitation, and precipitation of both the driest month and driest quarter. Figure 3.3 is a PCA plot with points representing individual collections coded by growth form. Lines connect the points to the mean value of their respective growth form. These mean values are clearly distinct from one another; however, there is considerable spread of the points within each category and overlap among them (Fig. 3.3).

Models of Growth Form Evolution — The likelihood ratio tests indicate that the OU model of adaptive growth form evolution in response to climatic niche within sect. *Euphorbia* explains the data significantly better than a pure drift model for PC2 but not for PC1, although the UO model was selected for PC1 using the ITS tree (Table 3.3). Akaike information criterion (AIC) scores give comparable results (Table 3.3). Estimates and confidence intervals for the OU model parameters alpha, which is a measure of selection strength, and sigma^2, which is an error term measuring the random variation not associated with selection are given in Table 3.4. Regardless of the phylogenetic reconstruction used to fit the model, the strength of selection (alpha) is strong and is generally greater for PC2 (Table 3.4). Estimates of alpha are greater using the two individual topologies than when the concatenated tree is used, but the estimates of sigma^2 are proportionally greater from these trees as well. The theta parameter of the OU model is an estimate of the optimal value for the PC scores (i.e., climatic niche) for each growth form. Estimates of theta for PC2 differ among growth forms, but the confidence intervals of theta for dwarf shrubs and trees broadly overlap, whereas the confidence interval for shrubs is distinct from those of the other two forms (Fig. 3.4, Table 3.4).

DISCUSSION

The widespread convergence of growth forms in *Euphorbia* demonstrated by Horn & al. (Horn & al., 2012) and others requires an evolutionary explanation. The results of this study provide a clearer, if somewhat complex, picture of the historical processes that likely shaped growth form evolution in sect. *Euphorbia*, the largest radiation of xerophytic species in the genus. Variation in stature, degree of succulence, and leaf size among species of sect. *Euphorbia*, along with the section's distribution in mesic forests of southern Asia to dry thickets and deserts of Africa, has led to the hypothesis that the present diversity of growth form is, in large part, the result of adaptations to variation in climatic parameters encountered during a historical migration from Asia to Africa (Carter, 1994). In this study we used this diverse group to investigate the roles of climatic niche and biogeography in shaping angiosperm growth form evolution. Based on our concatenated phylogeny, as discussed below, we suggest that the diversity of growth forms in sect. *Euphorbia* is the result of selection by a dynamic paleoclimate across the group's ancestral range, which led to localized diversification. We further suggest that the evolution of growth form within the clade, both convergent and divergent, has been facilitated by both stabilizing selection for optima along a niche axis of seasonal drought and a wide tolerance about that optimal niche which has led to a high rate of morphological evolution.

***Phylogenetics of the spine-shield euphorbias* —** Our concatenated matrix of ITS and chloroplast sequence data represents the largest sampling effort to date, with respect to both number of taxa and characters, for a phylogenetic study of sect. *Euphorbia* (Table 3.1). The ML phylogeny produced from these data shows that sect. *Euphorbia* consists of a basal grade subtending two major clades (Fig. 3.2). The backbone of this tree (Fig. 3.2, nodes 1-10 excluding node 5) is generally well-supported, with all but two nodes having bootstrap support of 85% or higher. These results differ in important ways from the most taxonomically inclusive study prior to this one (Bruyns & al., 2011), which was based on concatenated ITS and *trn*L-F spacer data, and is most congruent with our ITS gene tree. Specifically, most of the species from the basal grades in our cpDNA and concatenated trees (Fig. 3.S2; Fig. 3.2A, nodes 2-7) are, according to Bruyns & al. (2011), placed in a single clade, with the Asian species in a nested position among those from South Tropical and Southern Africa. Not surprisingly, the placement of the Asian species, using our expanded data set, as the two earliest lineages to diverge within sect. *Euphorbia*, rather than in a more derived position, has important consequences for the estimation of ancestral areas and the origin of the Asian species.

Bruyns & al. (2011) reported no well-supported conflict between the chloroplast and nuclear data in their study. However, with the inclusion of four separate chloroplast regions, both coding and non-coding, we find some incongruence in phylogenetic signal between the two genomes (Figs. 3.2, 3.S1—3.S2) as was found by Dorsey & al. (in press). Still, our ML analysis of the concatenated data set produced a phylogeny that is more resolved, and better supported overall, than either of the two phylogenies based on individual gene regions. There is evidence that concatenation of separate gene regions can either increase the robustness of the results (Rokas & al., 2003; Rokas & Carroll, 2005) or, under certain conditions, can be positively misleading in the estimation of phylogeny (Degnan & Rosenberg, 2006; Kubatko & Degnan, 2007). We attempted to estimate a species tree using a method explicitly designed to deal with incongruence among gene trees (*BEAST, Heled & Drummond, 2010; data not shown), but individual runs failed to converge, likely due to the size of our data set and the lack of multiple accessions per species. While our individual phylogenies are gene trees representing the individual histories of the chloroplast genome and the ITS region of the nuclear genome, the concatenated phylogeny is our best estimate of the species tree for sect. *Euphorbia*. Moreover, given the overall congruence of our subsequent analyses utilizing each of these separate trees, we feel the concatenated tree adequately serves as a working hypothesis for the relationships among species in sect. *Euphorbia*.

Estimates of divergence dates across *Euphorbia* (Bruyns & al., 2011; J. Horn & al., in prep.), indicate a crown age of approximately 12 my for the spine-shield euphorbias of sect. *Euphorbia*. These genus-wide date estimates allow us to place our phylogenetic results in a temporal and paleoclimatic context. Accordingly, we discuss our results assuming that sect. *Euphorbia* originated in the middle to upper Miocene and diversified during the late Cenozoic.

***Biogeographic History* —** Our reconstruction of ancestral areas shows that the ancestor of sect. *Euphorbia* was widespread from South Tropical Africa through East Tropical and Northeast Tropical Africa to South Asia (Fig. 3.2). This ancestral distribution and the subsequent range evolution discussed below reveal a more complicated biogeographic history for sect. *Euphorbia* than Carter's (1994) Asian-origin hypothesis and contradict Bruyns & al.'s (2011) hypothesis of a recent colonization of that region. Our reconstructions indicate that the Asian species are the result of two vicariance events in the middle to late Miocene and thus represent some of the oldest lineages in the section. Subsequent to the divergence of the Asian species, another early vicariance event occurred at the opposite end of the ancestral range, which resulted in a clade of mostly Southern and South Tropical African species (Fig. 3.2, node 3). This was followed by the expansion of several species to western Africa.

While the early history of sect. *Euphorbia* involved vicariance at the fringes of a widespread range, which resulted in a contraction of that range, later speciation and range evolution involved the divergence of lineages within areas occupied by their common ancestor. Two patterns characterize the later history of sect. *Euphorbia*: divergence of localized clades within a widespread ancestral range, and species diversification mostly within a single area. The first pattern is evident in clade 9 of Fig. 3.2. The ancestor at node 9 inherited a widespread range stretching from Northeast Tropical Africa through East Tropical Africa to Southern Africa, and this entire range was occupied throughout the history of the clade. From this widespread ancestor, a series of clades diverged, each of which occupied only single areas initially. The second pattern is predominant in clade 10, where the MRCA is initially present only in Northeast Tropical Africa and the majority of speciation occurred either within Northeast or East Tropical Africa.

This overall pattern of speciation within an ancestral range suggests that there were ample opportunities for isolation of lineages. A possible explanation for this can be found in the pattern of climatic change in eastern Africa during the upper Cenozoic. While an overall drying trend is evident from the middle Miocene on, evidence from a variety of sources suggests that the extent and timing of this drying was heterogeneous across Africa (Senut & al., 2009). At the broadest scale, aridification of Africa during the Neogene began in southwestern Africa followed by northern and finally eastern Africa (Senut & al., 2009). Precipitation patterns in eastern Africa during the Plio-Pleistocene are thought to have been temporally quite heterogeneous (Bonnefille & al., 2004; Trauth & al., 2005) due to the effect of glacial cycles (Hamilton & Taylor, 1991) and spatially variable, due to tectonic uplift (Sepulchre & al., 2006). A temporally and spatially heterogeneous climatic regime across eastern Africa would have created a patchwork of favorable and inhospitable areas that broke up the contiguous ancestral range at various times and in different locations leading to speciation of isolated populations. This is consistent with studies of Evans & al. (2009) and Knowles (2000), which found evidence of speciation in evening primroses and crickets, respectively, associated with the dynamic Pleistocene paleoclimate of western North America.

***Adaptive Landscape of Climatic Niche and Growth Form* —** Our reconstruction of ancestral growth form reveals a mixed pattern of convergent and divergent evolution within sect. *Euphorbia*. At the broadest scale we find that the ancestor of the section was a tree, and this form was conserved through the first several cladogenetic events, followed by a switch to the shrub form at node 8 (Fig. 3.2). The pattern observed among extant species where all three growth forms are distributed across the phylogeny, is the result of many instances of convergent evolution in sect. *Euphorbia*. Reversion to the ancestral tree form from shrub ancestors occurs ten times, and dwarf shrubs have evolved at least twenty times from shrubs and several times from trees. It is evident from this pattern of convergence that there has been a rapid turnover of forms throughout the history of this relatively young clade, especially after node 8 (Fig. 3.2).

The comparison of the OU model to a Brownian motion model shows that growth form evolution in sect. *Euphorbia* has been an adaptive process related to variation in climatic tolerances (Table 3.3). The estimates of alpha indicate that the attraction toward an optimal niche, as defined by the PC2 axis, has been strong (Table 3.4). The PC2 axis is associated with the amount of precipitation during the driest months as well as the variation (i.e., seasonality) of precipitation, so we interpret this as an axis of seasonal drought (Fig. 3.3). The PC2 optima (theta) estimated for the three growth forms suggest that each form has been selected by different intensities of seasonal drought (Table 3.4, Fig. 3.3). However, contrary to our predictions, the confidence intervals for the optima of trees and dwarf shrubs broadly overlap (Table 3.4, Fig. 3.4). The confidence interval for the shrub optimum is distinct from the other two but, again contrary to predictions, the estimate is the lowest of the three, indicating that these species experience the most extreme seasonal drought. These findings support the possibility that water availability has affected growth form evolution throughout the section but do not support Carter's (1994) hypothesis that the dwarf shrubs represent the form best adapted to drought in sect. *Euphorbia*.

On a macroevolutionary timescale, the OU model describes shifting adaptive peaks through time with the ratio of sigma^2 to alpha giving a measure of the overall shape of the peak for each selection regime (Hansen, 1997; Butler & King, 2004). In sect. *Euphorbia*, the estimates of sigma^2 were five times that of alpha (Table 3.4) and these parameters describe a selective landscape in which three distinct but wide hills separated by shallow valleys representing two or three optimal climatic niches. Sigma^2 measures variation in climatic parameters not attributable to growth form in our model (e.g. other forces of selection, which are not captured by our data, or random drift; Hansen, 1997). The high estimates of this parameter relative to the force of selection parameter (alpha) indicate that, while selection by climatic parameters has been important in the evolution of growth form, it is not the whole story. The effect of other potentially selective factors not included in our model (e.g. soil type, competition, herbivory), or perhaps drift, is also evident in the amount of variation in climatic niche that we found among species and growth forms in sect. *Euphorbia*. Figure 3.3 provides a visual account of this variation, and while we can discern three distinct mean values for PC2 among the three growth forms, there is substantial spread within and overlap among the groups. This spread of points shows that species of sect. *Euphorbia* inhabit a range of intensities of seasonal drought, regardless of growth form. This pattern is consistent with selection to an optimal niche tempered by the effect of other factors. Moreover, this pattern combined with the adaptive landscape described above can help to explain the high turnover of growth forms we inferred during much of the history of sect. *Euphorbia*. We suggest that the shape of the adaptive landscape has allowed species of a particular growth form to remain in habitats that may be relatively far from their respective optimal adaptive peak. For species that were near the valleys of overlap between distinct growth form optima when the adaptive landscape (i.e., set of climatic parameters) changed, it is likely that they found themselves on the slope of an alternate peak. In this case, they would have been drawn toward that alternative peak and likely evolved the corresponding growth form. The dynamic climatic regime that prevailed across the range of sect. *Euphorbia* during the last 12 my would have facilitated switching among forms. In other words, growth form evolution in sect. *Euphorbia* may have been unable to keep strict pace with the changing climate of eastern Africa during the Plio-Pleistocene such that there was not a strict correspondence between form and climatic parameters, but this lag in adaptive evolution was less detrimental than might be expected because of wide climatic tolerances.

It is important to remember that our estimation of climatic niche is not the same as quantifying a species' entire niche and so it is expected that other factors such as soil type, topology, and competition have also played a role in both range limitation and character evolution of sect. *Euphorbia*. However, given that the species of sect. *Euphorbia* nearly all occur in relatively hot and dry habitats, the fact that we detected any difference in climatic niche among growth forms is quite significant and indicates that it has been an important factor in the evolution of this clade.

We suggest two alternative hypotheses that may explain the pattern of optimal niches among growth forms that we observe. First, it may simply be that climatic niche affected the switch from trees to shrubs but not the evolution of dwarf shrubs. The distinct, lower optimum estimated for the shrub species suggests that when this form first evolved (Fig. 3.2, node 8) it was associated with a switch in climatic niche and that shrub species in sect. *Euphorbia* have occupied a similar niche since that point. However, while becoming smaller (tree to shrub) may have conferred an advantage in drought-prone habitats up to a point, the further reduction in size to dwarfs could have been a response to other selective events. If this is the case, it would be informative to ask what other niche dimensions are common among dwarfs and compare these to their shrub relatives. Alternatively, it may still be that water availability played a significant role in the evolution of dwarf shrubs, but that the scale of our data does not detect the signal of this history. Water availability is ultimately due to precipitation and temperature, but proximal factors, such as microsite edaphic conditions, can limit the water immediately available to plants even though it may be present in relatively high levels in the surrounding landscape. We can envision a scenario similar to the one we proposed above for the isolating effect of climate change at a regional scale, but which operated at a very local scale and was driven by microsite characteristics. This would have involved selection for smaller variants of shrub species that dispersed to microsites within the ancestral range, such as rock outcrops or especially sandy soils, which had particularly low soil water potential. If the evolution of dwarf shrubs was the result of *in situ* adaptation to microsite water availability, we would expect that these dwarf species would inhabit microsites of lower soil water potential than their shrub relatives. We would also expect that the speciation events that preceded the evolution of dwarf shrubs would be within the range of their shrub ancestors rather than the result of range expansion and subsequent vicariance. Our data do not provide information regarding the first prediction, but the second prediction is supported by the distribution of dwarf shrub species in our phylogeny of sect. *Euphorbia* and by the pattern of range evolution. All but three of the 29 unambiguously reconstructed instances of the evolution of the dwarf shrub habit across sect. *Euphorbia* are associated with the contraction to, or the inheritance of, a single area from within the range of their respective ancestors. While this pattern is still at a fairly large geographic scale, it is consistent with our scenario of microsite selection. Further work on this problem should include finer scale measurements of water availability (i.e., soil water potentials) and hydraulic traits of species representing the shrub and dwarf shrub growth forms to elucidate any differences that would support this hypothesis.

CONCLUSIONS

This study is the first to link ecological differences to growth form evolution in the very diverse genus *Euphorbia* by incorporating estimates of species' climatic niches with the biogeographic history and growth form evolution in a clade of spiny stem-succulent species (*Euphorbia* sect. *Euphorbia*). Furthermore, the methods employed here are an example of our increasing ability to explicitly test for adaptive evolution of specific characters on a macroevolutionary scale, by incorporating a model-based approach into the modern comparative method. This approach allowed us to reject the previous hypothesis that sect. *Euphorbia* originated in Asia and that species became progressively smaller as they encountered dryer habitats during their spread across Africa. Instead we find that the ancestral range was widespread from Northeast Tropical Africa to Southern Africa and that the biogeographic history and patterns of growth form evolution in sect. *Euphorbia* are quite complex. We have shown that even though most species occur in generally warm and dry habitats, climatic niches and specifically the severity of seasonal drought differ among growth forms, and selection by optimal climatic niche parameters has been instrumental in producing the patterns of growth form evolution we observe in this clade. While this integrative approach gives us a more complete picture of the evolution of sect. *Euphorbia*, it emphasizes the multidimensionality of species' niches and the fact that the morphological evolution of any species is a balance between multiple, dynamic selective filters that may not be equally detectable at a given spatial scale. Further work should focus on the other dimensions of species' niches in sect. *Euphorbia*, as well as ecophysiological traits that may be under selection to clarify the role of niche differences in the evolutionary history of the group.

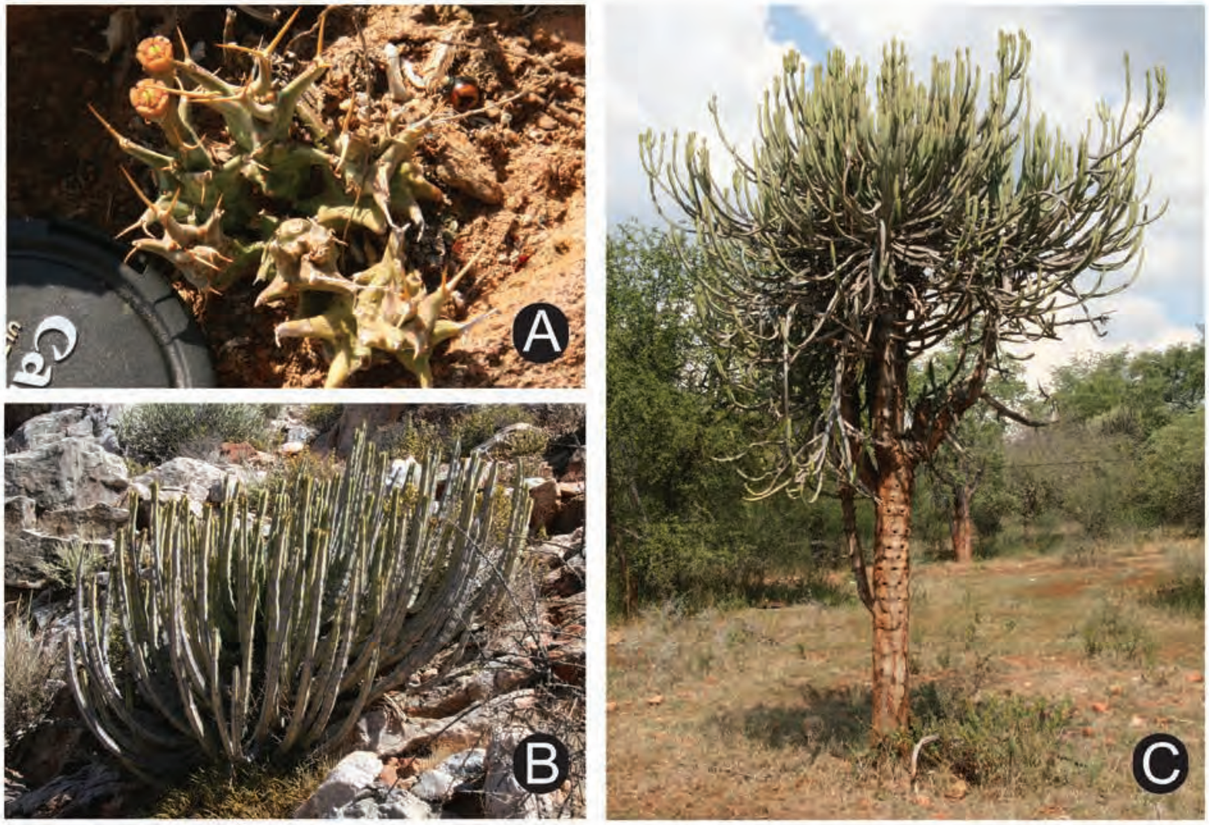


Fig. 3.1. Examples of three main growth forms found in *Euphorbia* sect. *Euphorbia* showing the extreme variation in stature. A, *E. groenwaldii*, a dwarf shrub; B, *E. hottentotta*, a shrub; C, *E. excelsa*, a tree. Photos: Jeffery Morawetz.

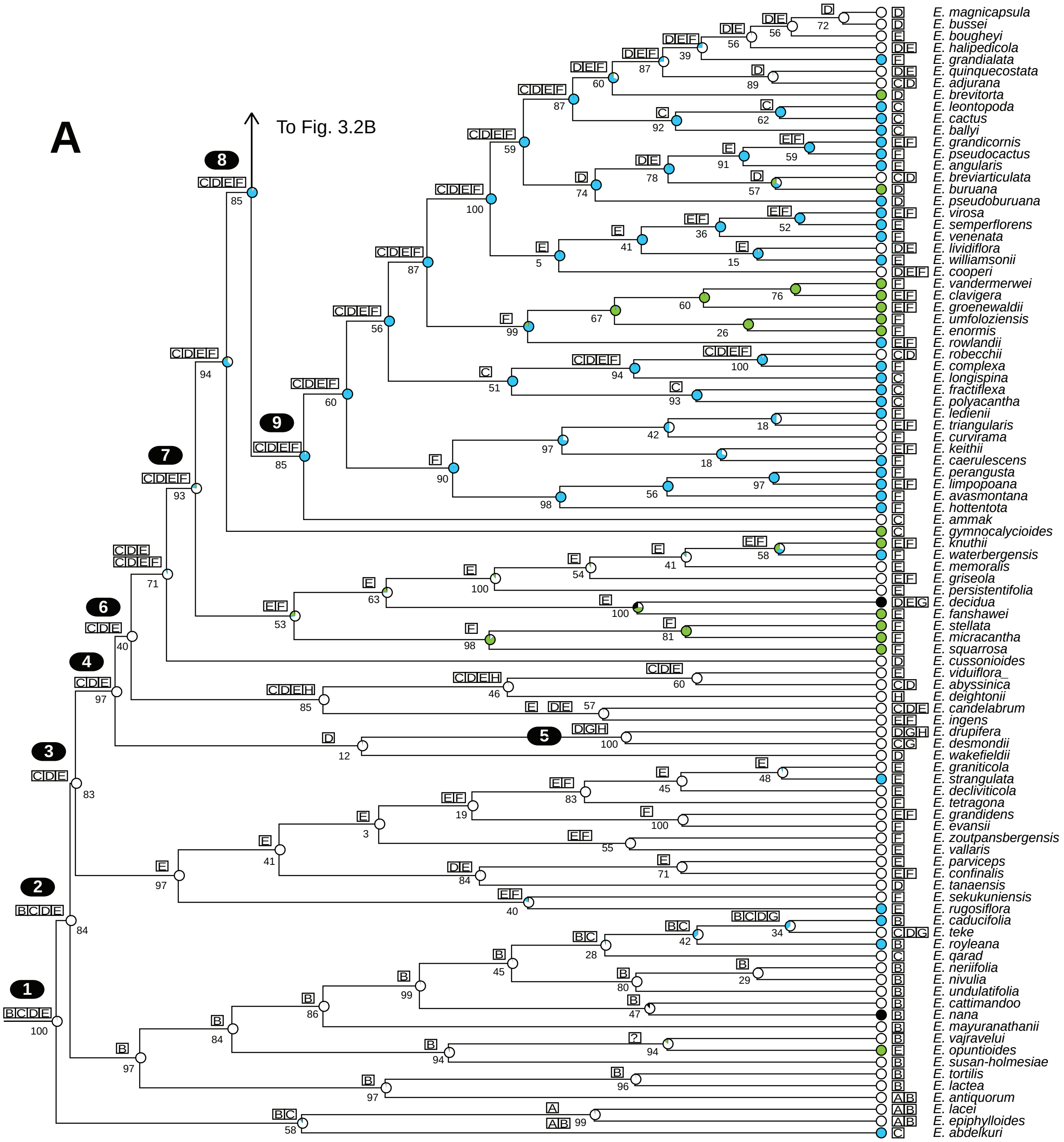


Fig. 3.2. Maximum likelihood phylogeny of *Euphorbia* sect. *Euphorbia* from RAxML analysis of concatenated ITS and cpDNA data and transformed into a relatively dated ultrametric tree using Penalized Likelihood. Numbers at nodes are BS values. Pie charts at nodes show the relative probabilities of ancestral states from ML reconstructions (see insert for color coding). Letters in boxes correspond to regions (see map insert): A, Southeast Asia; B, South Asia; C, Northeast Tropical Africa; D, East Tropical Africa; E, South Tropical Africa; F, Southern Africa; G, West-Central Tropical Africa; H, West Tropical Africa; I, Northern Africa. Boxes at the tips indicate ranges of extant species and those at nodes indicate reconstructed ancestral ranges from LaGrange analysis. Alternative ranges are given at nodes with ambiguous reconstructions. Numbers in black ovals indicate nodes/clades discussed in the text.

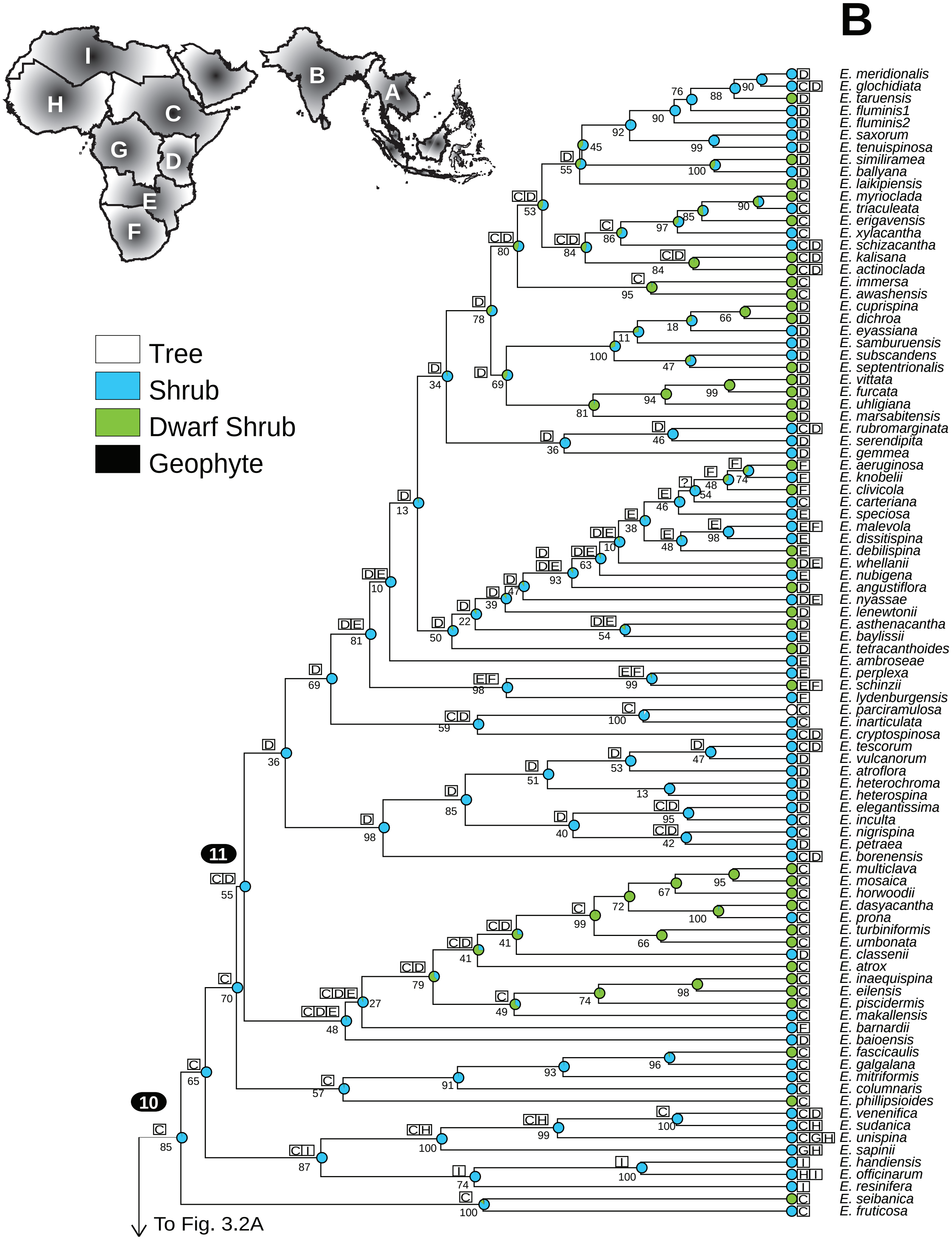
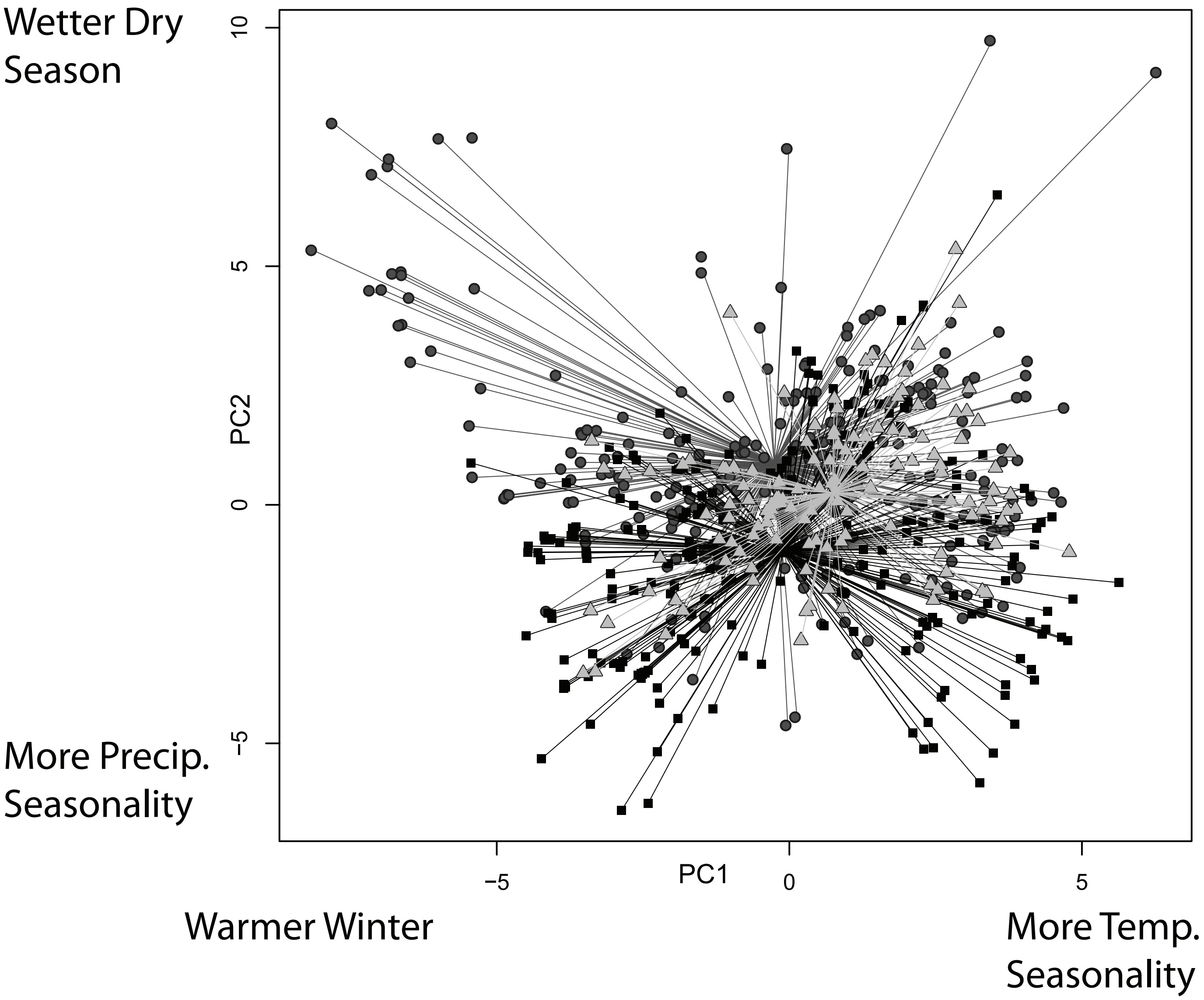

B
I
H
C
B
A
G
D
E
F
Tree
Shrub
Dwarf Shrub
Geophyte
D E. meridionalis
CD E. glochidiata
D E. taruensis
D E. fluminis1
D E. fluminis2
D E. saxorum
D E. tenuispinosa
D E. similiramea
D E. ballyana
D E. laikipiensis
C E. myrioclada
C E. triaculeata
C E. erigavensis
C E. xylacantha
CD E. schizacantha
CD E. kalisana
CD E. actinoclada
C E. immersa
C E. awashensis
D E. cuprispina
D E. dichroa
D E. eyassiana
D E. samburuensis
D E. subscandens
D E. septentrionalis
D E. vittata
D E. furcata
D E. uhligiana
D E. marsabitensis
CD E. rubromarginata
D E. serendipita
D E. gemmea
F E. aeruginosa
F E. knobelii
F E. clivicola
C E. carteriana
E E. speciosa
EF E. malevola
E E. dissitispina
E E. debilispina
DE E. whellanii
E E. nubigena
D E. angustiflora
DE E. nyassae
D E. lenewtonii
D E. asthenacantha
E E. baylissii
D E. tetracanthoides
E E. ambroseae
E E. perplexa
EF E. schinzii
F E. lydenburgensis
C E. parciramulosa
C E. inarticulata
CD E. cryptospinosa
CD E. tescorum
D E. vulcanorum
D E. atroflora
D E. heterochroma
D E. heterospina
D E. elegantissima
C E. inculta
C E. nigrispina
D E. petraea
CD E. borenensis
C E. multiclava
C E. mosaica
C E. horwoodii
C E. dasyacantha
C E. prona
C E. turbiniformis
C E. umbonata
D E. classenii
C E. atrox
C E. inaequispina
C E. eilensis
C E. piscidermis
C E. makallensis
F E. barnardii
D E. baioensis
C E. fascicaulis
C E. galgalana
C E. mitriformis
C E. columnaris
C E. phillipsioides
CD E. venenifica
CH E. sudanica
CGH E. unispina
GH E. sapinii
I E. handiensis
HI E. officinarum
I E. resinifera
C E. seibanica
C E. fruticosa
11
10
To Fig. 3.2A

Fig. 3.3. Plot of scores from the first two axes of the PCA analysis of 19 bioclimatic variables for 650 collections of sect. *Euphorbia* species. Dark-grey circles = Trees, Black squares = Shrubs, Light-grey triangles = Dwarf shrubs. Lines connect points of each growth form to their respective centroids. The x-axis (PC1) is related to winter temperatures and temperature seasonality and the y-axis (PC2) is related to rainfall in the dry season and precipitation seasonality (i.e., seasonal drought).



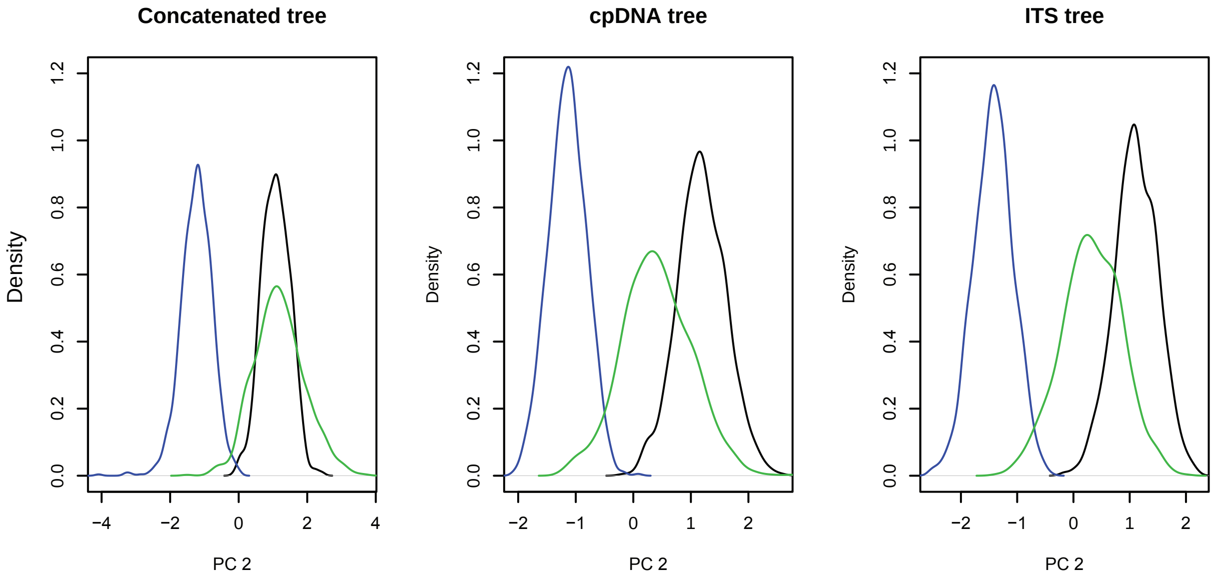


Fig. 3.4. Kernel density plots of confidence intervals for the optimal value (i.e., theta) of PC2 for three growth forms, derived from the parametric bootstrap analysis of the OU model for phylogenies of sect. *Euphorbia* based on three data sets. Black = Trees, Blue = Shrubs, Green = Dwarf shrubs.

Fig. 3.5 ML phylogeny from the concatenated data set for subg. *Euphorbia* (pruned to include only taxa with climatic niche data) showing the three hypothesized selection regimes based on ancestral state reconstructions of growth form. Lineages with different colors were hypothesized to have experienced a different selection regime defined by climatic parameters. See inset for color indications.

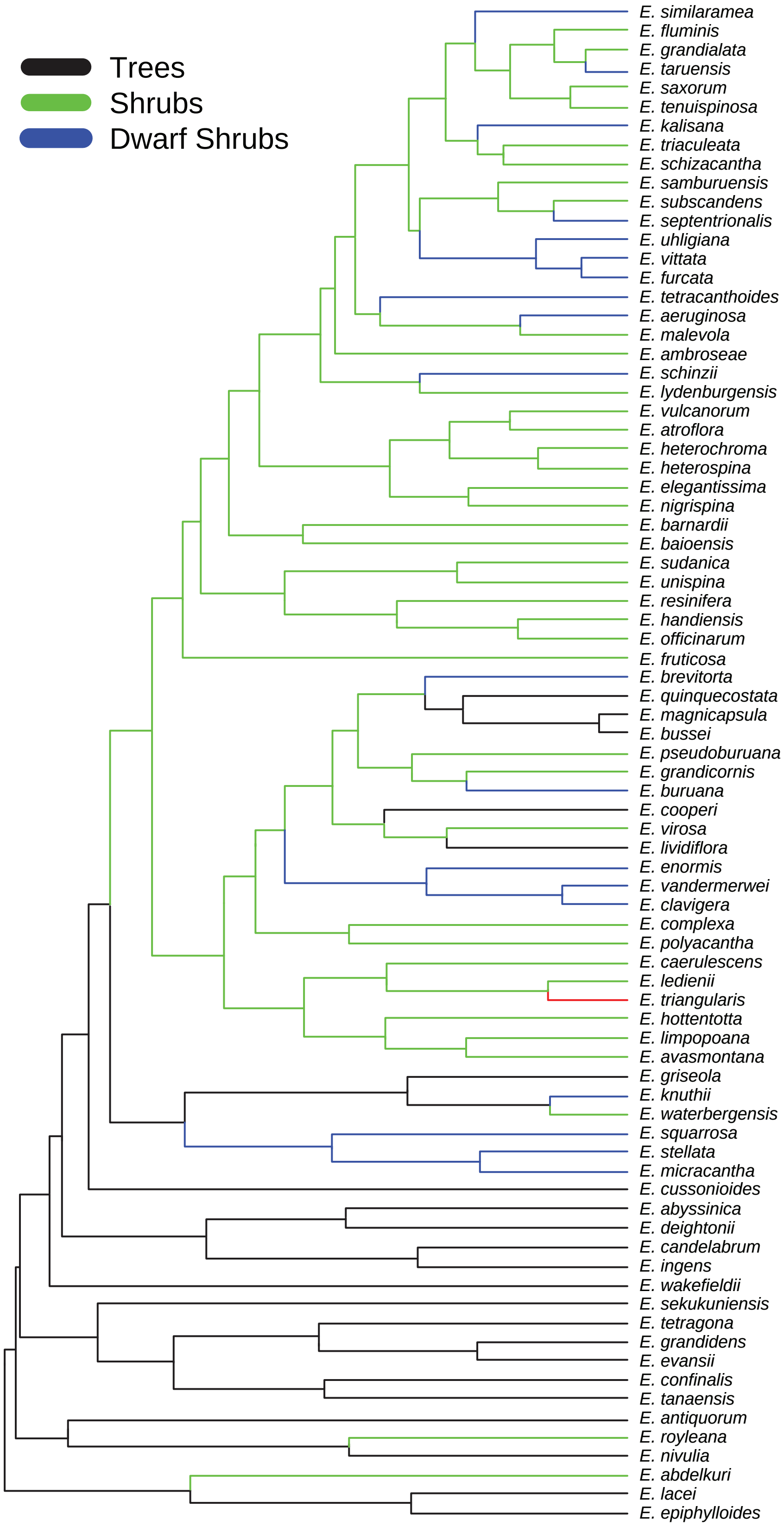


Fig. 3.S1. Relatively dated MCC phylogeny of *Euphorbia* sect. *Euphorbia* from BEAST analysis of ITS data (excluding outgroup taxa). Numbers at nodes are posterior probabilities. Pie charts at nodes show the relative probabilities of ancestral states from ML reconstructions. See insert for growth form color coding. Bars to right of taxa indicate species that are placed in different positions in the cpDNA phylogeny (Fig. 3.S2). Numbers in black ovals indicate nodes/clades discussed in the text.

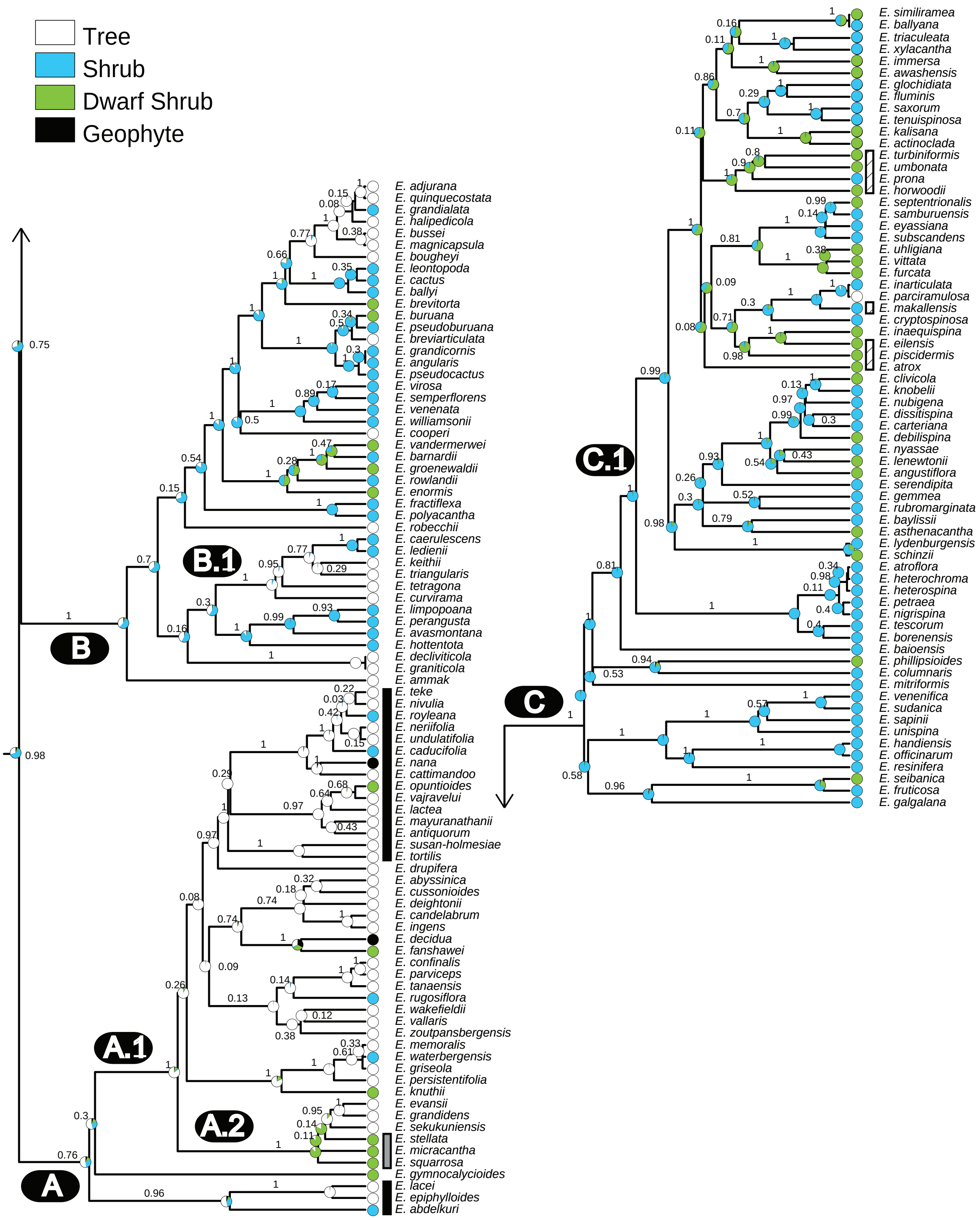
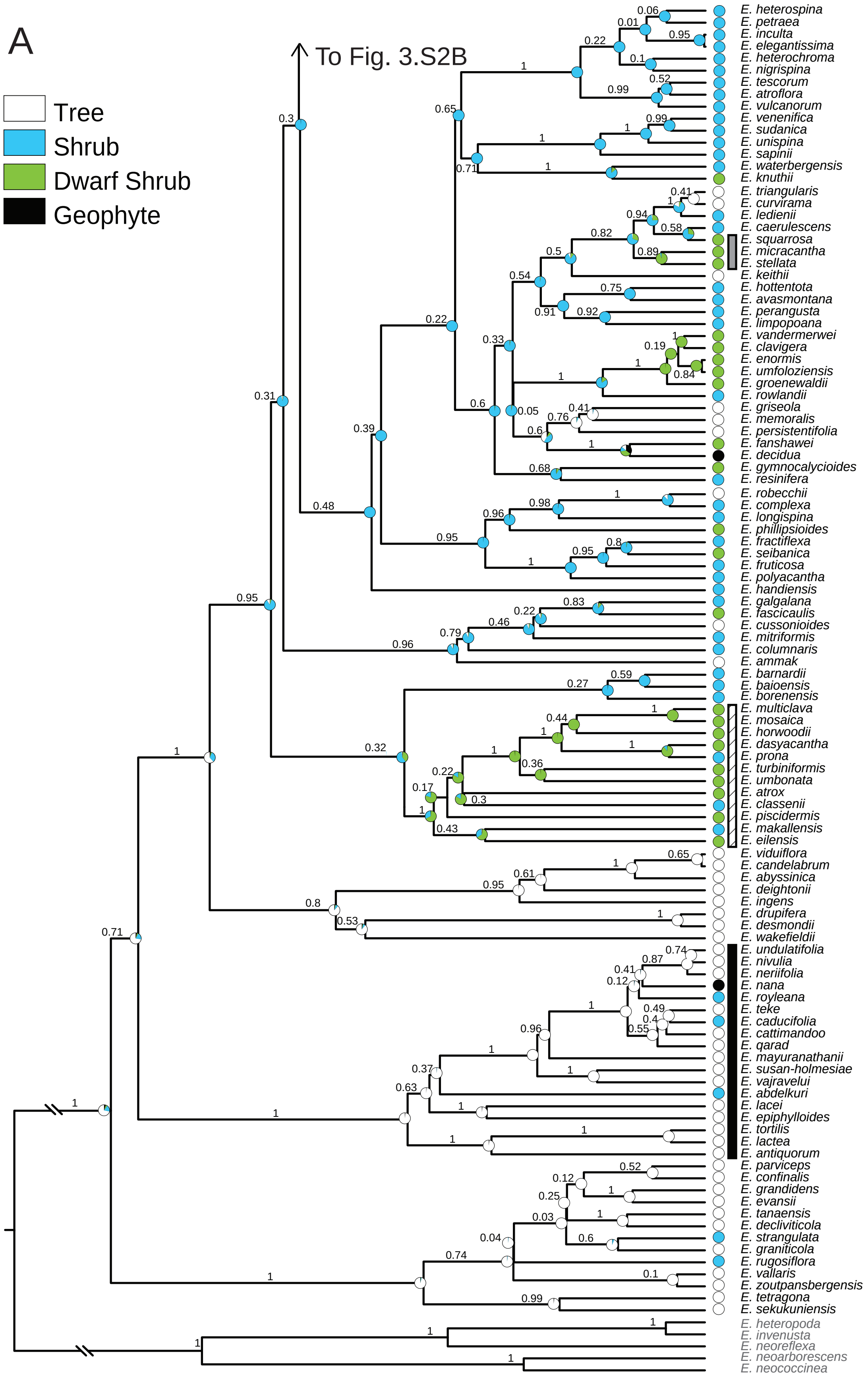

Tree
Shrub
Dwarf Shrub
Geophyte
A
A.1
A.2
B
B.1
C
C.1
E. adjurana
E. quinquecostata
E. grandialata
E. halipedicola
E. bussei
E. magnicapsula
E. bougheyi
E. leontopoda
E. cactus
E. ballyi
E. brevitorta
E. buruana
E. pseudoburuana
E. breviarticulata
E. grandicornis
E. angularis
E. pseudocactus
E. virosa
E. semperflorens
E. venenata
E. williamsonii
E. cooperi
E. vandermerwei
E. barnardii
E. groenewaldii
E. rowlandii
E. enormis
E. fractiflexa
E. polyacantha
E. robecchii
E. caerulescens
E. ledienii
E. keithii
E. triangularis
E. tetragona
E. curvirama
E. limpopoana
E. perangusta
E. avasmontana
E. hottentota
E. decliviticola
E. graniticola
E. ammak
E. teke
E. nivulia
E. royleana
E. neriifolia
E. undulatifolia
E. caducifolia
E. nana
E. cattimandoo
E. opuntioides
E. vajravelui
E. lactea
E. mayuranathanii
E. antiquorum
E. susan-holmesiae
E. tortilis
E. drupifera
E. abyssinica
E. cussonioides
E. deightonii
E. candelabrum
E. ingens
E. decidua
E. fanshawei
E. confinalis
E. parviceps
E. tanaensis
E. rugosiflora
E. wakefieldii
E. vallaris
E. zoutpansbergensis
E. memoralis
E. waterbergensis
E. griseola
E. persistentifolia
E. knuthii
E. evansii
E. grandidens
E. sekukuniensis
E. stellata
E. micracantha
E. squarrosa
E. gymnocalycioides
E. lacei
E. epiphylloides
E. abdelkuri
E. similiramea
E. ballyana
E. triaculeata
E. xylacantha
E. immersa
E. awashensis
E. glochidiata
E. fluminis
E. saxorum
E. tenuispinosa
E. kalisana
E. actinoclada
E. turbiniformis
E. umbonata
E. prona
E. horwoodii
E. septentrionalis
E. samburuensis
E. eyassiana
E. subscandens
E. uhligiana
E. vittata
E. furcata
E. inarticulata
E. parciramulosa
E. makallensis
E. cryptospinosa
E. inaequispina
E. eilensis
E. piscidermis
E. atrox
E. clivicola
E. knobelii
E. nubigena
E. dissitispina
E. carteriana
E. debilispina
E. nyassae
E. lenewtonii
E. angustiflora
E. serendipita
E. gemmea
E. rubromarginata
E. baylissii
E. asthenacantha
E. lydenburgensis
E. schinzii
E. atroflora
E. heterochroma
E. heterospina
E. petraea
E. nigrispina
E. tescorum
E. borenensis
E. baiensis
E. phillipsioides
E. columnaris
E. mitriformis
E. venenifica
E. sudanica
E. sapinii
E. unispina
E. handiensis
E. officinarum
E. resinifera
E. seibanica
E. fruticosa
E. galgalana

Fig. 3.S2. Relatively dated MCC phylogeny of *Euphorbia* sect. *Euphorbia* from BEAST analysis of cpDNA data. Outgroup taxa are in grey, ingroup taxa in black. Annotations as in Fig. 3.S1.


A
To Fig. 3.S2B
Tree
Shrub
Dwarf Shrub
Geophyte
E. heterospina
E. petraea
E. inculta
E. elegantissima
E. heterochroma
E. nigrispina
E. tescorum
E. atroflora
E. vulcanorum
E. venenifica
E. sudanica
E. unispina
E. sapinii
E. waterbergensis
E. knuthii
E. triangularis
E. curvirama
E. ledienii
E. caerulescens
E. squarrosa
E. micracantha
E. stellata
E. keithii
E. hottentota
E. avasmontana
E. perangusta
E. limpopoana
E. vandermerwei
E. clavigera
E. enormis
E. umfoloziensis
E. groenewaldii
E. rowlandii
E. griseola
E. memoralis
E. persistentifolia
E. fanshawei
E. decidua
E. gymnocalycioides
E. resinifera
E. robecchii
E. complexa
E. longispina
E. phillipsioides
E. fractiflexa
E. seibanica
E. fruticosa
E. polyacantha
E. handiensis
E. galgalana
E. fascicaulis
E. cussonioides
E. mitriformis
E. columnaris
E. ammak
E. barnardii
E. baioensis
E. borenensis
E. multiclava
E. mosaica
E. horwoodii
E. dasyacantha
E. prona
E. turbiniformis
E. umbonata
E. atrox
E. classenii
E. piscidermis
E. makallensis
E. eilensis
E. viduiflora
E. candelabrum
E. abyssinica
E. deightonii
E. ingens
E. drupifera
E. desmondii
E. wakefieldii
E. undulatifolia
E. nivulia
E. neriifolia
E. nana
E. royleana
E. teke
E. caducifolia
E. cattimandoo
E. qarad
E. mayuranathanii
E. susan-holmesiae
E. vajravelui
E. abdelkuri
E. lacei
E. epiphylloides
E. tortilis
E. lactea
E. antiquorum
E. parviceps
E. confinalis
E. grandidens
E. evansii
E. tanaensis
E. decliviticola
E. strangulata
E. graniticola
E. rugosiflora
E. vallaris
E. zoutpansbergensis
E. tetragona
E. sekukuniensis
E. heteropoda
E. invenusta
E. neoreflexa
E. neoarborescens
E. neococcinea

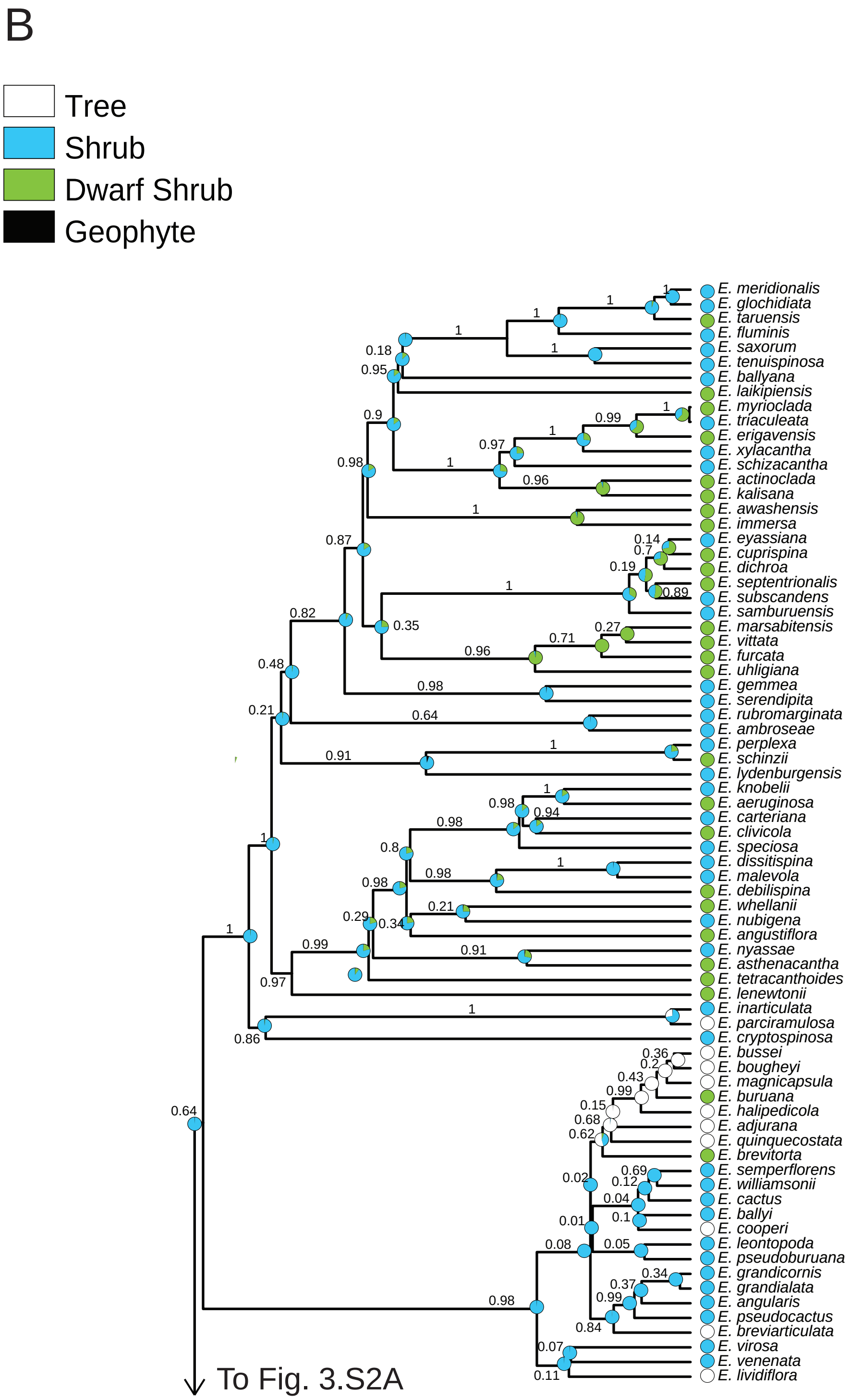
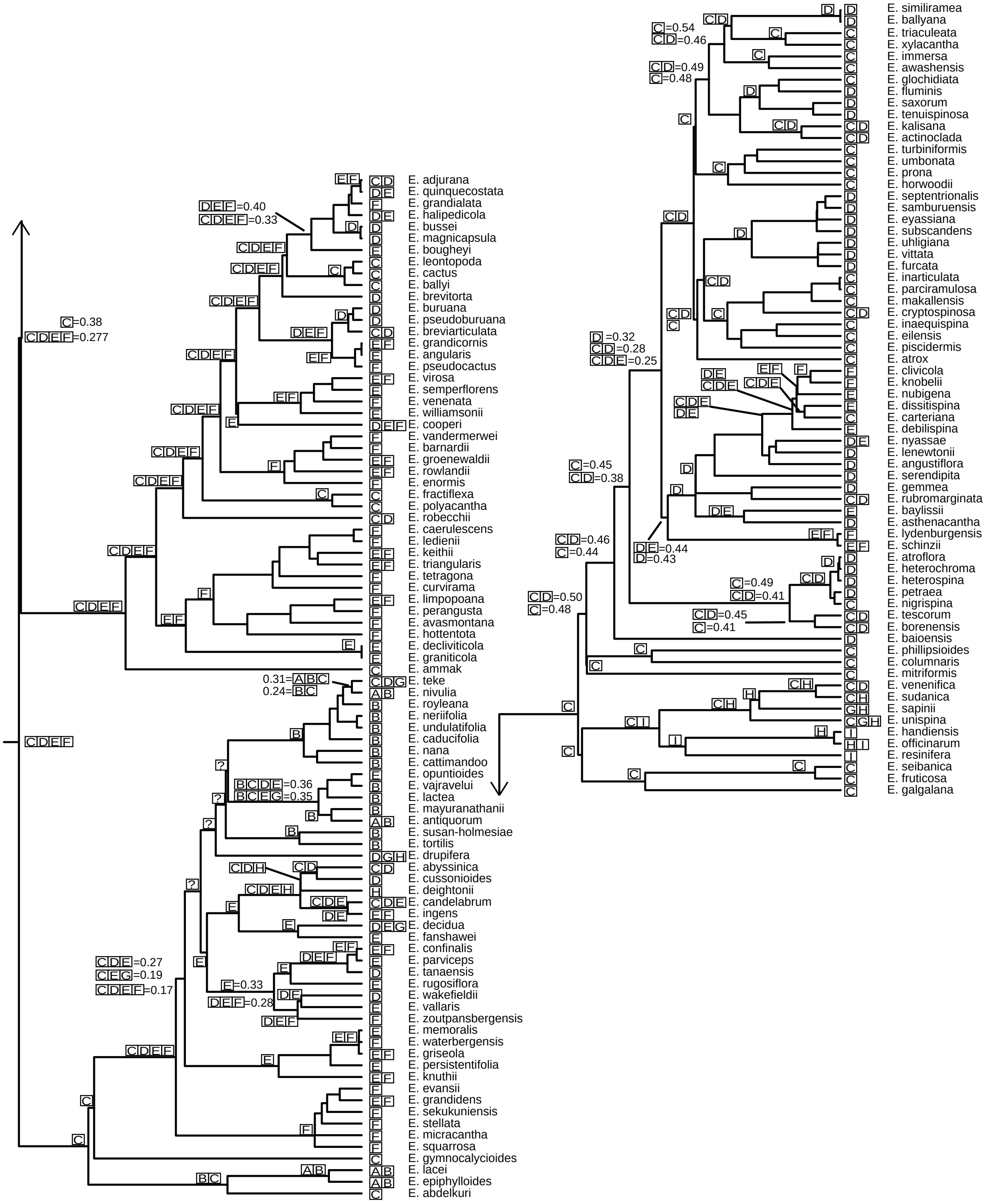
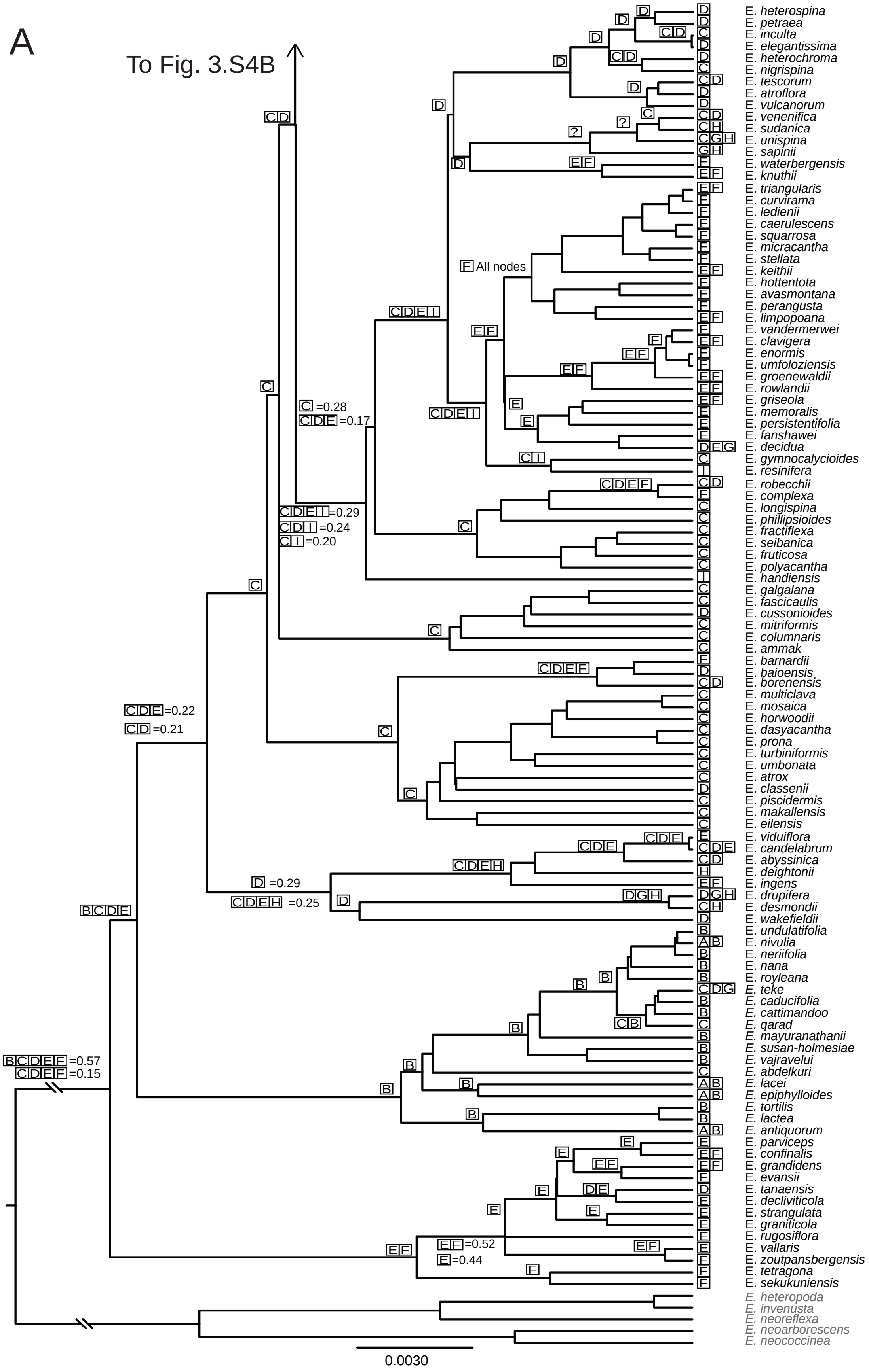
B
Tree
Shrub
Dwarf Shrub
Geophyte
E. meridionalis
E. glochidiata
E. taruensis
E. fluminis
E. saxorum
E. tenuispinosa
E. ballyana
E. laikipiensis
E. myrioclada
E. triaculeata
E. erigavensis
E. xylacantha
E. schizacantha
E. actinoclada
E. kalisana
E. awashensis
E. immersa
E. eyassiana
E. cuprispina
E. dichroa
E. septentrionalis
E. subscandens
E. samburuensis
E. marsabitensis
E. vittata
E. furcata
E. uhligiana
E. gemmea
E. serendipita
E. rubromarginata
E. ambroseae
E. perplexa
E. schinzii
E. lydenburgensis
E. knobelii
E. aeruginosa
E. carteriana
E. clivicola
E. speciosa
E. dissitispina
E. malevola
E. debilispina
E. whellanii
E. nubigena
E. angustiflora
E. nyassae
E. asthenacantha
E. tetracanthoides
E. lenewtonii
E. inarticulata
E. parciramulosa
E. cryptospinosa
E. bussei
E. bougheyi
E. magnicapsula
E. buruana
E. halipedicola
E. adjurana
E. quinquecostata
E. brevitorta
E. semperflorens
E. williamsonii
E. cactus
E. ballyi
E. cooperi
E. leontopoda
E. pseudoburuana
E. grandicornis
E. grandialata
E. angularis
E. pseudocactus
E. breviarticulata
E. virosa
E. venenata
E. lividiflora
To Fig. 3.S2A

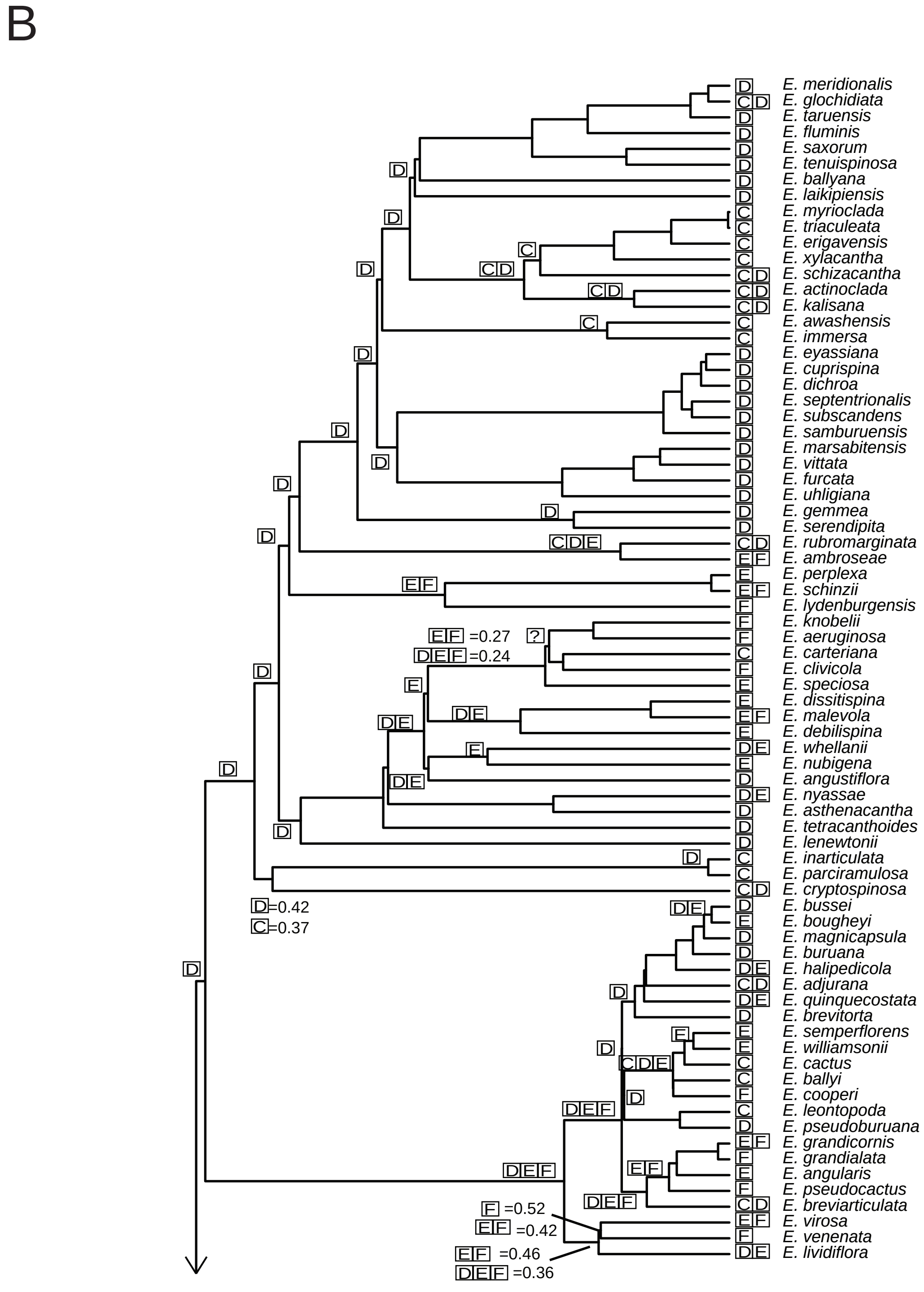
Fig. 3.S3. Ancestral area reconstructions of sect. *Euphorbia* based on the ITS phylogeny (excluding outgroup taxa). Lettered boxes as in Fig. 3.2. Alternative ranges are shown along with their relative probabilities (where space allows) at nodes with ambiguous reconstructions.



C=0.38
CDEF=0.277
CDEF
DEF=0.40
CDEF=0.33
0.31=ABC
0.24=BC
BCDE=0.36
BCEG=0.35
CDE=0.27
CEG=0.19
CDEF=0.17
E=0.33
DEF=0.28
CD E. adjurana
DE E. quinquecostata
F E. grandialata
DE E. halipedicola
D E. bussei
D E. magnicapsula
F E. bougheyi
C E. leontopoda
C E. cactus
C E. ballyi
D E. brevitorta
D E. buruana
D E. pseudoburuana
CD E. breviarticulata
EF E. grandicornis
E E. angularis
F E. pseudocactus
EF E. virosa
E E. semperflorens
F E. venenata
E E. williamsonii
DEF E. cooperi
F E. vandermerwei
F E. barnardii
EF E. groenewaldii
EF E. rowlandii
F E. enormis
C E. fractiflexa
C E. polyacantha
CD E. robecchii
F E. caerulescens
F E. ledienii
EF E. keithii
EF E. triangularis
F E. tetragona
F E. curvirama
EF E. limpopoana
F E. perangusta
F E. avasmontana
F E. hottentota
E E. decliviticola
E E. graniticola
C E. ammak
CDG E. teke
AB E. nivulia
B E. royleana
B E. neriifolia
B E. undulatifolia
B E. caducifolia
B E. nana
B E. cattimandoo
E E. opuntioides
B E. vajravelui
B E. lactea
B E. mayuranathanii
AB E. antiquorum
B E. susan-holmesiae
B E. tortilis
DGH E. drupifera
CD E. abyssinica
D E. cussonioides
H E. deightonii
CDE E. candelabrum
EF E. ingens
DEG E. decidua
E E. fanshawei
EF E. confinalis
E E. parviceps
D E. tanaensis
E E. rugosiflora
D E. wakefieldii
E E. vallaris
F E. zoutpansbergensis
E E. memoralis
F E. waterbergensis
EF E. griseola
E E. persistentifolia
EF E. knuthii
F E. evansii
EF E. grandidens
F E. sekukuniensis
F E. stellata
F E. micracantha
F E. squarrosa
C E. gymnocalycioides
AB E. lacei
AB E. epiphylloides
C E. abdelkuri
C=0.54
CD=0.46
CD=0.49
C=0.48
D=0.32
CD=0.28
CDE=0.25
C=0.45
CD=0.38
CD=0.46
C=0.44
DE=0.44
D=0.43
C=0.49
CD=0.41
CD=0.45
C=0.41
CD=0.50
C=0.48
D E. similiramea
D E. ballyana
C E. triaculeata
C E. xylacantha
C E. immersa
C E. awashensis
C E. glochidiata
D E. fluminis
D E. saxorum
D E. tenuispinosa
CD E. kalisana
CD E. actinoclada
C E. turbiniformis
C E. umbonata
C E. prona
C E. horwoodii
D E. septentrionalis
D E. samburuensis
D E. eyassiana
D E. subscandens
D E. uhligiana
D E. vittata
D E. furcata
C E. inarticulata
C E. parciramulosa
C E. makallensis
CD E. cryptospinosa
C E. inaequispina
C E. eilensis
C E. piscidermis
C E. atrox
F E. clivicola
F E. knobelii
E E. nubigena
E E. dissitispina
C E. carteriana
E E. debilispina
DE E. nyassae
D E. lenewtonii
D E. angustiflora
D E. serendipita
D E. gemmea
CD E. rubromarginata
E E. baylissii
D E. asthenacantha
F E. lydenburgensis
EF E. schinzii
D E. atroflora
D E. heterochroma
D E. heterospina
D E. petraea
C E. nigrispina
CD E. tescorum
CD E. borenensis
D E. baioensis
C E. phillipsioides
C E. columnaris
C E. mitriformis
CD E. venenifica
CH E. sudanica
GH E. sapinii
CGH E. unispina
I E. handiensis
HI E. officinarum
I E. resinifera
C E. seibanica
C E. fruticosa
C E. galgalana

Fig. 3.S4. Ancestral area reconstructions of sect. *Euphorbia* based on the cpDNA phylogeny. Lettered boxes as in Fig. 3.2. Alternative ranges are shown along with their relative probabilities (where space allows) at nodes with ambiguous reconstructions.


A
To Fig. 3.S4B
CD
C =0.28
CDE =0.17
CDEI =0.29
CDI =0.24
CI =0.20
CDE =0.22
CD =0.21
D =0.29
CDEH =0.25
BCDE
BCDEF =0.57
CDEF =0.15
EF =0.52
E =0.44
F All nodes
0.0030
E. heterospina
E. petraea
E. inculta
E. elegantissima
E. heterochroma
E. nigrispina
E. tescorum
E. atroflora
E. vulcanorum
E. venenifica
E. sudanica
E. unispina
E. sapinii
E. waterbergensis
E. knuthii
E. triangularis
E. curvirama
E. ledienii
E. caerulescens
E. squarrosa
E. micracantha
E. stellata
E. keithii
E. hottentota
E. avasmontana
E. perangusta
E. limpopoana
E. vandermerwei
E. clavigera
E. enormis
E. umfoloziensis
E. groenewaldii
E. rowlandii
E. griseola
E. memoralis
E. persistentifolia
E. fanshawei
E. decidua
E. gymnocalycioides
E. resinifera
E. robecchii
E. complexa
E. longispina
E. phillipsioides
E. fractiflexa
E. seibanica
E. fruticosa
E. polyacantha
E. handiensis
E. galgalana
E. fascicaulis
E. cussonioides
E. mitriformis
E. columnaris
E. ammak
E. barnardii
E. baioensis
E. borenensis
E. multiclava
E. mosaica
E. horwoodii
E. dasyacantha
E. prona
E. turbiniformis
E. umbonata
E. atrox
E. classenii
E. piscidermis
E. makallensis
E. eilensis
E. viduiflora
E. candelabrum
E. abyssinica
E. deightonii
E. ingens
E. drupifera
E. desmondii
E. wakefieldii
E. undulatifolia
E. nivulia
E. neriifolia
E. nana
E. royleana
E. teke
E. caducifolia
E. cattimandoo
E. qarad
E. mayuranathanii
E. susan-holmesiae
E. vajravelui
E. abdelkuri
E. lacei
E. epiphylloides
E. tortilis
E. lactea
E. antiquorum
E. parviceps
E. confinalis
E. grandidens
E. evansii
E. tanaensis
E. decliviticola
E. strangulata
E. graniticola
E. rugosiflora
E. vallaris
E. zoutpansbergensis
E. tetragona
E. sekukuniensis
E. heteropoda
E. invenusta
E. neoreflexa
E. neoarborescens
E. neococcinea


B
D E. meridionalis
C D E. glochidiata
D E. taruensis
D E. fluminis
D E. saxorum
D E. tenuispinosa
D E. ballyana
D E. laikipiensis
C E. myrioclada
C E. triaculeata
C E. erigavensis
C E. xylacantha
C D E. schizacantha
C D E. actinoclada
C D E. kalisana
C E. awashensis
C E. immersa
D E. eyassiana
D E. cuprispina
D E. dichroa
D E. septentrionalis
D E. subscandens
D E. samburuensis
D E. marsabitensis
D E. vittata
D E. furcata
D E. uhligiana
D E. gemmea
D E. serendipita
C D E. rubromarginata
E F E. ambroseae
E E. perplexa
E F E. schinzii
F E. lydenburgensis
F E. knobelii
F E. aeruginosa
C E. carteriana
F E. clivicola
E E. speciosa
E E. dissitispina
E F E. malevola
E E. debilispina
D E E. whellanii
E E. nubigena
D E. angustiflora
D E E. nyassae
D E. asthenacantha
D E. tetracanthoides
D E. lenewtonii
C E. inarticulata
C E. parciramulosa
C D E. cryptospinosa
D E. bussei
E E. bougheyi
D E. magnicapsula
D E. buruana
D E E. halipedicola
C D E. adjurana
D E E. quinquecostata
D E. brevitorta
E E. semperflorens
E E. williamsonii
C E. cactus
C E. ballyi
F E. cooperi
C E. leontopoda
D E. pseudoburuana
E F E. grandicornis
F E. grandialata
E E. angularis
F E. pseudocactus
C D E. breviarticulata
E F E. virosa
F E. venenata
D E E. lividiflora
E F =0.27
D E F =0.24
?
D=0.42
C=0.37
F =0.52
E F =0.42
E F =0.46
D E F =0.36

To Fig. 3.S4A

Table 3.1. Summary statistics of data sets used for phylogenetic inference of *Euphorbia* sect. *Euphorbia*. The cpDNA matrix consists of *ndh*F, *mat*K, *psb*B-H, and *trn*S-G-G regions combined and the concatenated matrix consist of the cpDNA plus ITS matrices.

	Matrix						
	*ndh*F	*mat*K	*psb*B-H	*trn*S-G-G	ITS	cpDNA	Concatenated
Number of accessions	85	164	127	108	161	189	195
Aligned sequence length	1685	2272	871	1758	646	6586	7232
Variable sites (%)	332 (20)	466 (21)	131 (15)	413 (23)	350 (54)	1342 (20)	1692 (23)
Parsimony-informative sites (%)	174 (10)	230 (10)	68 (8)	108 (6)	273 (42)	580 (9)	853 (12)

Table 3.2. Comparison of five DEC models of ancestral area reconstructions for sect. *Euphorbia* showing that the 59 range model was the best fitting model across topologies.

Model	**Ranges allowed**	**Max. Areas**	**-LnL**		
			Concatenated Matrix	**ITS**	**cpDNA**
502 range	unconstrained	9	456.581	411.239	460.413
246 range	unconstrained	4	457.492	411.526	459.858
81 range	contiguous + 1 disjunction	3	444.679	393.351	445.449
59 range	contiguous only	4	**432.038**	**387.349**	**438.332**
31 range	contiguous only	3	435.674	390.552	458.805

Table 3.3. Comparison of two models of growth form evolution in *Euphorbia* sect. *Euphorbia*. The OU model describes adaptive evolution in response to variation in climatic niche parameters and fits the data significantly better than the Brownian motion random drift model for those parameters associated with PC2. The two models were compared on each phylogeny inferred from the ITS, cpDNA, and concatenated data sets.

Model	**-LnL**			**AIC**		
PC1	**Concatenated**	**ITS**	**cpDNA**	**Concatenated matrix**	**ITS**	**cpDNA**
Brownian	169.13	168.46	165.60	342.25	340.93	335.21
OU	168.14	155.08	163.18	346.29	320.16	336.36
P-value	0.42	6.61E-06	0.18			
PC2						
Brownian	164.90	152.85	166.93	342.25	309.70	337.86
OU*	151.98	128.67	149.32	313.97	267.33	308.64
P-value	1.04E-05	1.78E-10	1.09E-07			

* = model significantly better fit to data.

Table 3.4. Parameter estimates and confidence intervals (CI) for the OU model of adaptive growth form evolution in response to climatic niche. Alpha measures the strength of selection, sigma^2 measures random variation (drift), and theta is the optimal PC score for each of three growth forms. Note that OU model for PC2 was significantly better than the null drift model (Table 3).

	PC1		**PC2**	
Full matrix	**estimate**	**CI**	**estimate**	**CI**
alpha	0.389	(0.19, 2.38)	3.060	(1.86, 9.00)
sigma^2	9.385	(6.84, 20.67)	17.347	(10.26, 48.06)
theta (trees)	-1.350	(-6.16, 3.30)	1.089	(0.28, 1.85)
theta (shrubs)	6.530	(-3.32, 15.06)	-1.274	(-2,14, -0.40)
theta (dwarfs)	10.196	(-2.73, 23.32)	1.232	(-0.13, 2.70)
ITS				
alpha	3.8163	(2.38, 9.46)	13.1791	(6.67, 37.71)
sigma^2	58.0855	(34.80, 108.53)	72.8813	(32.91 , 179.82)
theta (trees)	-1.1282	(-2.83, 0.44)	1.1140	(0.33, 1.85)
theta (shrubs)	0.7318	(-0.62, 1.97)	-1.4262	(-2.13, -0.75)
theta (dwarfs)	0.0913	(-2.58, 2.48)	0.3327	(-0.76, 1.37)
cpDNA				
alpha	1.234	(0.67, 3.78)	6.290	(3.58, 21.01)
sigma^2	18.184	(12.44, 33.91)	36.603	(19.41, 120.24)
theta (trees)	-1.424	(-3.88, 1.11)	1.222	(0.32, 2.04)
theta (shrubs)	1.767	(-1.69, 5.13)	-1.143	(-1.77, -0.53)
theta (dwarfs)	2.164	(-2.76, 7.98)	0.428	(-0.76, 1.56)

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Chapter IV

The evolutionary loss of leaves and its adaptive significance in *Euphorbia* subg. *Euphorbia*

INTRODUCTION

While all plants possess adaptive features to deal with water stress, in the most arid environments, major morphological and anatomical specializations have evolved. One putative adaptation to limited water is a reduction in leaf size since leaves are the primary source of water loss. Arid environments tend to not be light limited so smaller, thicker leaves limit water loss while still maintaining adequate surface area to capture light. In a few groups of plants, this is taken to the extreme by the extreme reduction of leaves such that they become basically non-functional, and utilization of the stem as the main photosynthetic organ. This has the advantage of decreasing the surface area:volume ratio of the photosynthetic organ, which aids in water conservation (Mauseth, 2000). Leaves are short-lived organs with high surface area:volume that allows excessive water loss when stomata are open. By transferring the photosynthetic role to long-lived stems, less of the hydrated tissue is exposed to the atmosphere resulting in less water loss per unit of time when stomata are open. The association of plants with rudimentary leaves and photosynthetic stems with arid habitats seems a self-evident case of adaptive evolution. However, adaptive explanations require explicit testing (Gould & Lewontin, 1979; Ackerly, 2004) and this hypothesis has not been tested in a phylogenetic context

Two prime examples of the evolutionary transition between an ancestral leafy plant and a specialized, leafless plant with stem-based photosynthesis are cacti of the New World and euphorbs of the Old World. This transfer of photosynthesis from the leaves to the stems has been investigated mainly in the Cactaceae (Mauseth & Sajeva, 1992; Mauseth, 1993; Mauseth, 1995; Edwards & Donoghue, 2006). While it is an impressive example of a xerophytic radiation, the Cactaceae has a single instance of this evolutionary transition and is therefore inappropriate for statistical tests of correlation between a shift in growth form and variation in environmental factors (Felsenstein, 1985; Edwards & al., 2005). In contrast, the genus *Euphorbia* is an ideal group in which to test for key variables that may have contributed to the origin and maintenance of the leafless growth form because such forms have evolved at least 14 times within this monophyletic group (Steinmann & Porter, 2002; Bruyns & al., 2006; Bruyns & al., 2011; Horn & al., 2012; Dorsey & al., in press). In fact, within *Euphorbia* subg. *Euphorbia*, leafless, semi-succulent species known as 'pencil-stem' plants have evolved independently at least seven times (Fig. 4.1; Dorsey & al., in press).

Euphorbia is a cosmopolitan genus of over 2000 species. It contains a very impressive array of growth forms from herbs and geophytes to trees and shrubs, including many xerophytic forms. The genus is defined morphologically by the cyathium, a highly reduced inflorescence that resembles a single flower. The species of *Euphorbia* are mostly distributed throughout the tropics although one subgenus (subg. *Esula*) is almost entirely north temperate. Horn & al. (2012) established the phylogenetic relationships of the four subgenera and reconstructed the evolution of a number of morphological traits including growth form. They showed that the ancestor of *Euphorbia* was most likely a leafy shrub or tree with terminal inflorescences and that specialized, xerophytic forms have evolved independently in each subgenus (Horn & al., 2012).

Subgenus *Euphorbia* is the largest and most morphologically diverse of the four subgenera within *Euphorbia* (Horn & al., 2012; Dorsey & al., in press). It contains a New World clade and two Old World clades, as well as a smaller Pacific clade distributed across Oceania (Dorsey & al., in press). The species of the New World are distributed from the southern USA through Central and South America to northern Chile (Fig. 4.3). The Old World species range from Papua New Guinea through South Asia and across Africa and Madagascar. The wide array of derived growth forms found in *Euphorbia* is present in each of the major clades of subg. *Euphorbia*, including cactiform succulents in sect. *Euphorbia*, herbs in several New World lineages, and geophytes scattered across the Old World clades. However, the most striking pattern of growth form evolution in the subgenus is among the 'pencil-stem' species. These trees and shrubs with rudimentary leaves and semi-succulent, photosynthetic stems have evolved at least seven times and are present in all four major clades in subg. *Euphorbia* (Fig.4.2). In the Old World, the pencil-stem species are found mainly in Madagascar and come from each of the two Old World clades (Figs. 4.2—4.3). This convergent pattern of evolution strongly suggests a common selective regime for these leafless groups and provides a natural experiment in which we can test the significance of an association between environmental variables and the evolution of this specialized form.

While species of *Euphorbia* with stem-based photosynthesis occur in generally dry habitats, closely related leafy shrubs often occur in the same regions and this pattern introduces an interesting complication into the scenario that the pencil-stem habit has been selected by very low water availability (Fig 4.3; Haevermans & Labat, 2004; Cacho & al., 2010; Dorsey & al., in press). Testing the hypothesis that the pencil-stem habit is an adaptation to low water availability requires precisely defined patterns of water availability and temperature encountered by species both with and without leaves, while accounting for the overlap in geographic distribution of these two growth form within subg. *Euphorbia*. This would provide a more complete understanding of which specific parameters of water availability and temperature are associated with the evolution of the leafless form. Utilizing the pattern of evolution of pencil-stem plants in subg. *Euphorbia* and estimates of the climatic parameters of species' niches, we tested the hypothesis that the evolutionary loss of leaves and switch to stem-based photosynthesis is an adaptation to specific niche parameters related to water availability.

MATERIALS AND METHODS

Taxonomic Sampling and Phylogenetic Analysis. — A reduced-taxon matrix of ITS, *mat*K, and *ndh*F sequences from Dorsey & al. (in press) that included only the pencil-stem species and their close relatives was used to obtain a phylogenetic framework for this study. A posterior distribution of trees and relative divergence times for this taxon set were obtained using BEAST (v1.7.2, Drummond & Rambaut, 2007) under an uncorrelated relaxed clock model assuming a log-normal prior on branch lengths (Drummond & al., 2006) and the GTR+gamma model of nucleotide evolution. Separate models of evolution were applied to each partition (defined by gene region), and two runs of $5x10^7$ generations each were performed. To determine an appropriate number of generations to discard as burn-in and to assess convergence of runs, posterior distributions of pertinent model parameters were compared using Tracer (Rambaut & Drummond, 2007).

Climatic Niche Analyses. — To estimate the climatic niche of each species, collection locality data (latitude and longitude) were compiled for multiple individuals of every species in our phylogenetic data set. These data were obtained from the Tolkin database of *Euphorbia* phylogenetic and taxonomic information (http://app.tolkin.org/projects/72, Riina & Berry, 2012) or from the Global Biodiversity Information Facility (data.gbif.org, accessed 2012-04-28 through 2012-05-09). The program DIVA-GIS (Hijmans & al., 2001) was used to extract values from the “Bioclim” data set for each locality. This data set is a collection of 19 biologically relevant variables derived from monthly precipitation and temperature data (at 30 arc-second resolution [= ~1km2 at the equator], Hijmans & al., 2005). Principal Components Analysis (PCA) implemented in R (R Core Team, 2012) was performed on the values of these 19 variables to account for covariation among parameters and to summarize the climatic niche of each species. The loadings of the original bioclimatic variables on each PCA axis were examined to identify those variables that were most strongly associated with the differences in PCA scores among species.

To test whether the evolutionary loss of leaves and a switch to stems as the primary photosynthetic organ was associated with differences in climatic niche in subg. *Euphorbia*, the phylogenetic logistic regression method of Ives & Garland (2010) was implemented by their Matlab® (Mathworks, Mathworks, 2011) code “PLogReg.m”. This program estimates regression coefficients between a binary dependent variable and one or more independent variables while accounting for the potential of non-independence of tip values among related species. It also provides an estimate of the strength of phylogenetic signal in the dependent variable either as the only output in the univariate case (no independent variable) or as part of the output from the multivariate case. For the multivariate analysis, the mean PCA scores for each species were used as independent variables and the presence/absence of functional leaves on each species were used as the dependent variable. The maximum clade credibility (MCC) tree from the posterior distribution of trees obtained from the BEAST analysis was used to calculate the variance-covariance matrix of the dependent variable among species, and the parametric bootstrapping function within PLogReg was used to calculate confidence intervals (CI) for estimates and p-values (Ives & Garland, 2010).

The phylogenetic ANOVA method of Garland & al. (1993) as implemented in the R package “geiger” (Harmon & al., 2009; R Core Team, 2012) was used as a second test of the correlation between climatic niche and the evolution of leafless photosynthetic stems, which incorporated phylogenetic uncertainty. This analysis performs a traditional ANOVA but derives a “phylogenetic” p-value for the F statistic based on a null distribution calculated from simulations of Brownian motion evolution of the dependent variable on a phylogenetic tree. Because ANOVA tests for significant difference between groups, the mean PCA scores of species as dependent variables and the presence/absence of functional leaves as the independent variable were used. To incorporate phylogenetic uncertainly, ANOVAs were performed using 100 randomly sampled trees form the combined posterior distribution of trees of the two BEAST runs and then the range and mean of phylogenetic p-values were calculated across all trees.

Loadings from the PCA analysis indicate original bioclimatic variables that are correlated with the principal components but do not provide tests of significance for this correlation. To assess this significance, phylogenetic ANOVAs were performed for each original variable with a loading score of 0.2 or greater on the axis that was significantly correlated to the dependent variable (see results). As above, these tests were performed on each of the 100 randomly sampled BEAST trees and the mean and range of p-values, as well as the percent of trees with $p < 0.05$ for each variable were calculated.

RESULTS

Phylogenetic framework — The two BEAST runs each reached stationarity by $5x10^6$ generations and all sampled trees prior to this were discarded as burn-in. Examination of the posterior distributions of parameters in Tracer indicated that the two runs converged so the trees from each rum were combined and used to calculate the MCC tree (Fig. 4.2). Posterior probabilities for most major clades in the MCC tree were greater than 0.9, and the topology is generally in accord with our current knowledge of the relationships within subg. *Euphorbia* (Dorsey & al., in press). The two Old World clades of Dorsey & al. are recovered with posterior probabilities (PP) of 1.0, as is the Pacific clade. *Euphorbia mandravioky*, representing sect. *Pachysanthae*, is placed outside the other Old World clades. The placement of the root in the MCC tree results in the New World species forming a grade rather than a clade but these species are still separated from the Old World species by strong PP (1.0).

Estimation of climatic tolerances — The climatic dataset used in this study represents 48 species within *Euphorbia* subg. *Euphorbia* with a total of 1134 collection localities (mean of 23.6 points per species; Fig. 4.3). The first two axes of the PCA of the bioclimatic variables explained over 72% of the variation among data points and the two groups (leafy vs. pencil-stem) occupy distinct areas of niche space, although there is some overlap between them (Fig. 4.4). Mean PCA scores for each species are given in Table 4.1 and the loadings on each principal component (PC) for each original variable are given in Table 4.2. According to these loadings, PC1 is associated with most measures of precipitation patterns as well as temperature seasonality, while PC2 is associated with most other measures of temperature.

The univariate phylogenetic logistic regression analysis indicates that there is significant phylogenetic signal in the distribution of the presence/absence of leaves in subg. *Euphorbia* (Table 4.3). Scores from the first two principal components were used as independent variables in the multivariate analysis, and the results indicate that the presence/absence of leaves is significantly correlated with PC1 but not PC2 (Table 4.3). The phylogenetic ANOVAs for PC1 and PC2 also showed a significant correlation between PC1 and the presence/absence of leaves using both the F distribution and the null distribution calculated from phylogenetic simulations (Table 4.4). The phylogenetic p-values for PC1 were <<0.05 whereas those for PC2 were >>0.05 across all trees (Table 4.4).

Of the 14 bioclimatic variables with loadings of 0.2 or higher on PC1, nine were significantly correlated with the presence/absence of leaves across all phylogenetic reconstructions, according to the individual ANOVAs (Tables 4.5—4.6). A small subset of topologies from the distribution (1—2 trees) produced significant relationships for two parameters, mean diurnal temperature range and isothermality, which were otherwise not correlated to the presence/absence of functional leaves. However, the mean p-values, across topologies, of each of these parameters were above 0.05 and the maximum value was well above this cutoff (Table 4.5).

DISCUSSION

This is the first study to investigate the adaptive significance of the evolution of stems as the main photosynthetic organ that explicitly tests for the environmental correlates of this significant switch in ecological strategy. Within subg. *Euphorbia* there is a pattern of repeated evolution of species with leafless, photosynthetic stems (Dorsey & al., in press) that suggests that this growth form is an adaptation to a common selective filter. The results of this study demonstrate that, in general, this is the case and the ‘pencil-stem’ species of subg. *Euphorbia* occupy a climatic niche that is different from their leafy relatives. This is despite the fact that species of the two alternative growth forms can have overlapping geographic distributions (Fig. 4.3). The significant difference between the climatic niche of pencil-stem species versus that of the leafy species across subg. *Euphorbia* complements the differences in niche parameters found by Dorsey & Berry (in prep) between growth forms in a large clade of cactiform succulents (*Euphorbia* sect. *Euphorbia*). In that study, the evolution of shrubs from tree ancestors in the history of sect. *Euphorbia* was shown to have been selected for by severity of seasonal drought. The great diversity of forms throughout *Euphorbia* is undoubtedly due to multiple factors acting throughout its history, but the significant correlation between small changes in niche parameters and a substantial change in morphology suggests that this could be an important determinant of patterns of diversification observed in the genus as a whole (Horn & al., 2012).

The pencil-stem growth form is not the only xerophytic or functionally leafless growth form in subg. *Euphorbia*, but it has evolved at least seven times independently, more times than any other growth form in the subgenus (Dorsey & al., in press). Pencil-stem species of subg. *Euphorbia* occur in both the Old World and the New World but are most diverse in Madagascar (Figs. 4.2--4.3, Table 4.1; Dorsey & al., in press). There are two main clades of pencil-stem species on Madagascar, sect. *Deuterocalli* with three species and sect. *Tirucalli*, with 25 species, along with a single pencil-stem species in sect. *Pervilleanae*. In the New World, the pencil-stem habit has evolved in at least three separate lineages, though these are not as speciose as the Madagascan clades (Figs. 4.2—4.3). In Australia there is a single pencil-stem species from subg. *Euphorbia*, which is well nested within a clade of leafy species that is distributed across Oceania (Figs. 4.2—4.3). This widespread pattern of convergence is also found across *Euphorbia*, as the pencil-stem habit has evolved in each of the other three subgenera, multiple times in subg. *Chamaesyce* and subg. *Rhizanthium* (Horn & al., 2012; Yang & al., 2012). This pattern of evolutionary lability suggests that the evolution of this form is relatively "easy" in *Euphorbia*.

Our estimates of climatic tolerances of leafy and pencil-stem species indicate that there is some overlap in climatic niche space among the two groups, but the pencil-stem species occupy a distinct and more narrowly defined region of that space (Fig. 4.4). The distinct climatic niche of the pencil-stem species is confirmed by both the logistic regression analysis and ANOVA for differences among these growth forms (Tables 4.3—4.4). The fact that we can detect a difference in the average niche of species with different growth forms is impressive, given the generally coarse resolution of our climatic data, relative to the habitat of individual plants, and the fact that many of these species are have overlapping distributions, especially in Madagascar. Ranges of pencil-stem and leafy species do not overlap as broadly in the New World or Pacific clades, so it is possible that the significant difference in niche detected in our analyses is mostly due to the climatic tolerances of leafy species from these regions. To test this, separate phylogenetic ANOVAs were performed on data sets including only the New World and Pacific species or the Old World species. The difference in climatic niche is still evident in these separate data sets (Table 4.7), indicating that despite the greater spatial overlap among Madagascan species, the pencil-stem species do indeed occupy a distinct climatic niche from leafy relatives.

The individual climatic parameters that correlate with PC1 and thus define the differences among leafy and leafless species are mostly measures of precipitation (Tables 4.5—4.6). Precipitation at sites occupied by leafless species of subg. *Euphorbia* is roughly half that of sites occupied by leafy species regardless of the season or temporal scale of the measurements (Table 4.6). These results support the hypothesis that the switch to stem-based photosynthesis is an adaptation that conserves water in arid habitats, presumably by reducing the surface area:volume ration of photosynthetic tissues (Mauseth, 2000). Interestingly, there is no signal in our data for pronounced seasonal drought as a major factor associated with the loss of leaves, as was found for the evolution of different growth forms in the spine-shield euphorbias of subg. *Euphorbia* (Dorsey & Berry, in prep). Nor do higher temperatures correlate with either growth form, but the variation in temperature, as measured by the seasonality index and the annual temperature range, is strongly correlated with PC1 (Tables 4.5—4.6). The precipitation parameters associated with the significant difference in climatic niche between leafy and leafless species in subg. *Euphorbia*, regardless of the phylogenetic sampling (Table 4.6), is strong evidence that the convergence on the pencil-stem habit by species across the subgenus is due to the selective filter of water conservation.

Given the fact that there are significant differences in climatic tolerances among leafy and pencil-stem species, how can we explain the overlap in the distributions of these groups on Madagascar? One explanation is that the individuals of different growth forms whose distributions overlap are partitioning the landscape based on water availability, but that this parameter is not determined solely by precipitation. It can be assumed that site factors such as soil type, slope, and vegetation cover will each have an effect on available water at a given site and these can change at relatively small spatial scales. An important limitation to this study is the fact that the data used for the estimation of climatic niche was based only on average precipitation and temperature measurements and not actual water availability for individual plants. Whereas the parameters we measured reflect general differences in the mean climatic tolerances of leafy vs. leafless species, it may be that the other factors mentioned above that can influence water availability play a larger role at the level of the realized niche of co-occurring individuals. Also, other functional traits not measured in this study, especially rooting depth, could play a significant role in allowing individuals of different growth forms to coexist despite differences in average climatic tolerances.

While there is a strong signal of convergent evolution among the pencil-stem species, the distribution of growth forms along the PC1 axis and the distribution of scores from this axis across the phylogeny suggest several more subtle patterns of niche differentiation. For example, when the 48 species in this study are ranked in ascending order according to their mean PC1 score, the species of sect. *Tirucalli* (all pencil-stem species) are distributed from the second to the 28th position (Table 4.1). Eleven leafy species from across the phylogeny of subg. *Euphorbia* that are interspersed among these species of sect. *Tirucalli*. Among these are three species of sect. *Tirucalli's* sister clade, sect. *Pervilleanae*, which includes one pencil-stem species among its mostly leafy shrubs and trees. The four species of sect. *Pervilleanae* included in this study occupy an intermediate range of the PC1 axis, from 12th to 35th (Table 4.1). Three of these have PC1 scores that are lower than those for five of the nine sect. *Tirucalli* species. Included in these three is the pencil stem species, *E. intisy*, but surprisingly it does not have the lowest PC1 score in the section. The amount of variation in PC1 scores within sects. *Tirucalli* and *Pervilleanae* and the overlap between them suggest that within each clade speciation has been associated with differentiation in precipitation tolerances. If this is the case it would help explain the coexistence of leafy and pencil-stem species on Madagascar. The original selective filter that favored the pencil-stem habit over leafy forms was likely lower water availability, as shown by our logistic regression and ANOVA results. However, the actual position of individual species on the gradient of water availability (PC1) suggests that, subsequent to the origination of this habit, species have diversified in their climatic niches, and a clear distinction between the two growth forms does not always hold at the species level.

Deviation from the general pattern of distinct niches found between pencil-stem and leafy species also occurs among species of the New World. As a group, the New World species of subg. *Euphorbia* exhibit perhaps the widest range in habitats in the subgenus, and this pattern is repeated within individual New World clades (Dorsey & al., in press). Four species of leafy shrubs and trees, from multiple New World sections, have some of the lowest PC1 scores among all species studied (Table 4.1). Three of these species, *E. lactiflua, E. calyculata,* and *E. tanquahuete*, are drought-deciduous trees or shrubs from lineages of only one or two species. *E. lactiflua* is endemic to the northern Atacama Desert, and *E. calyculata* and *E. tanquahuete* are native to dry sub-tropical forests of Mexico. It is clear from the positions of these species on the PC1 axis and their native ranges that multiple strategies for survival in water-limited habitats have evolved in the New World clades of subg. *Euphorbia*. However, these drought-deciduous species represent species-poor lineages, whereas each pencil-stem species in the New World is nested within a larger clade of leafy trees or shrubs. In each instance of the evolution of the pencil-stem habit in the New World clades, there is an associated shift to a lower PC1 value, again indicating that while it is not the only water conserving strategy, it does represent an adaptation to drier habitats.

Edwards et al. (2006), found that species of *Pereskia*, basal members of the Cactaceae, had very conservative water use strategies, comparable to their succulent relatives, even though they are leafy trees with woody stems. They also reconstructed ancestral states of water use traits and climatic parameters for two nodes in their *Pereskia* phylogeny and found that traits associated with the cactus life form and ecophysiology, including conservative stomatal behavior, high water use efficiency, and low maximum transpiration rates, were present in the ancestor of the entire family and did not originate in the highly succulent core cacti. Moreover, their reconstructed habitats for the ancestor of the two *Pereskia* clades indicate that they occupied a similar semi-arid niche as extant species. Given the variation in climatic parameters among leafy species of subg. *Euphorbia*, it is possible that a similar pattern may hold within this subgenus. The range and mean of annual precipitation among leafy species of subg. *Euphorbia* from this study are comparable to those of *Pereskia* species (Table 4.6; Edwards & Donoghue, 2006). Indeed, most species of subg. *Euphorbia*, especially in the Old World, live in semi-arid habitats whether they are leafy or not. Whether the leafy species of subg. *Euphorbia* have encroached on the climatic tolerances of pencil-stem species or vice versa we cannot say at this point. Next steps in the investigation of the adaptive significance of leafless plants in this group will focus on ancestral niche reconstructions and the tempo of niche evolution. This will allow us to better characterize the history of niche differentiation among growth forms within this group.

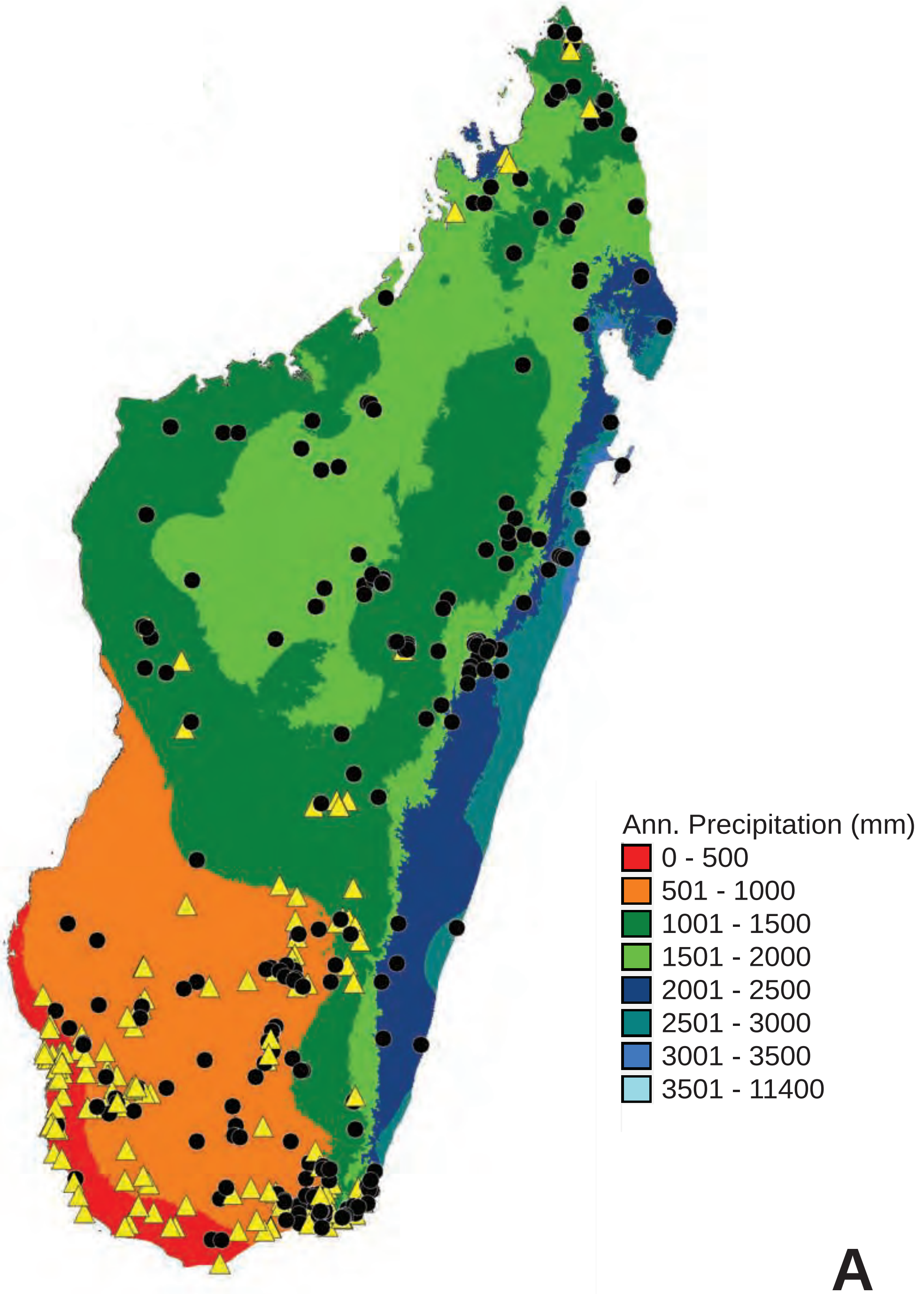
CONCLUSIONS

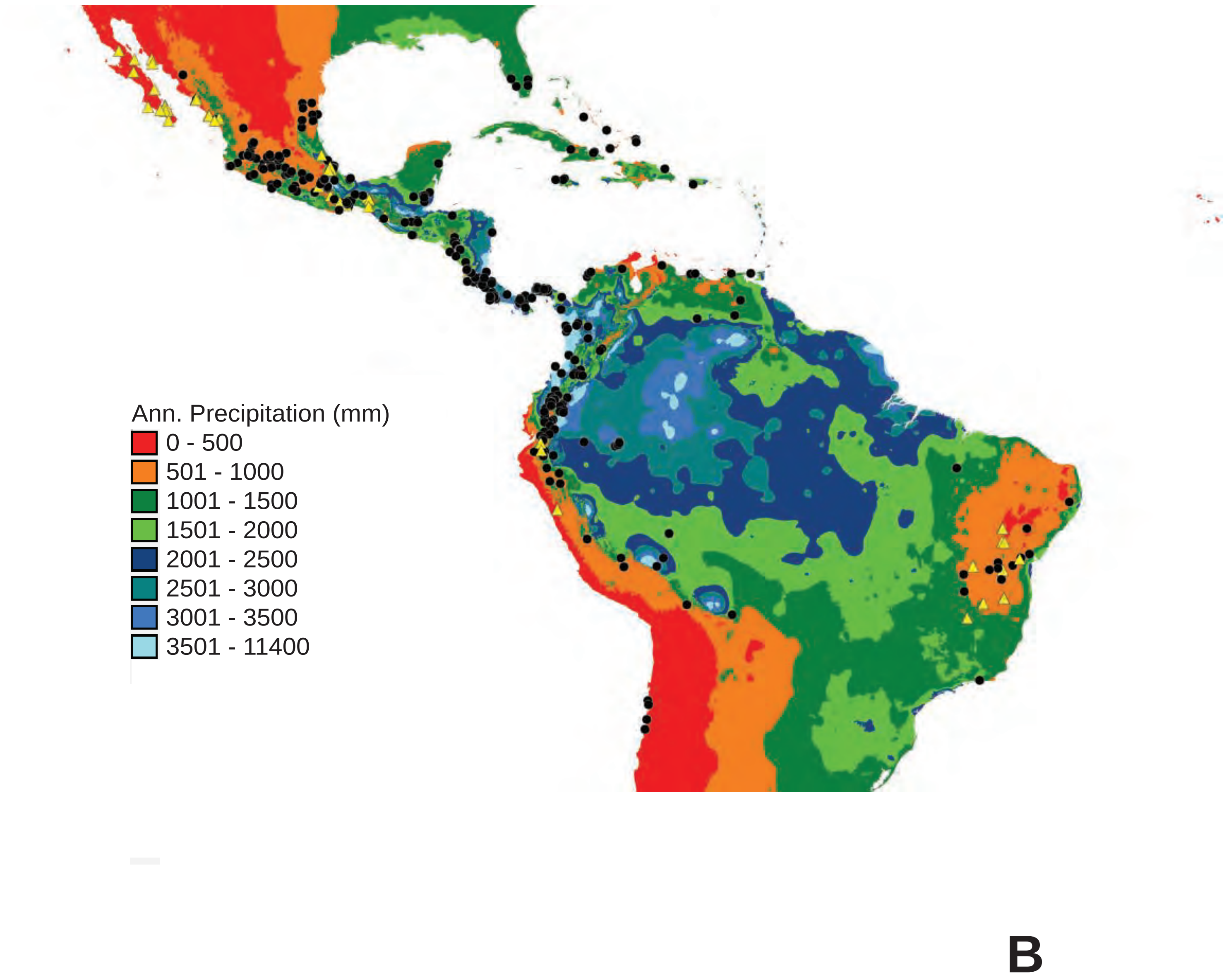
Species in *Euphorbia* subg. *Euphorbia* have converged on the pencil-stem habit at least seven times, so the subgenus represents a prime model for examining the role of adaptation in morphological evolution. This study has shown that selection by habitats with low precipitation has favored this leafless growth form across the subgenus. However, the distribution of precipitation tolerances across the phylogeny of subg. *Euphorbia* suggests that this general trend may break down at the level of individual species where divergence of climatic niches has likely occurred among closely related species (i.e., within sections). This niche differentiation is one explanation for the co-occurrence of leafy and leafless species on Madagascar. Further work to test this hypothesis should focus on the timing of niche evolution within the subgenus to see whether bursts of niche evolution can be detected within individual sections, or whether this diversification has proceeded at a steady rate. Data for other morphological and ecophysiological traits that could also influence water uptake and use, such as rooting depth, water use efficiency, and minimum water potential, could elucidate other axes of functional variation between species of subg. *Euphorbia* and improve our understanding of both the history of niche differentiation and their current distributions.

Figure 4.1. Examples of pencil-stem and leafy species in subg. *Euphorbia*. A) *E. tirucalli* (sect. *Tirucalli*), B) *E. decorsei* (sect. *Tirucalli*), C) *E. rauhii* (sect. *Pervilleanae*), D) *E.* sp. (sect. *Denisophorbia*)

Figure 4.2. Maximum clade credibility tree of *Euphorbia* subg. *Euphorbia* from the posterior distribution of trees inferred from a reduced-taxon data set of ITS, *mat*K, and *ndh*F sequences from Dorsey et al. (in press). Green braches lead to pencil-stem species. The general distribution of major clades is given to the right of the taxa. Numbers at branches are posterior probabilities >=0.9.

Fig. 4.3. Three main centers of diversity for species of subg. *Euphorbia* included in this study. A) Madagascar, B) New World, C) Pacific Region (Hawaii not shown). Localities of individuals used to extract climate data are shown by symbols and the annual precipitation is shown by colors. Yellow triangles = pencil-stem species, Black circles = leafy shrubs and trees. See insert for colors.




Ann. Precipitation (mm)
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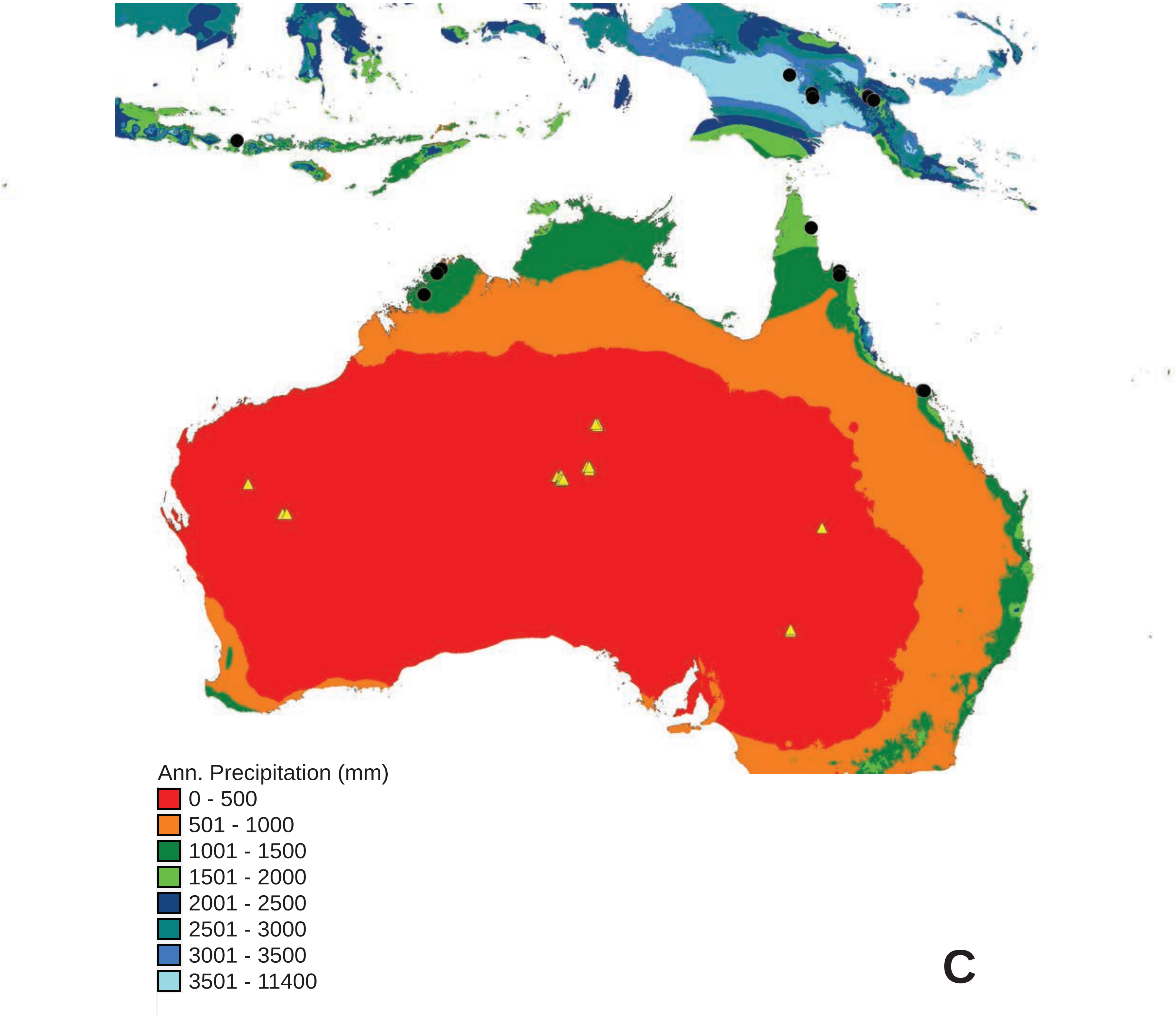
🟦 3501 - 11400
B


Ann. Precipitation (mm)
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🟦 3501 - 11400
C

Fig. 4.4. Position of 1134 individuals from 48 species in subg. *Euphorbia* on the first two principal components of variation among 19 bioclimatic variables. Black diamonds = pencil-stem species, Grey diamonds = leafy shrub/tree species. Lines connect individual points to the centroid of their respective growth form. PC1 is significantly correlated with growth form and with measures of precipitation.

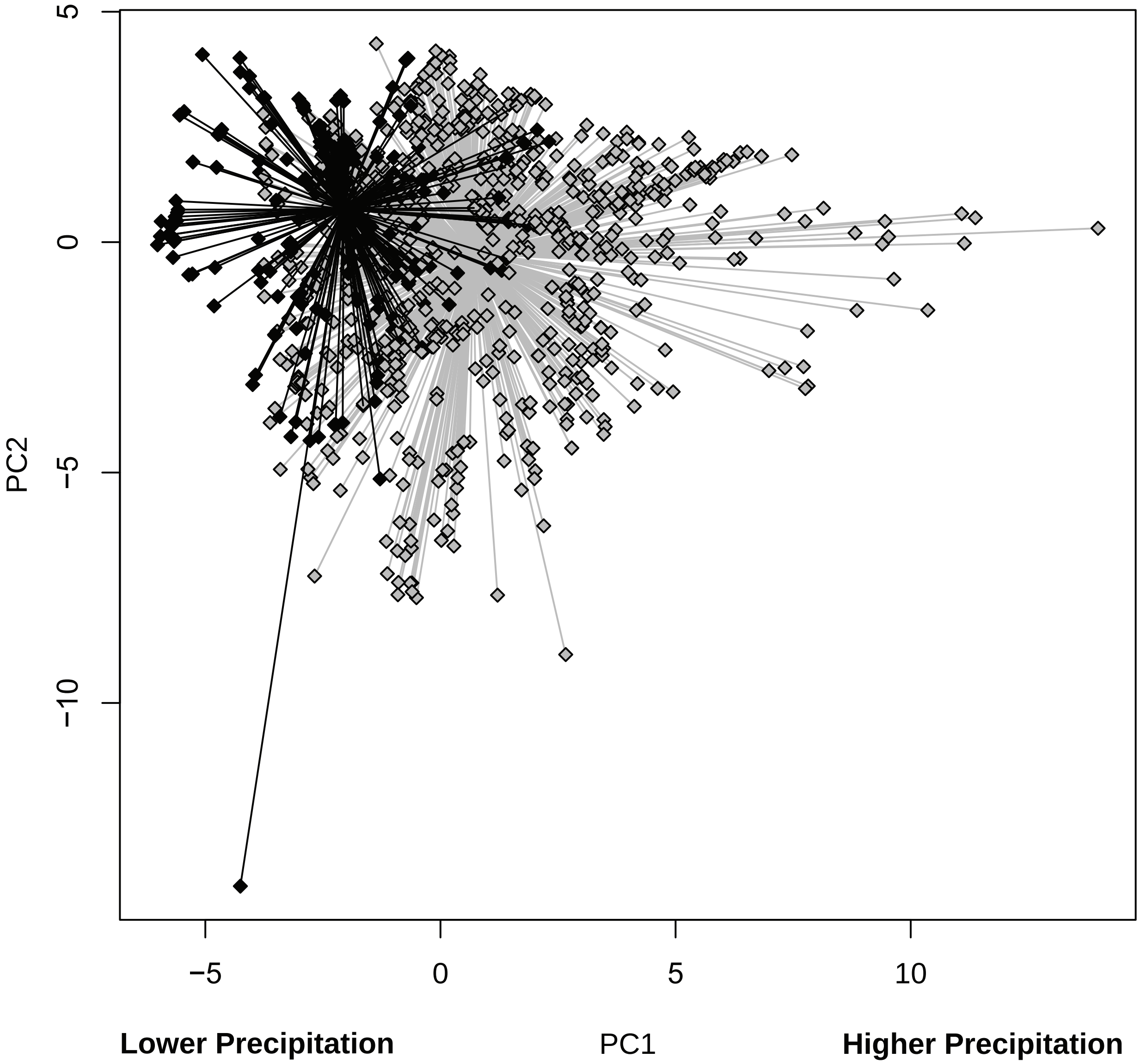

Table 4.1. Sectional classification, general distribution, presence/absence of functional leaves, and mean PCA scores of 48 species from subg. *Euphorbia* used in this study. Species are ranked by mean score of PC1.

Species	Section	Distribution[a]	Functional leaves	Mean Principal Component score	
			presence (1) absence (0)	Axis 1	Axis 2
E. sarcostemmoides	*Pacificae*	P	0	-5.396	1.098
E. gummifera	*Tirucalli*	OW	0	-3.777	-2.497
E. damarana	*Tirucalli*	OW	0	-3.392	-0.730
E. lomelii	*Crepidaria*	NW	0	-3.275	2.253
E. bracteata	*Crepidaria*	NW	1	-3.109	1.367
E. lactiflua	*Lactifluae*	NW	1	-2.803	-1.601
E. calyculata	*Calyculatae*	NW	1	-2.438	-2.343
E. famatamboay	*Deuterocalli*	OW	0	-2.259	1.938
E. fiherenensis	*Tirucalli*	OW	0	-2.137	1.992
E. tanquahuete	*Tanquahuete*	NW	1	-1.956	0.193
E. stenoclada	*Tirucalli*	OW	0	-1.941	1.113
E. pervilleana	*Pervilleanae*	OW	1	-1.926	0.929
E. horombensis	*Goniostema*	OW	1	-1.912	-0.771
E. beharensis	*Goniostema*	OW	1	-1.872	1.683
E. didiereoides	*Goniostema*	OW	1	-1.781	0.805
E. croizatii	*Goniostema*	OW	1	-1.749	1.386
E. alluaudii	*Deuterocalli*	OW	0	-1.698	0.929
E. hedyotoides	*Denisophorbia*	OW	1	-1.637	1.512
E. enterophora	*Tirucalli*	OW	0	-1.615	0.413
E. intisy	*Pervilleanae*	OW	0	-1.601	0.748
E. arahaka	*Tirucalli*	OW	0	-1.068	1.850
E. rauhii	*Pervilleanae*	OW	1	-1.024	0.482
E. weberbaueri	*Euphorbiastrum*	NW	1	-0.942	-5.069
E. arbuscula	*Tirucalli*	OW	0	-0.941	2.752
E. pteroneura	*Euphorbiastrum*	NW	0	-0.888	0.313
E. attastoma	*Brasillienses*	NW	0	-0.846	-0.593
E. tirucalli	*Tirucalli*	OW	0	-0.832	2.144
E. decorsei	*Tirucalli*	OW	0	-0.815	1.337
E. phosphorea	*Brasillienses*	NW	0	-0.615	-0.482
E. milii	*Goniostema*	OW	1	-0.565	-0.431
E. pedilanthoides	*Goniostema*	OW	1	-0.506	2.542
E. heterodoxa	*Stachydium*	NW	1	-0.170	-0.593
E. comosa	*Stachydium*	NW	1	0.013	0.950
E. tetraptera	*Pervilleanae*	OW	1	0.559	-0.999
E. viguieri	*Goniostema*	OW	1	0.616	2.880
E. tithymaloides	*Goniostema*	OW	1	0.618	1.924
E. gymnonota	*Cubanthus*	NW	1	0.625	2.658
E. laurifolia	*Euphorbiastrum*	NW	1	0.643	-5.136
E. haeleeleana	*Pacificae*	P	1	0.645	-0.912
E. helenae	*Cubanthus*	NW	1	1.274	1.902
E. geroldii	*Goniostema*	OW	1	1.562	1.329
E. mandravioky	*Pachysanthae*	OW	1	1.564	1.945
E. hoffmanniana	*Euphorbiastrum*	NW	1	1.935	-2.376
E. plumerioides	*Pacificae*	P	1	2.193	-0.440
E. lophogona	*Goniostema*	OW	1	2.297	0.361
E. cestrifolia	*Euphorbiastrum*	NW	1	2.311	-3.928
E. punicea	*Cubanthus*	NW	1	2.671	0.376
E. sinclairiana	*Mesophyllae*	NW	1	4.470	0.032

[a]OW = Old World (Madacascar, Africa, Arabia), NW = New World, P = Pacific (Australia, Hawaii)

Table 4.2. Loadings on the first two axes of the PCA for each bioclimatic variable.

	Loading	
Bioclimatic Variable	PC1	PC2
Annual Mean Temperature	0.090	0.404
Mean Diurnal Range (Mean of monthly (max temp - min temp))	-0.253	0.014
Isothermality (BIO2/BIO7) (* 100)	0.222	-0.155
Temperature Seasonality (standard deviation *100)	-0.258	0.121
Max Temperature of Warmest Month	-0.076	0.391
Min Temperature of Coldest Month	0.252	0.254
Temperature Annual Range (BIO5-BIO6)	-0.291	0.075
Mean Temperature of Wettest Quarter	-0.013	0.400
Mean Temperature of Driest Quarter	0.168	0.339
Mean Temperature of Warmest Quarter	-0.004	0.413
Mean Temperature of Coldest Quarter	0.195	0.325
Annual Precipitation	0.326	-0.043
Precipitation of Wettest Month	0.287	-0.010
Precipitation of Driest Month	0.265	-0.059
Precipitation Seasonality (Coefficient of Variation)	-0.199	0.107
Precipitation of Wettest Quarter	0.292	-0.026
Precipitation of Driest Quarter	0.276	-0.059
Precipitation of Warmest Quarter	0.201	-0.048
Precipitation of Coldest Quarter	0.301	-0.017

Table 4.3. Phylogenetic logistic regression parameter estimates for phylogenetic signal and the effect of mean scores from the first two PCA axes on the presence or absence or functional leaves in 48 species of subg. *Euphorbia*. (CI = confidence interval)

	Parameter	Estimate	SE	T-score	P-value	Bootstrap Mean	Bootstrap CI	Bootstrap P-value
Univariate (phylogenetic signal)	a: (phylogenetic signal)	-1.345				-1.215	(-3.77, 0.812)	0.02
	b0 (intercept)	1.117	0.499	2.239	0.03	1.034	(0.185, 2.158)	0.03
Multivariate (effect of PCA scores)	a: (phylogenetic signal)	-0.963				-1.109	(-4, 3.99)	0.09
	b0 (intercept)	1.074	0.573	1.873	0.067	1.026	(-0.09, 2.538)	0.07
	b1 (PC1)	1.1909	0.454	2.623	0.012	1.229	(0.418, 2.74)	0.002
	b2 (PC2)	-0.114	0.373	-0.305	0.76	-0.109	(-1.04, 0.68)	0.81

Table 4.4. Phylogenetic ANOVA for first two PCA scores between leafy and pencil-stem species of *Euphorbia* subg. *Euphorbia*. Mean (range) of phylogenetic p-values based on null distributions of simulated Brownian motion evolution of response variables on 100 trees sampled from the posterior distribution of trees from BEAST analysis.

	df	Sum Sq.	Mean Sq	F-value	P(>F)	Mean phylogenetic p-value (range)
Response: PC 1 Scores						
group	1	41.013	41.013	14.218	0.00046	0.001 (0.0009-0.002)
residuals	46	132.693	2.885			
Response: PC 2 Scores						
group	1	1.281	1.281	0.3541	0.55	0.64 (0.59-0.67)
residuals	46	46	166.45	3.6185		

Table 4.5. Loadings from the PCA and p-values from phylogenetic ANOVAs of individual Bioclim variables associated with PC1. Phylogenetic P-values are based on null distributions of simulated Brownian motion evolution of the response variables on 100 trees sampled from the posterior distribution of trees from the BEAST analysis.

		ANOVA Phylogenetic p-values			
Bioclim variable	PC1 loading	mean	min.	max.	% trees with p <0.5
Mean Diurnal Range	-0.253	0.070	0.045	0.095	2
Isothermality	0.222	0.120	0.014	0.158	1
Temp. Seasonality	-0.258	0.013	0.006	0.022	100
Min Temp. Cold Month	0.252	0.092	0.060	0.118	0
Ann. Temp. Range	-0.291	0.018	0.008	0.031	100
Mean Temp. Coldest Quarter	0.195	0.236	0.204	0.279	0
Ann. Precipitation	0.326	0.001	0.001	0.003	100
Prec. Wettest Month	0.287	0.001	0.001	0.004	100
Prec. Driest Month	0.265	0.007	0.002	0.013	100
Prec. Seasonality	-0.199	0.524	0.484	0.570	0
Prec. Wettest Quarter	0.292	0.001	0.001	0.004	100
Prec. Dries Quarter	0.276	0.006	0.001	0.015	100
Prec. Warmest Quarter	0.201	0.016	0.010	0.031	100
Prec. Coldest Quarter	0.301	0.019	0.008	0.032	100

Table 4.6. Mean values of bioclimatic variables significantly correlated with PC1 for pencil-stem and leafy species. (T = temperature in °C, P = precipitation in mm.)

Growth Form	T seasonality[a]	Ann. T range	Ann. P	P wettest month	P driest month	P wettest quarter	P driest quarter	P warmest quarter	P coldest quarter
pencil-stem	260.31	19.83	641.82	132.25	10.70	348.76	40.04	289.55	65.25
leafy	183.09	16.68	1262.74	234.05	28.14	616.21	102.98	442.80	182.24

[a]Standard deviation of monthly mean temperature multiplied by 100

Table 4.7. Phylogenetic ANOVA for PC1 scores between leafy and pencil-stem species of *Euphorbia* subg. *Euphorbia* from the Old World and the New World.

	df	Sum Sq.	Mean Sq	F-value	P(>F)	Phylogenetic p-value
New World Species						
group	1	34.99	34.99	8.16	0.009	0.003
residuals	21	90.05	4.29			
Old World Species						
group		8.997	8.9966	5.8629	0.02376	0.0169
residuals	23	35.294	1.5345			

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Chapter V

Conclusion

The past decade has seen great improvement in our understanding of the evolutionary relationships within the giant genus *Euphorbia* (Steinmann & Porter, 2002; Bruyns & al., 2006; Barres & al., 2011; Bruyns & al., 2011; Yang & Berry, 2011; Horn & al., 2012; Yang & al., 2012). We are now beginning to use this phylogenetic framework to investigate the group's biogeographic history and the impressive patterns of diversification that make *Euphorbia* such an interesting and important angiosperm genus. This dissertation has added to that ongoing work by focusing on subgenus *Euphorbia*, one of four subgenera in *Euphorbia*.

Chapter two represents the most taxonomically and geographically comprehensive phylogenetic study of *Euphorbia* subg. *Euphorbia* to date. Sequence data from multiple regions representing the nuclear and plastid genomes allowed us to infer phylogenetic relationships across the subgenus, and they support four major lineages within the subgenus that can be characterized by their distributions. Our results clearly establish the monophyly of all New World species in subg. *Euphorbia*, define the species composition of the New World Clade, and suggest an early split between the Old World and New World early in the history of the subgenus. While the relationships among major clades in subg. *Euphorbia* remain unresolved, our data do resolve many smaller clades with good support, which we designate as sections. In total, we recognize 21 sections, nine of which are newly circumscribed here. Our phylogeny also supports the hypothesis of at least two independent lineages in Madagascar within subg.

Euphorbia. Included in these is sect. *Tirucalli*, which is recircumbscribed as the clade of pencil-stemmed species within subg. *Euphorbia* that includes *E. tirucalli*. Also included in these Madagascan clades are three sections of leafy shrubs and trees (sect. *Denisophorbia*, sect. *Pervilleanae*, and sect. *Pachysanthae*) that will require more thorough study to better understand their circumscriptions and the delimitations of species within them.

Consistent with the overall evolutionary lability of growth form in *Euphorbia* as a whole, several of the sections in subg. *Euphorbia* contain a wide range of growth forms for their small size. The pencil-stem growth form is particularly homoplasious within the subgenus, just as it is across the genus as a whole. These patterns are investigated more thoroughly in chapters three and four.

Chapter three is the first study to link ecological differences to growth form evolution in *Euphorbia* by incorporating estimates of species' climatic niches with the biogeographic history and growth form evolution in a clade of spiny stem-succulent species (*Euphorbia* sect. *Euphorbia*). This approach allowed us to reject the previous hypothesis that sect. *Euphorbia* originated in Asia and that species became progressively smaller as they encountered dryer habitats during their spread across Africa (Carter, 1994). Instead we find that the ancestral range was widespread from Northeast Tropical Africa to Southern Africa and that the biogeographic history and patterns of growth form evolution in sect. *Euphorbia* are quite complex. We have shown that even though most species occur in generally warm and dry habitats, climatic niches and specifically the severity of seasonal drought differ among growth forms, and selection by optimal climatic niche parameters has been instrumental in producing the patterns of growth form evolution we observe in this clade.

In chapter four we investigated the role of water availability in the evolution of a leafless, stem-photosynthetic growth form, termed ‘pencil-stem’ plants, which have evolved repeatedly in *Euphorbia*. Using estimates of climatic parameters among closely related leafy and leafless species, we have shown that selection by habitats with low precipitation has favored the leafless ‘pencil-stem’ growth form across the subgenus. However, the distribution of precipitation tolerances across the phylogeny of subg. *Euphorbia* suggests that this general trend may break down at the level of individual species where divergence of climatic niches has likely occurred among closely related species (i.e., within sections). This niche differentiation is one explanation for the co-occurrence of leafy and leafless species on Madagascar.

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