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## Insights on the Evolution of Plant Succulence from a Remarkable Radiation in Madagascar (*Euphorbia*)

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**Abstract.**—Patterns of adaptation in response to environmental variation are central to our understanding of biodiversity, but predictions of how and when broad-scale environmental conditions such as climate affect organismal form and function remain incomplete. Succulent plants have evolved in response to arid conditions repeatedly, with various plant organs such as leaves, stems, and roots physically modified to increase water storage. Here, we investigate the role played by climate conditions in shaping the evolution of succulent forms in a plant clade endemic to Madagascar and the surrounding islands, part of the hyper-diverse genus *Euphorbia* (Euphorbiaceae). We used multivariate ordination of 19 climate variables to identify links between particular climate variables and three major forms of succulence—succulent leaves, cactiform stem succulence, and tubers. We then tested the relationship between climatic conditions and succulence, using comparative methods that account for shared evolutionary history. We confirm that plant water storage is associated with the two components of aridity, temperature, and precipitation. Cactiform stem succulence, however, is not prevalent in the driest environments, countering the widely held view of cactiforms as desert icons. Instead, leaf succulence and tubers are significantly associated with the lowest levels of precipitation. Our findings provide a clear link between broad-scale climatic conditions and adaptation in land plants, and new insights into the climatic conditions favoring different forms of succulence. This evidence for adaptation to climate raises concern over the evolutionary future of succulent plants as they, along with other organisms, face anthropogenic climate change. [Adaptation; climate; comparative analysis; *Euphorbia*; ordination; phylogeny.]

Succulent plants have such diverse, downright bizarre growth forms that they make an irresistible target for evolutionary studies of the relationship between form and function, between organism and environment (Nobel 1988; Edwards and Donoghue 2006; Hearn 2006; Ogburn and Edwards 2009). Succulence is thought to be an adaptation to arid conditions (Futuyma 1997; Niklas 1997; Arakaki et al. 2011). Indeed, the radiation of succulent plants in arid regions on two continents, in two distantly related families, cacti (Cactaceae) in the New World and spurges (Euphorbiaceae) in the Old World, is used as a textbook example of convergent adaptive evolution (Raven et al. 1986; Futuyma 1997; Niklas 1997; Stearns and Hoekstra 2005). Surprisingly then, there are very few comparative studies of the relationship between succulence and climate (Hearn 2004; 2013). In particular, there are no studies of, much less prediction about, how climate influence the tremendous variety of forms of succulence among land plants, ranging from leaf succulents such as agaves (*Agave*) and living stones (*Lithops*), to stem succulents such as cacti and bottle trees (*Adansonia* and *Pachypodium*), to root or root-like succulents (see Fig. 1c,e). Arguably, the most interesting groups of succulent plants are those where water storage occurs in radically different parts of the

plant body among close relatives (Fig. 1, Hearn et al. 2013), suggesting evolutionary lability, and begging the question of whether there is adaptive value in such striking changes in morphology.

Here, we examine the relationship between climate and different forms of succulence in a subclade of the giant genus *Euphorbia* (comprising sections *Goniostema*, *Denisophorbia*, and *Deuterocallia*; hereafter, *Euphorbia* GDD) that is fantastically varied in form (Fig. 1) and endemic to Madagascar and surrounding islands. The *Euphorbia* GDD clade thus carries another kind of significance: it is representative of the endemism that places Madagascar in the top tier of global biodiversity hotspots (Myers et al. 2000; Ganzhorn et al. 2001; Mittermeier et al. 2005; Phillipson et al. 2006). We focus on three kinds of succulence: (i) leaf succulence, (ii) cactiform stem succulence, and (iii) below-ground water-storing organs, either a caudex (a swollen, perennial stem at or near-ground level) or tuber (a more general term referring to a storage organ derived from stem or root tissue). Cactiform stem succulents are considered iconic of deserts, but in fact they are not prevalent in places subject to very long or unpredictable drought. Instead, they store water above ground, fully exposed to the evaporative powers of the sun and wind, making

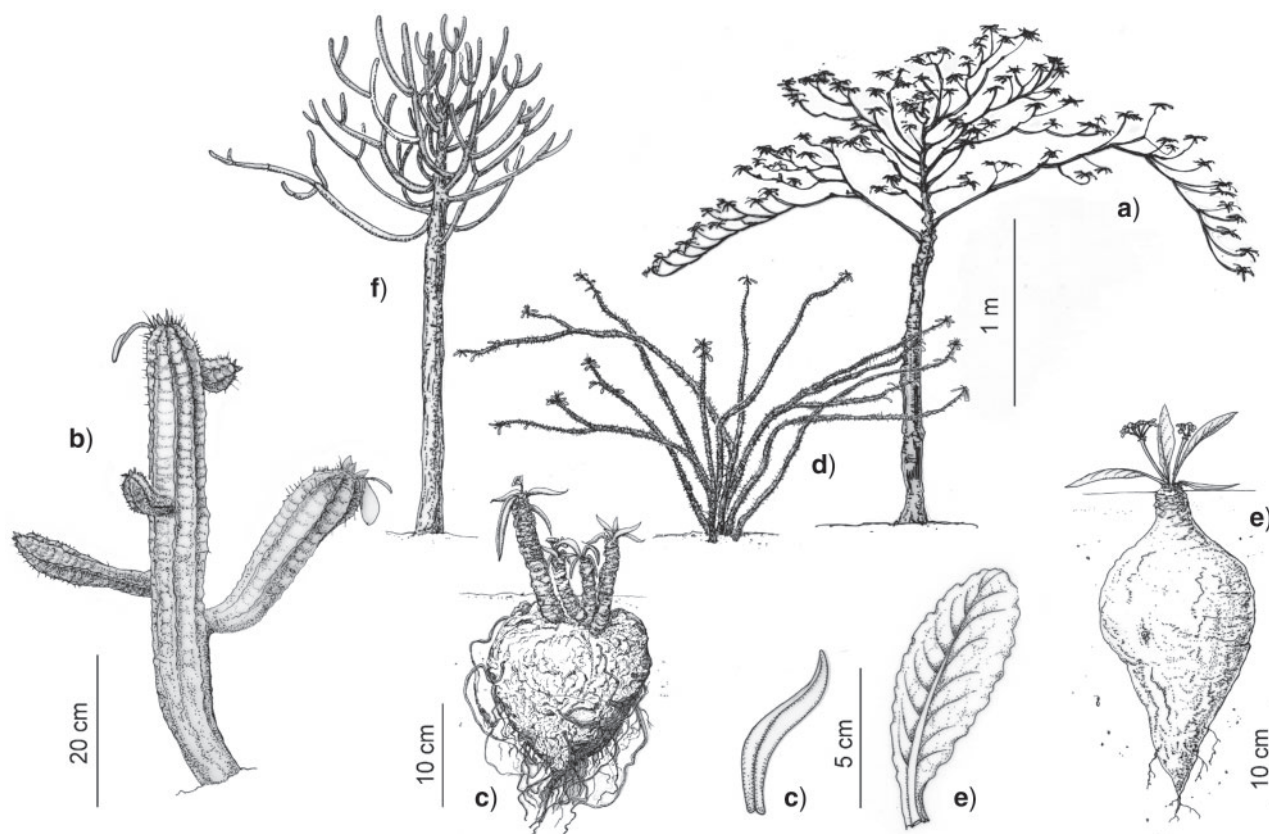


FIGURE 1. Growth form diversity in the *Euphorbia* clade GDD (sections *Goniostema*, *Denisophorbia*, and *Deuterocalli*): a) *E. aff. pyrifolia*, a non-succulent tree, b) *E. capmanambatoensis*, a cactiform, c) *E. cylindrifolia*, a dwarf chamaephyte with highly succulent leaves (left leaf), d) *E. mahafalensis*, a shrub, e) *E. primulifolia* var. *primulifolia*, a true geophyte with non-succulent leaves (right leaf), and f) *E. alluaudii*, a coraliform tree. These represent mature specimens, with the exception of *E. alluaudii*, which reaches a maximum height of 10 m. Drawings by A. Haevermans.

them relatively vulnerable to water loss; they are most prevalent in areas with seasonal drought, but a reliable season of precipitation (Gibson and Nobel 1986; Burgess and Shmida 1988; Burgess 1995; Eggli and Nyffeler 2009; Ogburn and Edwards 2010). In contrast, we suggest that in very dry climates, water storage should occur below ground or near the soil surface, where evaporative potential is lower. We argue that below-ground succulents are more narrowly adapted to extremely dry environments than cactiform species, since tubers are highly susceptible to rot, a fact well known to succulent enthusiasts and Irish-American immigrants alike (Hearn 2004). We also expect below-ground or near-ground forms of succulence to be associated with cooler temperatures, both because risk of frost damage selects for these forms in cold climates, and ground-hugging growth forms put photosynthetic structures in a warmer microenvironment (near the soil surface), where they can be more efficient. Cactiform stem succulents, by contrast, are known to be limited by cold conditions (Shreve 1911; Steenbergh and Lowe 1977; Nobel 1980; Gibson and Nobel 1986; Pierson and Turner 1998; Godinez-Álvarez et al. 2003; Ogburn and Edwards 2010). Thus, we propose that there are two fundamental categories of succulence—those positioning water-storing tissues below ground or near

the surface versus above ground—associated with very dry and relatively cool conditions versus moderately dry and warm conditions, respectively.

We addressed these predictions about climate and different forms of succulence in the *Euphorbia* GDD clade using a two-step analysis. The first is an exploratory phase. Wet/dry and cold/warm gradients can be described with a variety of summary statistics (mean, maximum, or minimum temperature and precipitation) at various time scales. Correlations between these variables are often high, making it inappropriate to include them in a single test (multicollinearity), yet multiple tests raise the risk of type 1 error. A key objective of the exploratory stage is thus to identify a suitable subset of climate variables for statistical testing. Toward this, we used multivariate ordination techniques to generate a reduced-dimension climate space in which we examined the distribution of succulence variables. This identified mean annual temperature (MAT) and mean annual precipitation (MAP) as suitable climate variables for statistical analysis. Second, we tested the relationship between succulence variables and MAT or MAP, taking into account the pseudoreplication that arises due to the relatedness of species (Felsenstein 1985). The results yield new insight into the climatic conditions favoring the evolution of different types of succulence in plants.

## MATERIALS AND METHODS

*Phylogeny*

*Euphorbia* GDD forms a well-supported and morphologically diversified clade of at least 90 species (123 taxa including infraspecific entities), with section *Goniostema* accounting for the majority of the clade (~75 species; Haevermans et al. 2009). Species discovery, circumscription, and description are not complete in this group, but recent progress provides a robust phylogenetic framework for analysis of trait evolution (Steinmann 2001; Steinmann and Porter 2002; Haevermans et al. 2004; Zimmerman et al. 2010; Aubriot 2012; Horn et al. 2012; Dorsey et al. 2013). Our phylogenetic reconstruction involved a sample of 279 individuals corresponding to 82 species, of which 30 samples represented outgroup taxa for the GDD clade, selected from four recently established subgenera of *Euphorbia* (Horn et al. 2012). *E.* sections *Goniostema*, *Denisophorbia* and *Deuterocalli* were represented, respectively, by 203, 34, and 12 samples, corresponding to approximately 63, 8, and 2 species, representing 90% of the estimated diversity in the *Euphorbia* GDD clade (Supplementary Fig. S1 and Table S1, available from <http://doi.org/10.5061/dryad.vq6mp>). DNA was sequenced for six chloroplast markers (*atpI-atpH*, *psbA-trnH*, *ndhA*, *matK*, *trnQ-5'rps16*, and *rbcL*) and two nuclear regions (ITS and ETS), resulting in a data matrix of 8507 bp (<1% missing data). Our approach was to use all of the available data (sequences) in order to verify operational taxonomic units (OTUs) and estimate phylogeny, then trim the trees to retain a single sample per ingroup OTU (i.e., removing duplicates), retaining all samples that were associated with both an unambiguous taxonomic circumscription and reliable locality data (Supplementary Fig. S1; <http://doi.org/10.5061/dryad.vq6mp>). Rerunning the phylogenetic analysis with one sample per final retained OTU (67 taxa) yields essentially the same topology. Sequence matrices were aligned using MUSCLE (version 3.8; Edgar 2004) and MAFFT (version 6; Katoh et al. 2002); the aligned data are available at [doi:10.5061/dryad.vq6mp](http://doi.org/10.5061/dryad.vq6mp). The best-fit model of nucleotide substitution evolution for each region was determined using Akaike's information criterion implemented in MrModelTest 2.2 (Nylander 2004): GTR+ $\Gamma$ +I for ITS, ETS, *atpI-atpH*, GTR+ $\Gamma$  for *psbA-trnH*, *ndhA*, *matK*, *trnQ-5'rps16*, and GTR+I for *rbcL*. Phylogenetic analysis of the nuclear and chloroplast markers separately revealed topological incongruence between them: chloroplast markers, which are lacking in sequence variation, do not recover as monophyletic *E.* sections *Goniostema* and *Denisophorbia*. The evidence suggests this incongruence is real, with unknown, potentially complex causes (e.g., incomplete lineage sorting and/or hybridization). We made the decision to concatenate all regions into a combined data set, because this offers a consensus tree most consistent with strong morphological data supporting monophyletic *E.* sections *Goniostema* and *Denisophorbia*—species of the

former are united by strictly bisexual cyathia, unique modifications of stipules into spines, prickles, or comb-like enations, and verrucose seeds, whereas species of the latter are united by uni- or bisexual cyathia, a unique habit with chandelier-like branching and plagiotropic branches (as in Fig. 1a), and smooth, unornamented seeds. Analyses based on the other most extensive sampling efforts to date (though substantially less than here; Haevermans et al. 2004; Zimmerman et al. 2010; Dorsey et al. 2013) come to the same conclusion—that is, that *E.* sections *Goniostema* and *Denisophorbia* are monophyletic. Topology reconstruction and relative divergence times were estimated simultaneously using BEAST v1.7.2 (Drummond et al. 2012), with the gene regions partitioned according to the best-fit models of evolution, and a Yule speciation tree prior (Drummond et al. 2007). Note that our analyses do not depend upon the absolute time scale of the *Euphorbia* GDD radiation; instead, dating served to produce credible ultrametric trees for visualizing trait evolution and phylogenetically corrected statistical tests of trait-climate relationships. Because the fossil record for *Euphorbia* is poor, we relied upon two dates estimated by Bruyns et al. 2011 as temporal constraints: the ages of subgenus *Euphorbia* (mean = 29.94 Myr, SD = 4.53 Myr; 95% HPD = 22.49–39.56) and our ingroup, *Euphorbia* GDD (mean = 12.65 Myr, SD = 3.0 Myr; 95% HPD = 7.099–19.62). Uncertainty regarding these dates was incorporated by assigning normal prior distributions to these two calibration points (Ho 2007; Couvreur et al. 2008; Bergh and Linder 2009; Su and Saunders 2009). Substitution models, rate heterogeneity, and base frequencies were unlinked across partitions. Divergence times were estimated under a relaxed uncorrelated molecular clock that allows rates to vary independently along branches according to a lognormal distribution (Drummond et al. 2007). Three independent Markov Chain Monte Carlo (MCMC) simulations were run on the CIPRES Science Gateway Web server (Miller et al. 2010), each 80 million generations, sampling every 4000 generations. MCMC samples were inspected for convergence and parameter stability (using Tracer 1.5; Rambaut and Drummond 2007). The first 25% of each chain was removed as burn-in, and chains were combined using LogCombiner 1.7.2. (Drummond et al. 2012).

Trimming trees yields a 67-tip phylogeny (Fig. 2) that accounts for approximately 74% of the species diversity in the *Euphorbia* GDD clade. In the process of trimming, we made the following decisions about problematic taxa. First, we split *E. pyrifolia*, a variable member of *E.* section *Denisophorbia* occurring on islands surrounding Madagascar (Mauritius, Assumption Island, Aldabra Atoll, and the Seychelles islands), into four distinct OTUs, to account for the significant morphological diversity it comprises. Second, many of the spiny, shrubby taxa related to *E. mahafalensis* and *E. milii* are very difficult to identify at the species level due to ambiguous descriptions and often fragmentary type material, hindering taxonomic clarity. Because



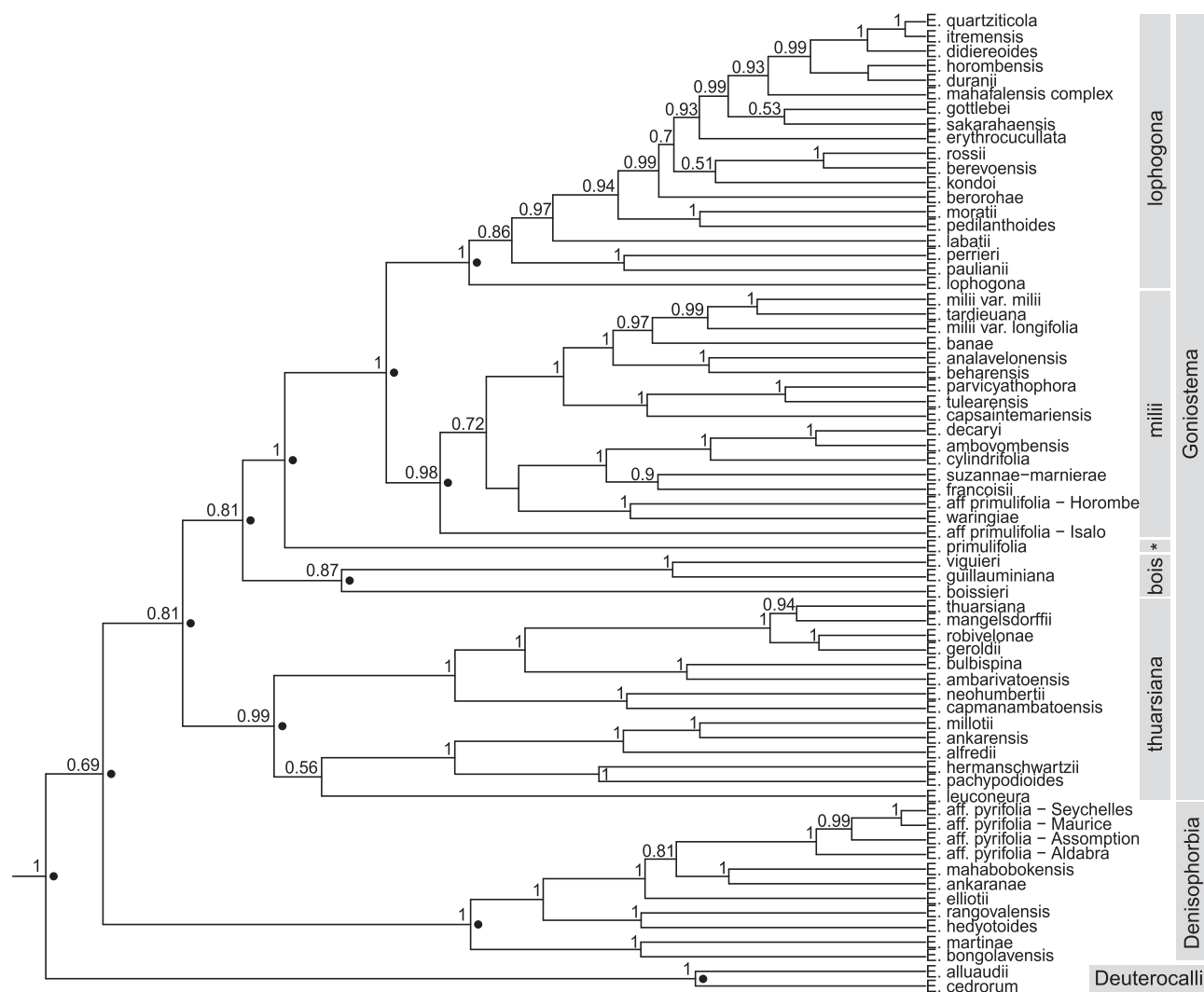


FIGURE 2. Trimmed phylogeny of *Euphorbia* GDD, derived from the maximum clade credibility tree. Node support is shown for all nodes with posterior probability greater than 0.50; support for the backbone and key nodes defining seven subclades (see Fig. 6) is highlighted with black points. Vertical bars indicate these seven subclades, plus *E. section Goniosstema*. The asterisk indicates the *E. primulifolia* clade.

the entities related to *E. mahafalensis* (*E. retrospina*, *E. razafindratsirae*, *E. croizatii*, *E. rubrostriata*, and *E. hoffstaetteri*) are quite homogeneous in overall morphology, geographic distribution, and ecological preferences (climate and substrate), we treated this group of putative species as constituting a single OTU, the "*E. mahafalensis* complex." The *E. milii* assemblage, which comprises numerous infraspecific concepts, is polyphyletic (Aubriot 2012). As a consequence, we reduced our sampling of *E. milii* to two monophyletic groups for which we had clear morphological and geographical data, *E. milii* var. *milii* from the northern part of Madagascar's central highlands and *E. milii* var. *longifolia* from the southern part.

Finally, we made decisions concerning the polyphyly and paraphyly, respectively, of two additional species, *Euphorbia primulifolia* and *E. alluaudii*. The three samples of *E. primulifolia* in our analysis, each from a distinct geographical area, were separated in the consensus

phylogeny by very strongly supported nodes, so we considered each to constitute a distinct OTU, according to their geographical provenance (*E. aff. primulifolia* var. *primulifolia*, *E. aff. primulifolia*—Isalo and *E. aff. primulifolia*—Horombe). In the small section *Deuterocalli*, composed of just *E. alluaudii* and *E. cedrorum*, we discovered that *E. alluaudii* is composed of two assemblages, one closer to *E. cedrorum* than to the other assemblage of *E. alluaudii*. Because all *E. alluaudii* specimens are very similar morphologically, it was impossible to associate them with one assemblage versus the other. Keeping two distinct OTUs would have drastically reduced the amount of occurrence data, since each of the OTUs would then have to be represented by the small number of individuals used for molecular analyses. In order to better reflect the very broad geographic distribution of what is traditionally referenced to as *E. alluaudii*, we selected one as representative of *E. alluaudii* and removed the other from

our data set. We regard our final selection of samples to be representative of the diversity of the *Euphorbia* GDD clade, especially since we included representatives from problematic taxa, as well as some of the “hidden” diversity within the clade, through the inclusion of samples from OTUs whose historical delimitation was shown to be paraphyletic or polyphyletic.

#### *Succulence and Life Form Data*

Some definitions of succulence exclude plants with a caudex or tuber, under the expectation that these structures primarily serve the function of storing carbon rather than water (Eggli and Nyffeler 2009). It is our view that the water and carbon storage value of tubers may be intimately intertwined in seasonal environments (see the “Discussion” section), and in at least some species it is clear that tubers do store water (Pate and Dixon 1981; Olson and Carlquist 2001; Eggli and Nyffeler 2009; Hearn 2009a, 2009b, 2013). For these reasons, we include tubers in our analysis of succulence and climate. The GDD clade includes a diversity of forms of succulence: trees and shrubs with stems and leaves exhibiting varying degrees of succulence, some with tuberous roots; “cactiform” species (single-stemmed or sparsely branched plants with highly succulent stems, bearing leaves only during the wet season); geophytes and dwarf chamaephytes with a highly developed underground caudex, some with succulent leaves; and thorny semisucculent shrubs such as *Euphorbia milii*, the famous “crown-of-thorns” (Fig. 1; Haevermans et al. 2004; Aubriot 2012). We tackled this diversity of degrees and forms of succulence by gathering data on three characters—leaf succulence, the presence of a tuber (including a caudex), and growth form—treated as categorical. The characters and character states were defined as follows:

1. Leaf succulence: (0) leaves weakly succulent, (1) leaves rigid, “semisucculent,” and (2) leaves highly succulent (Fig. 1).

Definition and coding of character states were based on visual inspection of the thickness of the leaf blade, visibility of veins, and leaf texture (viz. leaves pliable or not). Weakly succulent leaves (0) have a thin blade with clearly visible veins, and are easily bent. Semisucculent leaves (1) have a slightly thick, rigid blade, typically with veins that are not prominent; they tend to break rather than bend. Highly succulent leaves (2) have a very thick, rigid, and inflexible blade, with inconspicuous veins.

2. Tuber: (0) nontuberous roots and (1) tuberous roots (Fig. 1c,e).

The absence (0) or presence (1) of a primarily subterranean caudex or swollen root system was captured using a binary scheme.

3. Growth form: (0) geophyte, (1) dwarf chamaephyte, (2) sparsely branched “cactiform,” (3) shrub, and (4) tree (Fig. 1).

Geophytes (0) are mostly subterranean with a large caudex and underground stems; only leaves and inflorescences are exposed above the surface. Dwarf chamaephytes (1) have a subterranean caudex that bears short, unbranched stems not exceeding 60 cm above the surface. Cactiform species (2) are unbranched or sparsely branched with highly succulent stems. Shrubs (3) lack a true trunk and have numerous basal branches. Trees (4) have a trunk, a central stem unbranched at the base.

These five categories were selected to capture the major growth forms in the GDD clade, while avoiding an excess of sparsely populated categories. For example, the two species of *E.* section *Deuterocalli* (*E. alluaudii* and *E. cedrorum*) in our sample are classified as succulent trees: they have a growth form described as “coraliform,” owing to the thickness of their stems and dense branching pattern (Fig. 1f). We did not attempt to model the evolution of this growth form, because it has a single origin in the GDD clade (convergent evolution of the coraliform habit has occurred elsewhere in *Euphorbia*).

Some species were difficult to categorize. For example, *Euphorbia didiereoides*, *E. duranii*, and *E. guillauminiana* have growth forms intermediate between the shrub and cactiform categories. We treated them as shrubs, preferring to err on the conservative side in our definition of cactiform. Ideally, continuous data on succulence should be collected (Ogburn and Edwards 2012), but this was not practical in a group that includes so many microendemics, including several examples where an accepted species was described long ago but has not been recorded since.

Coding for these three characters drew upon multiple sources of information: observations in the field, examination of living collections in greenhouses (at the MNHN arboretum in Chèvreloup, France; Heidelberg Botanical Garden in Germany; and Wageningen Botanical Garden in the Netherlands) and outdoor collections in Madagascar (Tsimbazaza Botanical Garden in Antananarivo; Antsokay Arboretum in Toliara), as well as herbarium specimens (mostly from MNHN and MBG). When living or dry specimens were not available, we used the primary literature as well as the expertise and personal experience of the authors and colleagues.

#### *Locality and Climate Data*

We assembled an initial database of 653 occurrences of members of the GDD clade, approximately 75% from museum collections, mainly the MNHN and MBG herbaria (available on SONNERAT [http://science.mnhn.fr/institution/mnhn/search, last accessed May 28, 2014] and TROPICOS [http://www.tropicos.org/, last accessed May 28, 2014]). The remaining localities were derived from monographs, unpublished records, and expert knowledge (Aubriot X., Haevermans T., unpublished data). All locality data were carefully evaluated; some records were discarded because of imprecision, species misidentification, or

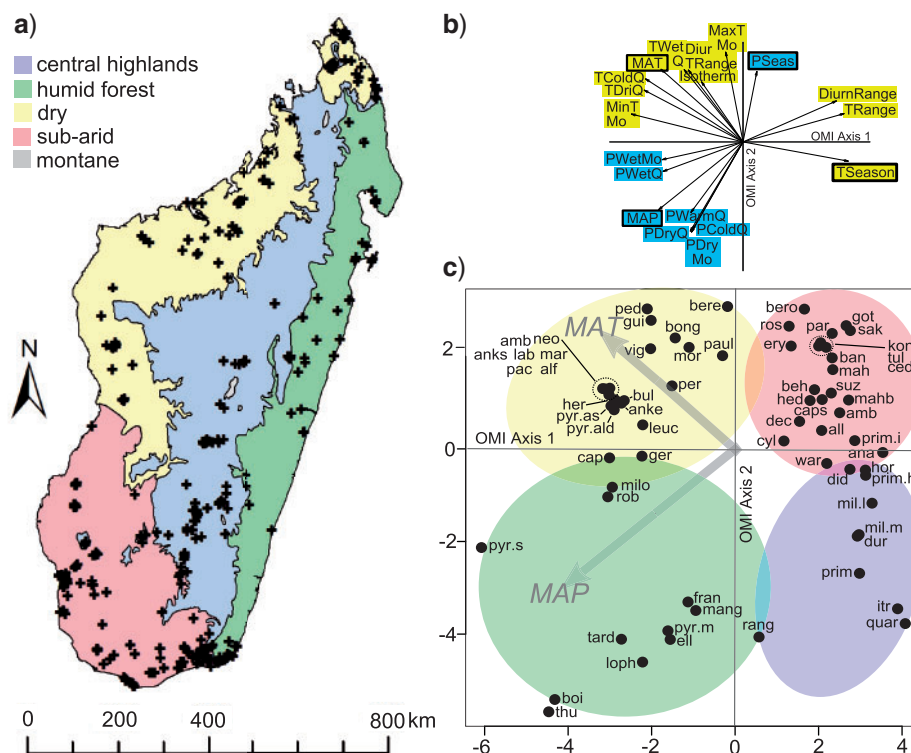


FIGURE 3. a) *Euphorbia* GDD localities used in this study (crosses), and major bioclimatic regions of Madagascar, as defined by Cornet (1974). b) Loading of the 19 Bioclim variables in the OMI 1 versus 2 climate space, with precipitation variables colored blue and temperature variables colored yellow. The variables are: MAT, mean diurnal temperature range (DiurTRange), isothermality (Isotherm), temperature seasonality (TSeason), maximum temperature of the warmest month (MaxTMo), minimum temperature of the coldest month (MinTMo), temperature annual range (TRange), mean temperature of the wettest quarter (TWetQ), mean temperature of the driest quarter (TDriQ), mean temperature of the warmest quarter (TWarmQ), mean temperature of the coldest quarter (TColdQ), MAP, precipitation of the wettest month (PWetMo), precipitation of the driest month (PDryMo), precipitation seasonality (PSeason), precipitation of the wettest quarter (PWetQ), precipitation of the driest quarter (PDryQ), precipitation of the warmest quarter (PWarmQ), precipitation of the coldest quarter (PColdQ). c) Ordination diagram of OMI axis 1 versus 2, with centroids for each species (points). Colored circles overlain here were placed to encompass species' geographic distributions, with colors corresponding to panel a.

duplication. The final data set contained 529 occurrences (Fig. 3a), which were used for climatic data extraction. Climate data were the 19 "Bioclim" variables available from worldclim (<http://www.worldclim.org/>, last accessed May 28, 2014; Hijmans et al. 2005), downloaded at the highest possible resolution ( $\sim 1 \text{ km}^2$ ) and extracted using ArcGIS version 9.3. These include 11 temperature-related variables (MAT, minimum temperature in the coldest month, mean temperature of the wettest and driest quarters, etc.) and 8 precipitation-related variables (MAP, precipitation of the driest and wettest quarters, precipitation seasonality, etc.).

### Ordination

We compared two different multivariate ordination techniques for reducing the dimensionality of the Bioclim data and examining the distribution of succulence characters: principal components analysis (PCA) and the outlying mean index (OMI; Dolédec et al. 2000). OMI is a type of discriminant analysis, so unlike PCA, it is designed to maximize separation of species' niches in the reduced-dimension niche space.

In particular, an OMI analysis produces a transformation of the original variables (here, the 19 Bioclim variables) that maximizes the distance between the centroid of a hypothetical species that occurs evenly across the study landscape (i.e., the mean environmental conditions among the sampled sites) and the centroids of the study species (Dolédec et al. 2000; Thuiller et al. 1994). This analysis was implemented using the R package ade4 (Thioulouse et al. 1997).

### Tests of Trait–Climate Relationships

Examination of the distribution of traits in climate space led us to select MAT and MAP for analyses of trait–climate relationships (Results). We used phylogenetic logistic regression to evaluate the ability of these dimensions of climate to predict species' character state. Each trait was modeled as a binomial response, converting the two traits with more than two categories (leaf succulence and growth form) into binary variables. Semisucculent leaves were grouped with nonsucculent leaves, yielding a variable that contrasts species with highly succulent

leaves against those without highly succulent leaves. Growth form was recoded into two variables: one contrasting species that are cactiform versus not, and another contrasting taxa that are geophytes or dwarf chamaephytes versus not, highlighting the most conspicuous forms of above-ground versus below-ground succulence, respectively. Our tests accounted for the relatedness of species by including a correlation structure derived from phylogenies (implemented using the MCMCglmm package in R; Hadfield 2010; Hadfield and Nakagawa 2010). Prior distributions for the climate effects were normally distributed centered on zero, with variance proportional to the estimated residual variance (Hadfield 2012). Based on convergence and autocorrelation statistics from initial simulations on the trimmed maximum clade credibility tree, we ran simulations of  $1.5 \times 10^6$  iterations, eliminating the first  $5 \times 10^5$  as burn-in, and thinning the chains to 1/1000 samples, yielding 1000 samples per chain. To account for phylogenetic uncertainty, including uncertainty about the monophyly of *Euphorbia* sections *Goniostema* and *Denisophorbia*, we repeated MCMCglmm tests (one simulation per tree) using a random sample of 10 000 trees taken from the post burn-in phase of the three BEAST runs described above. A climate effect was considered significant if at least 95% of its posterior distribution fell on one side of zero.

## RESULTS

### Phylogeny

The aligned data matrix comprised 8507 characters, of which 2478 were variable and 1919 parsimony informative. Bayesian analysis conducted with BEAST yielded a well-resolved phylogeny (Fig. 2) in which posterior probability support was generally strong, except for some relationships in the deeper nodes. The analysis adds support for the monophyly of the *Euphorbia* GDD and all three of its constituent clades (*E.* sections *Goniostema*, *Denisophorbia*, and *Deuterocalli*), corroborating earlier results (Haevermans et al. 2004; Zimmerman et al. 2010; Dorsey et al. 2013), and questioning the suggestion made by Bruyins et al. (2006) and Horn et al. (2012) that *E.* sections *Goniostema* and *Denisophorbia* may be paraphyletic. These differences in topology likely result from differences in taxon sampling and in DNA regions examined (Aubriot 2012). Many of the relationships within *E.* section *Goniostema* are strongly supported and are consistent with previous studies (Haevermans et al. 2004; Zimmerman et al. 2010). These relationships, considered along with ecomorphological features, allowed us to identify five well-supported subclades within *E.* section *Goniostema* (Aubriot 2012).

### Ordination of Climate

PCA and OMI eigenvectors were oriented similarly, but differed in magnitude (Fig. 3c vs. Supplementary

Fig. S2b; <http://doi.org/10.5061/dryad.vq6mp>). We preferred the output from the OMI analysis over PCA because it nicely captured the four major bioclimatic regions defined by Cornet (1974)—the humid, warm eastern forest; the cool central highlands; the subarid south and southwest; and the climatically heterogeneous west—each one falling into one-quadrant of the OMI axis 1 versus 2 climate space (Fig. 3b). Further, species were better separated from one another in OMI axis 1 versus 2 space, compared with PCA axis 1 versus 2 space (Fig. 3b vs. Supplementary Fig. S2a; <http://doi.org/10.5061/dryad.vq6mp>). MAT is highest in the upper left and lowest in the lower right of the OMI ordination diagram, whereas MAP is highest in the lower left and lowest in the upper right (Fig. 3b). The driest region of the ordination diagram has the strongest precipitation seasonality, and the coolest region has the greatest temperature seasonality (Fig. 3b); thus, we take MAT and MAP as proxies for seasonality of temperature and precipitation, respectively. Species of *Euphorbia* belonging to the GDD clade are found throughout Madagascar, with multiple representatives in each of Cornet (1974) bioclimatic regions (Fig. 3a,c).

### Distribution of Succulence Characters in Climate Space

Examination of the distribution of character states in OMI axis 1 versus 2 climate space allowed us to select two climate variables for trait–climate tests: MAT and MAP. The prevalence of highly succulent leaves (orange, Fig. 4a) and tubers (red, Fig. 4b) varies along the diagonal of the OMI ordination diagram stretching from the upper right to lower left. Prevalence of the cactiform growth form varies along the upper left to lower right diagonal (blue, Fig. 4c). Prevalence of geophytes and dwarf chamaephytes varies along both of these diagonals (green and orange, Fig. 4c), either individually or taken together. The upper right to lower left diagonal is a precipitation gradient, from dry and seasonal precipitation to mesic and even precipitation (Fig. 3b), whereas the upper left to lower right diagonal is a temperature gradient, from warm and even temperature to cooler and seasonal temperature variation (Fig. 3b). MAT and MAP, which load most directly on these diagonals (Fig. 3b), were selected for tests of the ability of climate variation to predict species' character state.

### Leaf Succulence

The majority of species have nonsucculent leaves (47 out of 67 total), with fewer having semisucculent leaves (8 species) or strongly succulent leaves (12 species). Those with nonsucculent leaves occur in all four quadrants of the climate diagram (green, Fig. 4a), whereas most with highly succulent leaves (10 out of 12) are found in the upper right quadrant (orange, Fig. 4a), corresponding to the subarid south of Madagascar. One species with highly succulent leaves (*E. francoisii*) and half of the species with semisucculent leaves



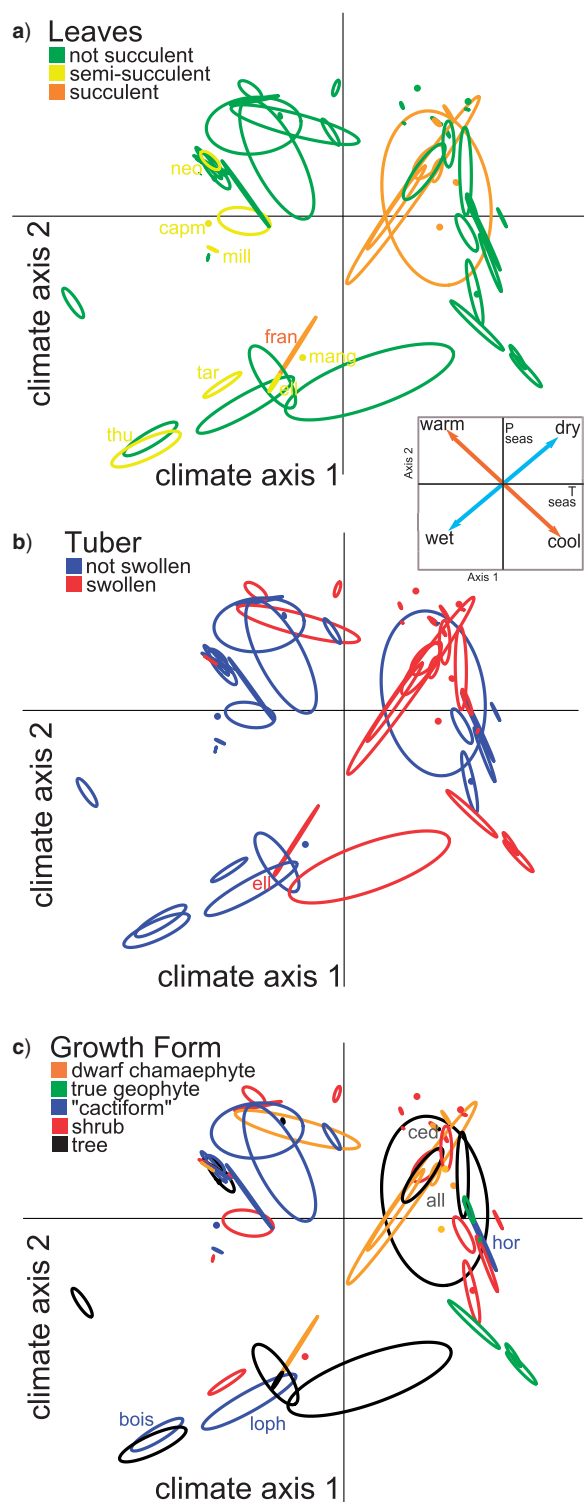


FIGURE 4. Distribution of traits: a) leaf succulence, b) tuber, and c) growth form—in the climate space defined by OMI axis 1 versus 2 (similar to PCA). Inset illustrates the interpretation of this climate space (see Fig. 3b)—warm versus cool, dry versus wet, as well as precipitation and temperature seasonality (P seas and T seas, respectively). Each taxon is represented by an ellipse of the 95% density of that taxon's localities; ellipses are colored according to the species' trait state (listed in Table 1). Full taxon names as well as abbreviations used here are listed in Table 1.

(*E. thuarsiana*, *E. tardieuana*, *E. elliotii*, and *E. mangelsdorfii*) are found in places that are surprisingly wet at the macroscale (eastern rainforest; Fig. 4a). Phylogenetic logistic regression indicates that MAP is significantly predictive of highly succulent leaves, which have a tendency to evolve in places with the least precipitation (Fig. 5a). This result is robust to phylogenetic uncertainty: MAP had a significant effect on the presence of highly succulent leaves on more than 99% of the 10 000 trees examined. At the lowest MAP observed among *Euphorbia* GDD species (376 mm per year), the predicted probability of having highly succulent leaves is 0.64 (Fig. 5a).

### Tubers

Nearly half of the *Euphorbia* GDD species in our sample have a tuber (31 out of 67; red, Fig. 4b). They are especially concentrated in the subarid south; just 2 of 21 taxa in the upper right quadrant of the ordination diagram lack a tuber (Fig. 4b). Phylogenetic logistic regression indicates that MAP is significantly related to the presence of a tuber; taxa occurring in places with less precipitation are more likely to have a tuber (Fig. 5b). This pattern was significant on 98% of 10 000 trees. At the lowest MAP observed among *Euphorbia* GDD species (376 mm per year), the predicted probability of having a tuber is 0.86 (Fig. 5b).

### Life Form

The 14 "cactiform" species tend to occur in the warmest region of the climate space (Fig. 4c and Supplementary Fig. S3a, <http://doi.org/10.5061/dryad.vq6mp>), with the notable exceptions of *Euphorbia horombensis*, *E. lophogona*, and *E. boissieri* (MAT = 20.5°C, 22.9°C, and 23.7°C, respectively). The effect of MAT on the cactiform life form is not statistically significant on any of 10 000 trees considered, nor is the effect of MAP. But the evolution of two growth forms with a majority of their biomass below ground—geophytes and dwarf chamaephytes—is significantly associated with low average annual precipitation and/or low average annual temperature. Evolution of geophytism is significantly associated with low MAT (on 100% of 10 000 trees), whereas evolution of the dwarf chamaephyte form is significantly associated with low MAP (on 88% of 10 000 trees). Evolution of these two growth forms considered together is significantly associated with low MAT (on 99% of 10 000 trees; Fig. 5c). At the lowest MAT observed among *Euphorbia* GDD species (16.6°C), the predicted probability of being either a geophyte or dwarf chamaephyte is 0.73 (Fig. 5c). Two other growth form categories, trees and shrubs, are found in all four quadrants of the climate space.

No statistically significant relationships were found between climate and the cactiform growth form. This included models with a linear term for MAP, a linear plus quadratic term, the preceding two models

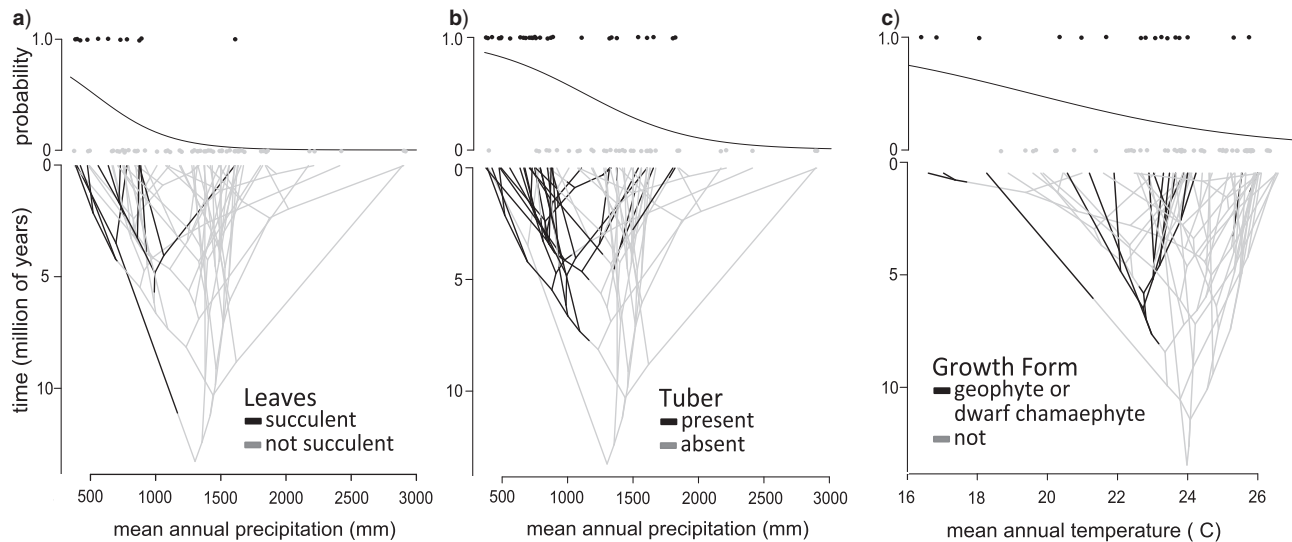


FIGURE 5. Lower panels: stochastic character mapping of three traits related to succulence on the trimmed maximum clade credibility tree, with the tree plotted in climate space. Climate data for the extant taxa are shown in Table 1. Climate at interior nodes was reconstructed using the function phenogram (package phytools, R; Revell 2013); stochastic character mapping was performed using make.simmap (also in the package phytools). A single stochastic realization of character mapping is shown for each trait. Upper panels: predicted probability of having the succulent character state as a function of climate, from a logistic regression not taking into account phylogeny. a) Leaf succulence converted to a binary trait; phylogeny plotted with respect to MAP. b) As in panel a, but the tuber character. c) Growth form converted to a binary trait, contrasting geophytes and dwarf chamaephytes against all other growth forms; phylogeny plotted with respect to MAT.

with a linear term for MAT, and the preceding models without two outliers with respect to MAP (*Euphorbia lophogona* and *E. boissieri*). This is in spite of trends in the data (Supplementary Fig. S3a,b; <http://doi.org/10.5061/dryad.vq6mp>). We suspect the lack of statistical significance is a consequence of low statistical power, due to a small number of transitions in and out of the cactiform state (Supplementary Fig. S3c; <http://doi.org/10.5061/dryad.vq6mp>).

## DISCUSSION

Although lingering phylogenetic uncertainty suggests some caution, our analyses confirm that succulence in the *Euphorbia* GDD clade—including leaf succulence, cactiform stem succulence, and tuber formation—is associated with aridity (warm, dry conditions; Figs. 4 and 5), providing new support for the notion that succulence is an adaptive response to aridity. Further, our results support the notion of two fundamental categories of succulence—those positioning water-storing tissues below ground or near the surface versus above ground—which are associated, respectively, with very dry and relatively cool conditions versus moderately dry and warm conditions. The association of tubers and dwarf chamaephytes (all of which have a tuber) with low MAP confirms the prediction that below-ground water storage should be favored in very dry environments. Hearn (2013) found a remarkably parallel result in *Adenia* (Passifloraceae), a clade centered in Madagascar and Africa with tremendous morphological diversity and expression of succulence in different parts

of the plant body: the evolution of tubers is significantly related to lower precipitation. The association between highly succulent leaves and low MAP in the *Euphorbia* GDD clade also confirms this prediction: 9 of the 12 species with highly succulent leaves are dwarf chamaephytes, thus they store water below ground, in a tuber, and their water-storing leaves are deployed near the soil surface (Fig. 1c). We also found the evolution of below-ground or near-ground succulence to be associated with cooler temperatures: geophytes, as well as geophytes plus dwarf chamaephytes combined, are more prevalent in cooler environments.

Contrary to a layperson's view of cactiforms, they are not prevalent in the driest places occupied by the *Euphorbia* GDD clade (upper right quadrant, Fig. 4c); instead, this growth form is most prevalent at intermediate levels of MAP (Supplementary Fig. S4b, <http://doi.org/10.5061/dryad.vq6mp>). Cactiform stem succulence is also associated with warm temperatures: 11 of the 14 cactiform species are found in places with MAT between 24°C and 26°C (Table 1 and Supplementary Fig. S3a, <http://doi.org/10.5061/dryad.vq6mp>). These patterns are consistent with the literature (Gibson and Nobel 1986; Burgess and Shmida 1988; Burgess 1995; Eggli and Nyffeler 2009), but they were not statistically significant, probably because of a lack of power (Supplementary Fig. S3c, <http://doi.org/10.5061/dryad.vq6mp>) and the presence of “climate outliers,” that is, *Euphorbia* GDD species that are found in surprisingly mesic places, given their morphological characteristics. For example, *E. lophogona* is cactiform, yet it occurs in the eastern mesic forest biome, where MAP is 2172 mm per year. However, many of these “climate

TABLE 1. Complete listing of the *Euphorbia* taxa included in this study, abbreviations used in figures, the average climatic conditions among localities—MAT (in °C); MAP (in mm); and scores on OMI axes 1 and 2—along with character states

Taxon	Abbreviation	MAT	MAP	OMI1	OMI2	Tuber	Leaves	Growth form	Localities
<i>E. alfredii</i>	alf <sup>R</sup>	26.0	1674	−3.09	1.32	Absent	Not	<b>Cactiform</b>	1
<i>E. alluaudii</i>	all	22.5	780	2.10	0.44	Absent	<b>Strong</b>	Tree	73
<i>E. ambarivatoensis</i>	amba <sup>R</sup>	26.0	1635	−3.13	1.33	Absent	Not	Shrub	9
<i>E. ambovombensis</i>	ambo	23.0	559	2.53	0.82	<b>Present</b>	<b>Strong</b>	Cham	5
<i>E. analavelonensis</i>	ana	20.5	760	3.56	−0.04	Absent	Not	Shrub	5
<i>E. ankaranae</i>	anke <sup>R</sup>	25.6	1486	−2.86	1.09	Absent	Not	Tree	15
<i>E. ankarensis</i>	anks	25.9	1502	−3.01	1.29	Absent	Not	<b>Cactiform</b>	10
<i>E. banae</i>	ban	24.0	484	2.35	2.00	<b>Present</b>	Not	Shrub	6
<i>E. beharensis</i>	beh	24.0	637	1.92	1.31	<b>Present</b>	<b>Strong</b>	Shrub	12
<i>E. berevoensis</i>	bere	26.5	1106	−0.16	3.10	<b>Present</b>	Not	Shrub	5
<i>E. berorohae</i>	bero	25.9	791	1.68	3.05	<b>Present</b>	Not	Shrub	1
<i>E. boissieri</i>	bois <sup>L</sup>	23.7	2894	−4.32	−5.39	Absent	Not	<b>Cactiform</b>	4
<i>E. bongolavensis</i>	bong	26.6	1500	−1.41	2.43	Absent	Not	Tree	6
<i>E. bulbispina</i>	bulb	25.3	1193	−2.64	1.09	Absent	Not	Shrub	4
<i>E. capmanambatoensis</i>	capm <sup>R</sup>	25.5	1370	−2.99	−0.17	Absent	Semi	<b>Cactiform</b>	5
<i>E. capsaintemariensis</i>	caps <sup>R</sup>	23.5	422	2.11	1.10	<b>Present</b>	<b>Strong</b>	Cham	10
<i>E. cedrorum</i>	ced	23.9	396	2.21	2.22	Absent	<b>Strong</b>	Tree	4
<i>E. cylindrifolia</i>	cyl	23.6	874	1.19	0.21	<b>Present</b>	<b>Strong</b>	Cham	7
<i>E. decaryi</i>	dec	23.9	730	1.57	0.63	<b>Present</b>	<b>Strong</b>	Cham	4
<i>E. didiereoides</i>	did	21.0	856	2.78	−0.40	Absent	Not	Shrub	9
<i>E. duranii</i>	dur	19.6	1010	2.95	−1.84	Absent	Not	Shrub	7
<i>E. elliotii</i>	ell <sup>S</sup>	22.7	1821	−1.54	−4.05	<b>Present</b>	Semi	Tree	6
<i>E. erythrocucullata</i>	ery	24.7	749	1.37	2.25	<b>Present</b>	Not	Shrub	5
<i>E. francoisii</i>	fran	22.9	1607	−1.10	−3.29	<b>Present</b>	<b>Strong</b>	Cham	9
<i>E. geroldii</i>	ger <sup>L</sup>	24.4	1396	−2.21	−0.12	Absent	Semi	Shrub	4
<i>E. gottlebei</i>	got	24.1	674	2.69	2.69	<b>Present</b>	Not	Shrub	1
<i>E. guillauminiana</i>	gui	26.5	1565	−1.99	2.80	Absent	Not	Shrub	6
<i>E. hedyotoides</i>	hed	23.8	708	1.82	1.08	<b>Present</b>	Not	Tree	13
<i>E. hermanschwartzii</i>	herm <sup>R</sup>	25.6	1603	−2.91	1.18	Absent	Not	<b>Cactiform</b>	6
<i>E. horombensis</i>	hor	20.5	840	3.14	−0.42	Absent	Not	<b>Cactiform</b>	6
<i>E. itremensis</i>	itr <sup>S</sup>	17.1	1322	3.92	−3.41	<b>Present</b>	Not	Geophyte	3
<i>E. kondoi</i>	kon	24.1	376	2.07	2.32	<b>Present</b>	Not	Shrub	8
<i>E. labatii</i>	lab <sup>L</sup>	26.0	1655	−3.15	1.29	<b>Present</b>	Not	Cham	3
<i>E. leuconeura</i>	leu	24.6	1278	−2.19	0.54	Absent	Not	<b>Cactiform</b>	10
<i>E. lophogona</i>	lop <sup>S</sup>	22.9	2172	−2.19	−4.57	Absent	Not	<b>Cactiform</b>	12
<i>E. mahabobokensis</i>	mahb	22.6	757	2.75	1.08	<b>Present</b>	Not	Tree	3
<i>E. mahafalensis</i>	mahf	24.0	493	2.36	1.75	<b>Present</b>	Not	Shrub	6
<i>E. mangelsdorffii</i>	mang <sup>L</sup>	21.6	1842	−0.92	−3.46	Absent	Semi	Shrub	1
<i>E. martinae</i>	mar <sup>R</sup>	25.9	1610	−3.04	1.30	Absent	Not	Tree	9
<i>E. milii</i> var. <i>longifolia</i>	mil.l	19.8	918	3.30	−1.13	Absent	Not	Shrub	4
<i>E. milii</i> var. <i>milii</i>	mil.m	18.9	1183	2.99	−1.81	Absent	Not	Shrub	2
<i>E. millotii</i>	mill <sup>S</sup>	25.2	1420	−2.92	−0.80	Absent	Semi	<b>Cactiform</b>	6
<i>E. moratii</i>	mor <sup>L</sup>	25.5	1320	−1.08	2.22	<b>Present</b>	Not	Cham	8
<i>E. neohumbertii</i>	neo <sup>R</sup>	25.9	1434	−2.97	1.35	Absent	Semi	<b>Cactiform</b>	12
<i>E. pachypodioides</i>	pach <sup>R</sup>	25.9	1627	−3.02	1.26	Absent	Not	<b>Cactiform</b>	10
<i>E. parvicynthophora</i>	parv	24.2	477	2.35	2.52	<b>Present</b>	<b>Strong</b>	Cham	5
<i>E. paulianii</i>	pau	25.1	1293	−0.28	2.04	Absent	Not	<b>Cactiform</b>	6
<i>E. pedilanthoides</i>	ped	26.5	1540	−2.08	3.05	<b>Present</b>	Not	Shrub	7
<i>E. perrieri</i>	per	24.6	1563	−1.48	1.38	Absent	Not	<b>Cactiform</b>	10
<i>E. aff. primulifolia</i> var. <i>primulifolia</i>	prim <sup>L</sup>	18.3	1376	3.00	−2.65	<b>Present</b>	Not	Geophyte	9
<i>E. aff. primulifolia</i> — <i>Horombe</i>	prim.h	20.6	847	3.14	−0.53	<b>Present</b>	Not	Geophyte	4
<i>E. aff. primulifolia</i> — <i>Isalo</i>	prim.i	21.2	792	2.89	0.22	<b>Present</b>	Not	Geophyte	3
<i>E. aff. pyrifolia</i> — <i>Aldabra</i>	pyr.ald	26.0	1077	−2.88	0.91	Absent	Not	Tree	11
<i>E. aff. pyrifolia</i> — <i>Assumption</i>	pyr.as	26.1	1085	−2.94	0.96	Absent	Not	Tree	7
<i>E. aff. pyrifolia</i> — <i>Maurice</i>	pyr.m	22.5	1852	−1.58	−3.89	Absent	Not	Tree	7
<i>E. aff. pyrifolia</i> — <i>Seychelles</i>	pyr.s <sup>R</sup>	26.0	2213	−6.05	−2.10	Absent	Not	Tree	11
<i>E. quartizicola</i>	qua <sup>S</sup>	16.6	1332	4.10	−3.73	<b>Present</b>	Not	Geophyte	20
<i>E. rangovalensis</i>	rang <sup>L</sup>	19.9	1804	0.59	−4.01	<b>Present</b>	Not	Tree	18
<i>E. robieltonae</i>	rob	25.3	1487	−3.02	−1.00	Absent	Not	Shrub	2
<i>E. rossii</i>	ros	25.2	717	1.32	2.68	<b>Present</b>	Not	Shrub	7
<i>E. sakarahaensis</i>	sak	24.1	666	2.78	2.59	<b>Present</b>	Not	Shrub	4
<i>E. suzannae</i> — <i>marnierae</i>	suz	23.3	875	2.33	1.25	<b>Present</b>	<b>Strong</b>	Cham	1
<i>E. tardieuana</i>	tar <sup>R</sup>	23.1	2412	−2.71	−4.09	Absent	Semi	Shrub	5
<i>E. thuarsiana</i>	thu <sup>S</sup>	23.6	2903	−4.41	−5.64	Absent	Semi	Tree	7
<i>E. tulearensis</i>	tul	24.0	387	2.11	2.27	<b>Present</b>	<b>Strong</b>	Cham	6
<i>E. viguieri</i>	vig	26.1	1324	−2.00	2.19	Absent	Not	<b>Cactiform</b>	23
<i>E. waringiae</i>	war	21.9	886	2.22	−0.27	<b>Present</b>	<b>Strong</b>	Cham	1

Note: Substrate is indicated with a superscript above the taxon abbreviation (R = rock outcropping, S = sand, L = laterite). Character states in bold are those contrasted against the other state(s) in phylogenetic logistic regression.

outliers" are found on edaphically arid substrates (Table 1): *E. lophogona* grows on sand, and thus, at a microenvironmental scale, is subjected to aridity, a phenomenon arising from the interaction between climate and substrate (Nobel 1983; McAuliffe 1994; Schwinning and Ehleringer 2001; Schwinning 2010). We return to this point below regarding niche conservatism versus lability.

There is still much to be learned about the resource strategies of succulent plants. In particular, water and carbon dynamics may be intimately intertwined in shaping growth form variation in environments with seasonal precipitation. In *Euphorbia* GDD, tubers are associated with the longest drought season and thus the shortest wet season—that is, they are associated with the need to mobilize both water and carbon rapidly to deploy temporary photosynthetic organs (drought-deciduous leaves) and upregulate the physiological processes necessary to take advantage of a short window of opportunity for resource acquisition, growth, and reproduction. That is, water stored in tubers may support photosynthesis on a seasonal time scale, as has recently been shown for stem-stored water in baobabs (Chapotin et al. 2006a, 2006b), rather than the diurnal time scale at which stem-stored water in cacti supports photosynthesis.

Herbivory and fire, in addition to climate, are thought to interact to shape growth form variation in arid and semiarid environments (Burgess 1995). However, herbivory seems an unlikely explanation for shifts from above-ground to below-ground water storage, since cactiform stem succulents have evolved successful defenses against herbivory, both physical and chemical (e.g., spines and toxic sap). Depending on fire intensity and frequency, cactiform stem succulents can be quite vulnerable to burning (Thomas 1991; Thomas and Goodson 1992; Pfab and Witkowski 1999), whereas below-ground forms of succulence are better designed to survive fire (Lesica 1999; Tyler and Borchert 2002; Proches et al. 2006). However, the prehuman fire regime of Madagascar is quite contentious (Lowry et al. 1997), making it difficult to conclude whether fire played a role in shaping growth form in *Euphorbia* GDD.

#### Climatic Niche Evolution versus Conservatism

At first glance, climatic niches seem quite labile in *Euphorbia* GDD. Although most species are found in places that are relatively warm (22–26°C, MAT) and dry (50–150 cm, MAP; Fig. 6), there are several exceptions: certain taxa live in cooler places, such as *E. quartziticola*, *E. itremensis*, *E. primulifolia* var. *primulifolia*, *E. milii* var. *milii*, and *E. milii* var. *longifolia* (of the central highlands), and others occur in wetter places, such as *E. boissieri*, *E. thuarsiana*, *E. tardieuana*, *E. aff. pyrifolia* Seychelles, and *E. lophogona* (of Madagascar's eastern forests and the Seychelles Islands). Across the *Euphorbia* GDD taxa that we sampled, MAP ranges from 38 cm per year to

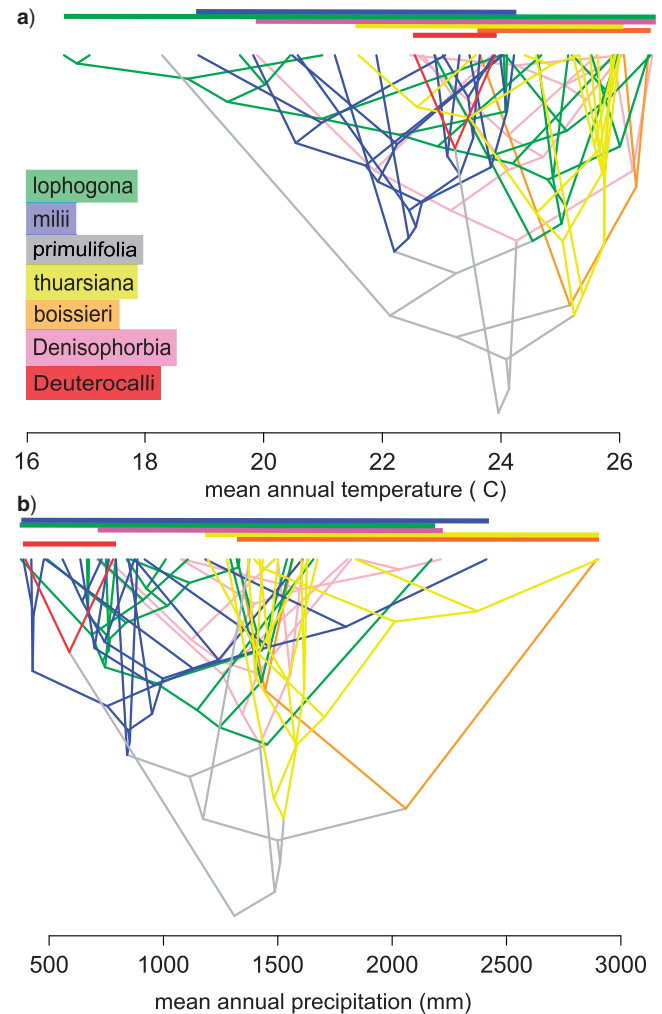


FIGURE 6. a) Trimmed phylogeny of *Euphorbia* GDD (as in Fig. 3) plotted with respect to MAT. MAT data for the extant taxa are shown in Table 1; ancestral reconstruction of MAT used the “pic” method in traitgram (package picante, R). Colors indicate the seven strongly supported subclades illustrated in Figure 3. Horizontal bars indicate the range of values for each subclade. b) As in a, but MAP.

more than 290 cm per year, nearly a 10-fold difference, and MAT from 17°C to 27°C (Table 1).

Multiple, independent colonizations of mesic environments are suggested by the fact that the species occupying these areas are derived from five separate, well-supported subclades (Figs. 2 and 6b): section *Deuterocalli* (red); section *Denisophorbia* (pink); a pair of sizeable clades with species in section *Goniostema* that are found in northern Madagascar, which we refer to as the “*thuarsiana*” clade and the “*boissieri*” clade (after the first-named species in each; yellow and orange, respectively); *E. primulifolia* var. *primulifolia* (gray); and another pair of clades in section *Goniostema* whose members are mostly found in southern Madagascar, hereafter the “*milii*” and “*lophogona*” clades (blue and green, respectively; also after the oldest named species in each; Fig. 6a). Multiple, independent colonizations



are also suggested, to a lesser degree, for the invasion of cooler environments (Fig. 6a).

This appears to paint a picture of niche lability, until one takes into consideration substrate and scale. Many of the *Euphorbia* GDD taxa occur on substrates—rock outcrops, sands, and laterite soils (Table 1)—that can be expected to yield limited pulses of available moisture, even in the face of considerable precipitation, given the shallow root profile of the plants. Further, temperature and precipitation estimated at the 1 km<sup>2</sup> scale (macroenvironmental conditions) can be profoundly affected not just by substrate, but also slope, aspect, and local topography (Kumar et al. 1997; Austin Van Niel 2011). We conclude that niche lability among members of *Euphorbia* GDD may not be as great as it first seems. Instead, we suspect microenvironmental specialization allows for a degree of niche conservatism (toward a warm, dry niche) in the face of considerable macroenvironmental variation. Conclusions about niche lability derived from macroscale habitat data should be viewed with caution.

#### Testing for Trait–Climate Relationships—A Methodological Note

OMI axes 1 and 2 were not significantly related to variation in succulence characters (results available from the authors). This raises an important point: species do not evolve in response to principal axes of climate variation constructed by multivariate ordination, but rather they respond to physiologically important climatic variation, which may or may not load parallel to such statistically constructed principal axes of climate variation. In some cases, traits may vary with principal axes of climate variation (e.g., Boucher et al. 2012); in other cases, they may not. Here, MAP and MAT load on the diagonals of the ordination diagram of OMI axis 1 versus 2 (Fig. 3b), and it is along these diagonals that traits related to succulence vary (Fig. 4). Tests of trait–climate relationships must navigate between the statistical rock of multicollinearity among climate variables and the statistical hard place of elevated type 1 error with multiple tests, but the solution of using principle axes of climate variation for trait–climate tests is not without pitfalls. Interesting and important trait–climate patterns can be overlooked if traits are only related to principal components. We suggest that examination of the distribution of traits in an ordination diagram is necessary for a robust analysis of trait–climate relationships, and that ordination should ultimately be used as an exploratory tool to refine hypotheses about trait–climate relationships.

#### CONCLUSION

Although it has long been understood that plants are shaped by their environment (Raunkiaer 1934; Daubenmire 1974; Niklas 1997), we still have much to

learn about how temperature, precipitation, and their seasonality interact with nonclimatic factors—such as fire, herbivory, and substrate—to shape plant form and function. Here, a study integrating trait, climate, and phylogenetic data for a remarkable radiation, in one of the world's most exciting laboratories of evolution (Madagascar), yields insight into the fascinating forms of succulent plants and the climatic conditions favoring different forms of succulence. This has implications for the conservation of succulent species, many of which are threatened: if different forms of succulence are favored by different climatic regimes—if their morphology indeed is the result of adaptation to environment—we can expect these iconic organisms to be affected by climate change. This is particularly significant in Madagascar, one of the world's most important biodiversity hotspots, where succulent and spiny *Euphorbia* are a dominant component of many vegetation types. In fact, climate effects on succulent *Euphorbia* seem to have already taken hold in South Africa, where there is widespread mortality of *Euphorbia ingens* trees (Van der Linde et al. 2012).

#### SUPPLEMENTARY MATERIAL

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.vq6mp>.

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