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Macroevolutionary trends and diversification dynamics in Atripliceae (Amaranthaceae s.l., Chenopodioideae): a first approach

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• **Background and aims** Atripliceae evolved and diversified by dispersals and radiations across continents in both hemispheres, colonizing similar semi-arid, saline–alkaline environments throughout the world. Meanwhile, its species developed different life forms, photosynthetic pathways, mono- or dioecy, and different morphological features in flowers, fruiting bracteoles and seeds. In this study, we introduce a first approach to the macroevolutionary patterns and diversification dynamics of the Atripliceae to understand how time, traits, speciation, extinction and new habitats influenced the evolution of this lineage.
• **Methods** We performed molecular phylogenetic analyses and clade age estimation of Atripliceae to apply time-, trait- and geographic-dependent diversification analyses and ancestral state reconstructions to explore diversification patterns within the tribe.
• **Key results** Opposite diversification dynamics within the two major clades of Atripliceae, the Archiatriplex and Atriplex clades, could explain the unbalanced species richness between them; we found low mean speciation rates in the Archiatriplex clade and one shift to higher speciation rates placed in the branch of the Atriplex core. This acceleration in diversification seems to have started before the transition between C_3 and C_4 metabolism and before the arrival of *Atriplex* in the Americas, and matches the Mid-Miocene Climatic Optimum. Besides, the American species of *Atriplex* exhibit slightly higher net diversification rates than the Australian and Eurasian ones. While time seems not to be associated with diversification, traits such as life form, photosynthetic pathway and plant sex may have played roles as diversification drivers.
• **Conclusions** Traits more than time played a key role in Atripliceae diversification, and we could speculate that climate changes could have triggered speciation. The extreme arid or saline environments where Atripliceae species prevail may explain its particular evolutionary trends and trait correlations compared with other angiosperms and highlight the importance of conservation efforts needed to preserve them as genetic resources to deal with climatic changes.

Key words: Amaranthaceae, America, ancestral state reconstruction, *Atriplex*, Atripliceae, BAMM, BiSSE, diversification, GeoHiSSE, HiSSE, MuSSE, macroevolution.

INTRODUCTION

Atripliceae Duby is one of the four tribes currently accepted within the subfamily Chenopodioideae Burnett (Fuentes-Bazán *et al.*, 2012*b*) in the frame of the most recent hypothesis of the Amaranthaceae/Chenopodiaceae alliance (Morales-Briones *et al.*, 2020); it was recircumscribed by Kadereit *et al.* (2010) based on molecular and morphological evidence, including *Atriplex* L., its sister genus *Halimione* Aellen, and nine other satellite genera (see below). Most recently, molecular phylogenetic analyses of Chenopodioideae, including a broad sampling of *Chenopodium* s.l. and allied genera (Fuentes-Bazán *et al.*, 2012*a*, *b*; Sukhorukov *et al.*, 2018), confirmed that *Chenopodium* L. is not monophyletic. Fuentes-Bazán *et al.* (2012*b*) extended the circumscription of Atripliceae to be monophyletic by including the genera *Chenopodium* s.str.,

Chenopodiastrum S. Fuentes, Uotila & Borsch, *Lipandra* Moq. and *Oxybasis* Kar. & Kir. Notwithstanding, we follow in this paper the tribe Atripliceae *sensu* Kadereit *et al.* (2010) (= Atripliceae s.str. according to Fuentes-Bazán *et al.*, 2012*b*) considering only the genus *Atriplex* and its related genera.

Like many other members of the subfamily, Atripliceae seems to have its geographic origin in Eurasia, ~32–31 Mya in the Early Oligocene (Kadereit *et al.*, 2010). Multiple radiations to other continents have occurred in the tribe, reaching Australia and the Americas at least two times each (Kadereit *et al.*, 2005, 2010; Brignone *et al.*, 2019).

Phylogenetic analyses using both nuclear and plastid loci show an early split in Atripliceae into two well-supported clades: the Archiatriplex and the Atriplex clades. The Archiatriplex clade includes monotypic or two- to four-species genera; four genera are native to northwest America: *Extriplex* E.H. Zacharias

(two species), *Proatriplex* (W.A. Weber) Stutz & G.L. Chu (one species), *Grayia* Hook. & Arn. (four species) and *Stutzia* E.H. Zacharias (two species); two monotypic genera are native to South Africa: *Exomis* Fenzl ex Moq. and *Manochlamys* Aellen; one genus is native to China: *Archiatriplex* G.L. Chu (one species); one genus to Tibet: *Microgynoecium* Hook. f. (one species); and one genus to South America: *Holmbergia* Hicken (one species). The Atriplex clade includes two genera:

Halimione (three species from Europe and Western Asia) and *Atriplex* (worldwide). The number of species in *Atriplex* is ~250–300 (Kadereit *et al.*, 2010; Zacharias and Baldwin, 2010; Brignone *et al.*, 2019); it is the core genus of the whole tribe and comprises 91 % of the species in Kadereit *et al.*'s (2010) tribe circumscription. Figures 1 and 2 show morphological variability of some species from the Archiatriplex and Atriplex clades, respectively.
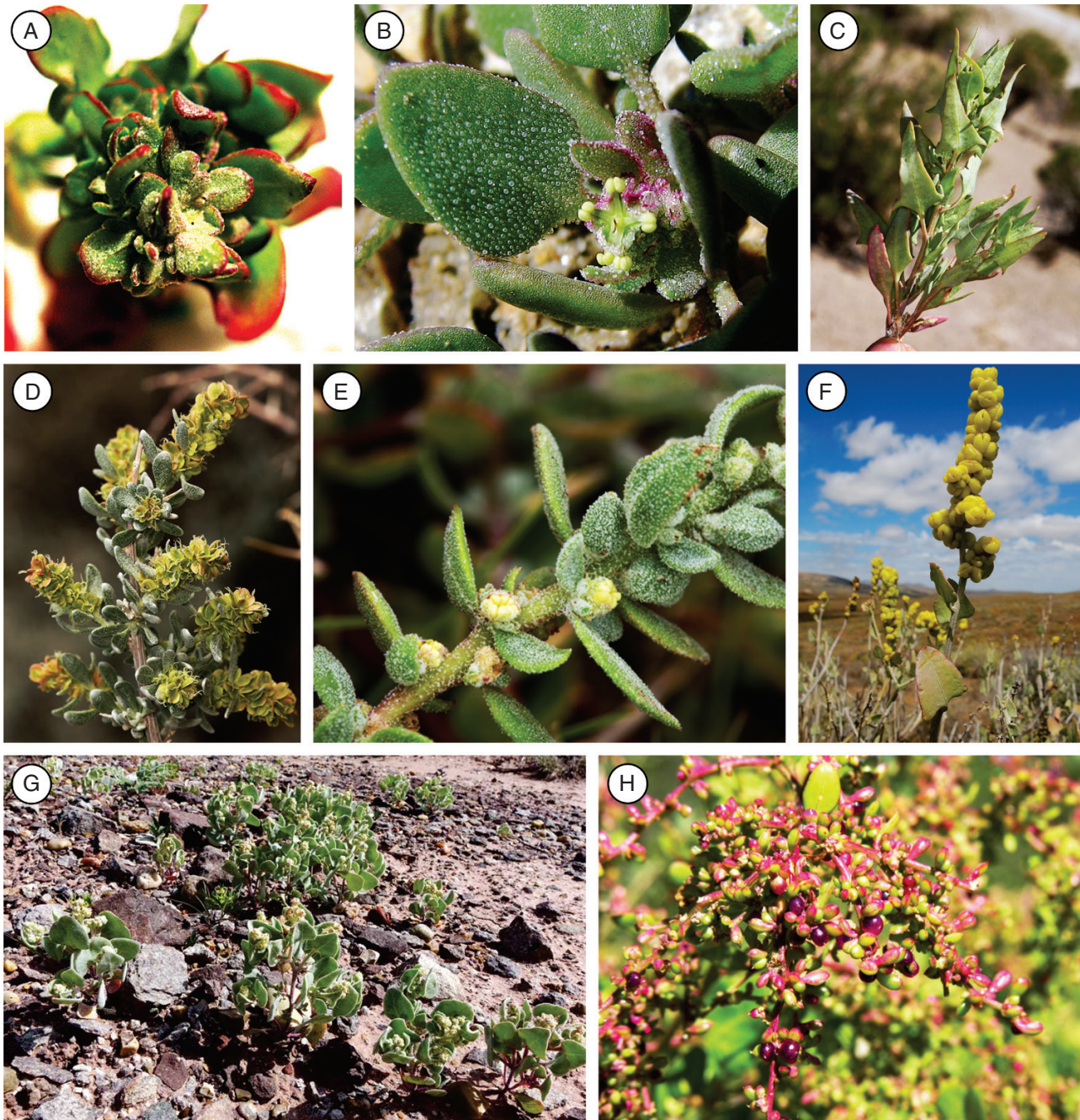

FIG. 1. Representative taxa of the Archiatriplex clade. (A) *Exomis microphylla*, Haarwegskloof Renosterveld Reserve, South Africa. (B) *Microgynoecium tibeticum*, near Tsokar Lake, India. (C) *Stutzia covillei*, California, USA. (D) *Grayia spinosa*, California, USA. (E) *Extriplex californica*, California, USA. (F) *Manochlamys albicans*, Namaqualand-Soebatsfontein, South Africa. (G) *Proatriplex pleiantha*, near Four Corners, USA. (H) *Holmbergia tweedii*, Santa Fe, Argentina. Credits: (A) Yvette van Wijk; (B) Prashant Awale; (C–E) Steve Matson; (F) Ute Schmiedel; (G) Al Schneider; (H) José F. Pensiero.
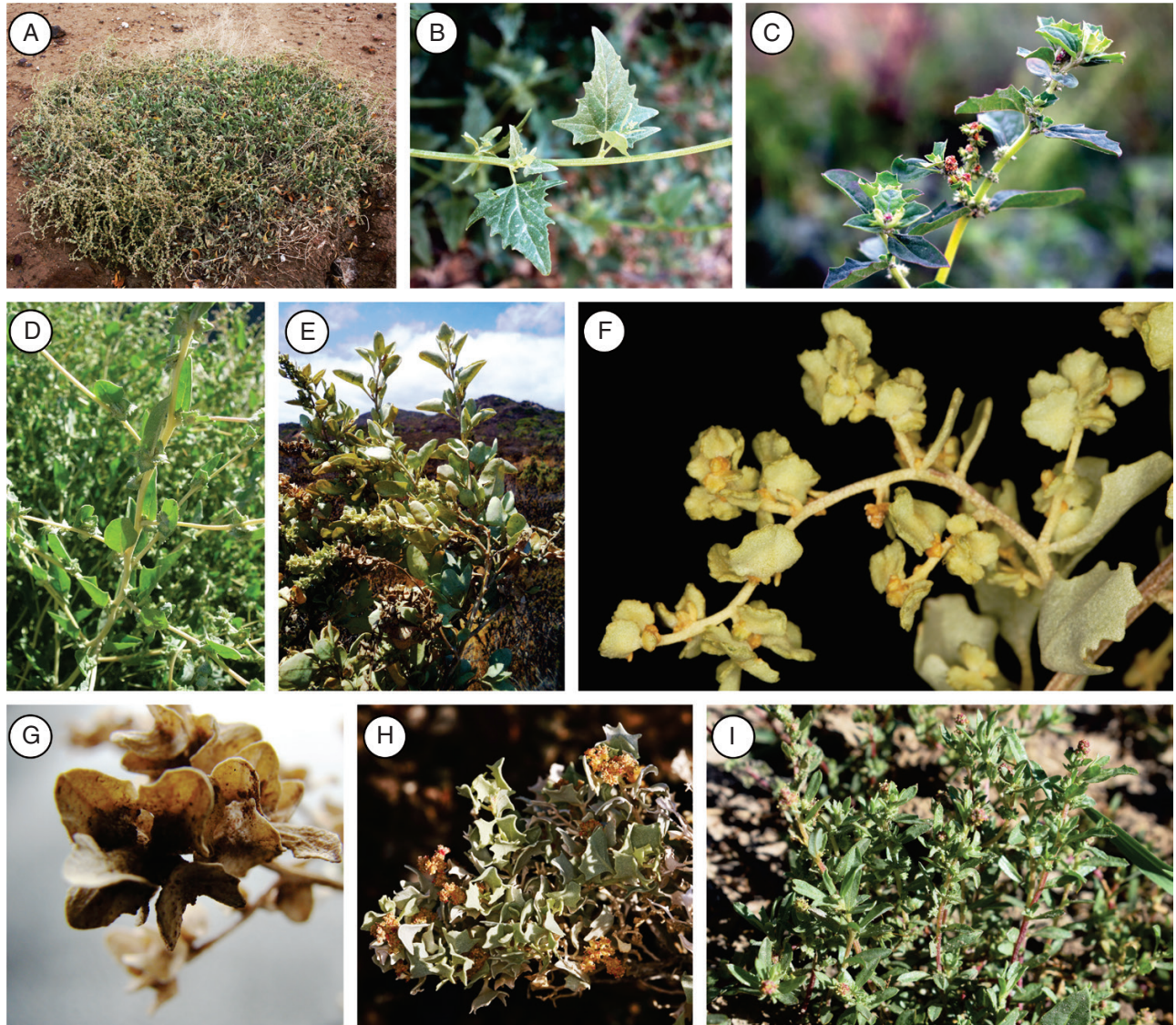
Fig. 2. Representative taxa of the Atriplex clade. (A) *Halimione verrucifera*, Orenburg province, Russia. (B) *Atriplex micrantha*, a C_3 species in Chubut, Argentina. (C) *Atriplex suberecta*, from the Australia 2 clade, growing in Santa Fe, Argentina. (D) *Atriplex rosea*, a Eurasian species, photographed in Chubut, Argentina; (E) *Atriplex nummularia*, from the Australia 1 clade, recorded in Coquimbo, Chile. (F) *Atriplex cordubensis*, from the SA2 clade, Salta, Argentina. (G) *Atriplex argentina*, from the SA1 clade, San Juan, Argentina. (H) *Atriplex hymenelytra*, a North American species, California, USA. (I) *Atriplex montevidensis*, from the SA3 clade, Santa Fe, Argentina. Credits: (A) Alexander Sukhorukov; (B, C, E, I) Nicolás F. Brignone; (D) Diego R. Quintana; (F) Christian Zanotti; (G) María E. Múlgura; (H) Steve Matson.

South American species of *Atriplex* (45) are almost fully included in the current phylogenies (Brignone *et al.*, 2019), North American species (~46) are less represented, and the sampling of Eurasian (~26 spp.) and Australian (~66 spp.) taxa needs to be increased and African representatives need to be incorporated. In Africa, the diversity of Atripliceae is less known so far; only a few species of *Atriplex* are native to tropical Africa, but a precise number of species has not been published yet (Sukhorukov, 2012; Sukhorukov *et al.*, 2016).

Within the Atriplex clade, Atriplex s.str. contains successive C_3 lineages that are sisters to the C_4-*Atriplex* clade. The C_4-*Atriplex* clade shows geographically structured internal clades: two Eurasian clades, two Australian clades and the American clade (Kadereit *et al.*, 2010; Zacharias and Baldwin, 2010, Brignone *et al.*, 2019). One of the Australian clades (Australia 1) is sister to the American clade. The American clade includes three South American lineages (SA1, SA2 and SA3) and a grade of isolated North American species. Brignone *et al.* (2019) revealed that *Atriplex* reached South America first (~10.4 Mya), where lineages SA1 and SA2 underwent *in situ* diversification and evolved sympatrically. North America was colonized by a South American *Atriplex* ancestor, and later one lineage returned to South America (SA3). As mentioned before, the American representatives, mainly from South America, are the best known in terms of taxonomy, phylogeny and biogeography (Zacharias, 2007; Zacharias and Baldwin, 2010; Brignone *et al.*, 2016, 2019; Brignone, 2020*a*, *b*).

Atriplex includes herbs, shrubs or subshrubs; species are annual or perennial, monoecious or dioecious, with representatives of the C_3 and C_4 photosynthetic pathways, and they usually inhabit arid or semi-arid regions, and saline or alkaline soil environments worldwide. The species of *Atriplex* are characterized by their high biomass productivity, high tolerance to drought and salinity, and high efficiency in the use of solar radiation (Múlgura de Romero, 1981; Zacharias and Baldwin, 2010). In several countries, some species of *Atriplex* are used to manage desertification, as alternative forage, for firewood or bioenergy production (Malcom, 2000; Mulas and Mulas, 2004; Paneque, 2018), and even for bioremediation of soils contaminated with heavy metals (Moray *et al.*, 2015; Vromman *et al.*, 2016, 2018).

Traditional traits considered in taxonomic delimitation within Atripliceae, such as the life form, the photosynthetic pathway (including Kranz or non-Kranz leaf anatomy), the degree of connation of the two modified tepal lobes when present (traditionally called 'accrescent bracteoles'), plant sex, and the orientation of the embryonic radicle, were studied for classification purposes and in a phylogenetic framework (Flores-Olvera and Davis, 2001; Sukhorukov, 2006; Flores-Olvera *et al.*, 2011; Brignone, 2020*a*). They mostly did not define monophyletic groups, and they even did not show phylogenetic signal. Even so, some of these features are of great importance in evolutionary biology, i.e. life form, sex systems and the photosynthesis pathway have been studied in the context of angiosperm evolution and diversification. The evolution from woody to herbaceous habit is considered a major trend in the angiosperms (Carlquist, 2013; Frankiewicz *et al.*, 2022); nevertheless, some hypotheses have been evoked to explain secondary woodiness (reversal from herbaceous to woody habit). Woody species (chamaephytes and phanerophytes life forms) could be better adapted to dry lands than their herbaceous relatives (therophytes and hemicryptophytes) because woody plants are more resistant to drought-induced embolism (cavitation theory), which can lead to lower photosynthetic rates (Lens *et al.*, 2013). Also, woodiness could have evolved in response to a stable, mild climate, allowing a prolonged life span (moderate climate theory; Carlquist, 1974). Concerning plant sex, dioecy has been related to increased extinction rates and lower species richness compared with non-dioecious sister clades, supporting the hypothesis that dioecy may be an evolutionary dead end (Heilbuth, 2000). But this hypothesis has been recently contradicted by macroevolutionary analyses that show a positive effect of dioecy on diversification (Kafer *et al.*, 2014; Muyle *et al.*, 2021). Dioecy is considered successful in dryer, semi-arid environments (Case and Barret, 2004), and it is also related to tropics and islands (Vamosi *et al.*, 2003). Additionally, dioecy has been correlated to traits such as woody habit, abiotic pollination and fleshy fruits (Vamosi *et al.*, 2003; Vamosi and Vamosi, 2004). Finally, the C_4 photosynthetic pathway has evolved as an adaptation to high light intensities, high temperatures and dryness (Edwards *et al.*, 2010), and the shift from C_3 to C_4 was surely a diversification driver in many plant groups. Currently, we do not have a clear understanding of how and when these traits could have shaped Atripliceae radiation. Also, time could have been crucial in the past evolutionary processes of the tribe; it must be considered that high species richness would be attributed to the age of the

group, allowing multiple speciation events (Frankiewicz *et al.*, 2022).

Spatiotemporal diversification for the tribe and subclades has not been studied so far, and we do not know the causes for the unbalance of species richness observed among sister groups. Identifying and understanding causes that drive the diversification and evolution of the worldwide Atripliceae may provide insights into the potential impacts of future climate changes with associated aridization and salinization. The diversification of different chenopods, both native and colonizing species, was certainly dependent on several factors, such as time, inherent genetic variability and the availability of suitable habitats (Kadereit *et al.*, 2005). Major goals in ecology and evolutionary biology are identifying the causes of species-rich rapid radiations, comprehending which traits trigger diversification, and understanding patterns of species richness among clades (Kozak and Wiens, 2016; Lagormarsino *et al.*, 2016).

The goal of this research is to elucidate, as a first approach, the diversification dynamics in Atripliceae, especially in the Americas, where sampling, and morphological, phylogenetic and biogeographic studies are more advanced. We look for diversification drivers in Atripliceae by time- and trait-dependent analyses and by ancestral-state reconstruction. We attempt to understand the impact of the C_3–C_4 transition in the macroevolutionary history of the tribe, and the role of dioecy and life form in its diversification.

MATERIALS AND METHODS

Taxon sampling

We based our analyses on data from the Atripliceae phylogeny of Brignone *et al.* (2019). Taxon sampling included 109 taxa: 107 representatives (~36 %) of Atripliceae *sensu* Kadereit *et al.* (2010) and two species of *Chenopodium* as outgroup. Of the 107 ingroup taxa, 13 correspond to members of the Archiatriplex clade, and the remaining 94 belong to the Atriplex clade. Only one voucher per species was included. Not all lineages in the tribe Atripliceae were equally represented; the Archiatriplex clade was fully sampled, and the Atriplex clade included ~50 % of its species. South American species were sampled in 93 %. To improve the inference precision of the evolutionary processes, we correct for incomplete taxon sampling in every diversification analysis performed. Supplementary Data Material S1 (Tables A–C) shows species richness data and the sampling fraction for each clade within Atripliceae.

Phylogenetic analyses and clade age estimation

Brignone *et al.* (2019) explored maximum parsimony, Bayesian inference and maximum likelihood approaches, and made independent analyses for nuclear (the ribosomal intergenic region, ITS, and the external transcribed spacer, ETS) and chloroplast (the *atp*B–*rbc*L spacer) data. They found that the nuclear phylogeny was much more resolved than the *atp*B–*rbc*L spacer tree, and that this poorly resolved tree precluded incongruence among partitions. To obtain a trustworthy phylogeny and support values, we here applied Bayesian

inference with the concatenated nuclear + chloroplast data matrix (ITS + ETS + *atp*B–*rbc*L spacer) using the parameters of Brignone *et al.* (2019) applied in MrBayes v.3.2.7a (Ronquist *et al.*, 2012) in the CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010). FigTree v.1.4.3 (available at http://tree.bio.ed.ac.uk/software/figtree/) was used to display the consensus topology and posterior probability (PP) values.

The time-calibrated phylogeny was estimated by Bayesian inference in BEAST2 v.2.5 (Bouckaert *et al.*, 2019) at the CIPRES Science Gateway using the nuclear sequences (ITS + ETS) comprising the same 109 taxa as in the MrBayes analysis. We did not use the *atp*B–*rbc*L spacer matrix because some critical topics have been raised regarding chloroplast-based dating (Bernhardt *et al.*, 2017; Vargas *et al.*, 2017). Additionally, Angelis *et al.* (2018) recommended that heterogeneous partitions or concatenated nuclear + chloroplast sequence matrixes should not be used for estimation of species divergence times. We followed Brignone *et al.* (2019) for secondary calibration points and for all analysis parameters, but as both nuclear markers are evolutionarily linked we analysed the combined nuclear ITS + ETS dataset (not only the ITS dataset). A maximum clade credibility (MCC) tree was constructed in TreeAnnotator v.2.5.1 and visualized in FigTree.

Trait coding

Trait-states were documented from herbarium specimens, plants in the field, photographs and online databases (JSTOR Global Plants database, https://plants.jstor.org/; Tropicos, https://tropicos.org/home), and were also obtained from an extensive search in relevant literature (Bassett *et al.*, 1983; Wilson, 1984; Chu, 1987; Stutz *et al.*, 1990; Flores Olvera, 1992, 2003; Flores Olvera and Davis, 2001; Welsh, 2003; Sukhorukov, 2006; Kadereit *et al.*, 2010; Zacharias and Baldwin, 2010; Brignone *et al.*, 2016; Brignone, 2020*b*). We scored two binary traits and one multistate trait for diversification analyses and ancestral-state reconstructions: photosynthesis pathway (C_3 or C_4), plant sex (dioecious or monoecious), and life form (therophytes, hemicryptophytes, chamaephytes and nanophanerophytes, according to Raunkiaer, 1934). Even though other plant sex conditions occur in the family, Atripliceae, as adopted here, contains only dioecious or monoecious species, with unisexual flowers (Kadereit *et al.*, 2010). As our primary focus is to elucidate the dioecy as a diversification driver in the tribe, we do not consider the way in which flowers achieve sexuality (Flores-Olvera *et al.*, 2011) and we assigned the state 'dioecious' to those species whose populations bear carpellate and staminate flowers on separate plants, and 'monoecious' to species that exhibit both carpellate and staminate flowers on the same plant. In this way, much plant sex evolution research is based on the binary trait dioecious/monoecious (e.g. Renner and Won, 2001; Flores Olvera, 2003). All trait data are provided in Supplementary Data Material S2.

Ancestral-state reconstructions

For ancestral-state reconstructions we conducted stochastic character mapping (SCM, Huelsenbeck *et al.*, 2003; Bollback,

2006) implemented in R v.4.0.2 (R Core Team, 2020) using the package *phytools* v0.7.47 (Revell, 2012) and the collection of trees from the posterior distribution obtained in BEAST2. For each binary trait we tested three alternative transition models: ER (equal rates), if all permitted transitions occur at the same rate; SYM (symmetric), if backward and forward transitions occur at the same rate; and ARD (all-rates-different), allowing transitions to occur at different rates. The best model was selected based on lowest Akaike information criterion (AIC) value (Burnham and Anderson, 2002). The ER transition model was used to life form. We performed 1000 SCM simulations and plotted the PP of each trait state on the selected nodes by averaging the state frequencies across all simulations (Revell, 2014). Reconstructions with PP < 0.7 were considered as ambiguous.

Time-dependent diversification analyses

We assessed the temporal accumulation of lineages in Atripliceae with a lineages-through-time plot using the packages *ape* v5.4.1 (Paradis and Schliep, 2019) and *phytools* implemented in R over the collection of trees from the posterior distribution obtained in BEAST2. Also, to explore the effect of age on diversity, we tested for a relationship between crown age and species richness of target clades (Supplementary Data Material S1, Table A), using Spearman's rank correlation test using the R package *mass* v7.3.51.6 (Venables and Ripley, 2002).

To estimate diversification rates through the time and to identify shifts in the speciation dynamics in Atripliceae we employed BAMM v2.5 (Rabosky, 2014), and the R package *BAMMtools* v2.1.7 (Rabosky *et al.*, 2014). Before analysing data, we pruned the tree to remove outgroups. BAMM implements the reversible jump Metropolis Coupled Markov Chain Monte Carlo method and allows for both time-dependent speciation rates as well as discrete shifts in the rate and pattern of diversification (Rabosky, 2014). The specific priors were estimated with the function *setBAMMpriors* in *BAMMtools*; the prior on the expected number of shifts was assigned to 1.0 due to the small number of tips (<500) in the tree, the lambdaInitPrior was 1.45078393713706, the lambdaShiftPrior was 0.0398808693880764, and the muInitPrior was 1.45078393713706. We ran BAMM for 10 million generations, sampling parameters every 1000 generations. After discarding 10 % of the output as burn-in, the likelihood of all sampled generations was plotted in R, and effective sample size (ESS) values values for the likelihood and the inferred numbers of shifts were calculated using the package *coda* v0.19.3 (Plummer *et al.*, 2006) to assess convergence (ESS >>200). The 9000 post-burn-in files were used in *BAMMtools*: (1) to calculate Bayes factors for the number of distinct rate regimes; (2) to generate the phylorate plot, with the purpose of visualizing the speciation on the tree branches; (3) to plot the marginal shifts probability tree; (4) to obtain the 95 % credible set of rate shift configurations and to identify the one with the highest PP; (5) to obtain mean speciation, extinction and net diversification rates and 95 % confidence intervals (CIs) for main clades; (6) to perform the macroevolutionary cohort analysis, and; (7) to generate the speciation-through-time plots for specific lineages.

Finally, we compared the BAMM results with results obtained applying MEDUSA v0.954, a maximum-likelihood approach for estimating diversification rate shifts (Alfaro *et al.*, 2009), using the R package *geiger* v2.0.7 (Pennell *et al.*, 2014). MEDUSA fits increasingly complex birth–death diversification models to the tree and uses a stepwise AIC corrected for small sample size (AICc) approach to identify the optimal number of shifts. We incorporated incomplete taxon sampling in the analysis by using a taxonomic approach, where the tips of the phylogeny represent clades with associated species richness. To this end, the 107-taxon tree was condensed to an 18-tip tree, preserving the maximum amount of phylogenetic resolution possible, but allowing us to assign species richness unambiguously to tip lineages (see the Results section). MEDUSA analysis was conducted under a mixed model (pure birth and birth–death), and the AICc values were used for model comparison.

Trait-dependent diversification analyses

Rate heterogeneity across clades could reflect the existence of trait-dependent diversification. We tested whether the life form, photosynthesis pathway or plant sex is associated with changes in diversification rates in Atripliceae. For this purpose, we used the binary and multi-state speciation and extinction (BiSSE and MuSSE, respectively) models (Maddison *et al.*, 2007; FitzJohn *et al.*, 2009) implemented with the R package *diversitree* v0.9.14 (FitzJohn, 2012). For each trait we evaluated eight BiSSE/MuSSE models using maximum likelihood searches: (1) null model: speciation (λ), extinction (μ) and transition (q) rates were constrained to be equal; (2) full model: all parameters are free to vary; (3) *lambda.free* model: λ was allowed to vary between character states while μ and q remained equal between states; (4) *mu.free* model: μ was allowed to vary while λ and q remained equal; (5) *q.free model*: q was allowed to vary while λ and μ remained equal; (6) *lambda.mu.free* model: λ and μ were allowed to vary while q rates were constrained to be equal; (7) *lambda.q.free* model: λ and q were allowed to vary while μ rates were constrained to be equal; and (8) *mu.q.free model*: μ and q were allowed to vary while λ rates were constrained to be equal. The best-fit model was selected considering the lowest AIC value. We conducted a Bayesian BiSSE/MuSSE analysis with the best-fit model for each trait, and we obtained the posterior distributions of the parameters with the purpose of examining the confidence interval of the estimations. This was run for 10 000 generations using an exponential prior (FitzJohn *et al.*, 2009), and the first 10 % were discarded as burn-in. PP distribution plots for λ, μ, q and net diversification (r) rates were computed. We caution that SSE-family models could lead to frequent Type I errors under some circumstances (Rabosky and Goldberg, 2015) or simply cannot discern the possibility of a complex diversification process involving many unmeasured and codistributed traits (Beaulieu and O'Meara, 2016). To avoid this, we applied hidden state speciation and extinction analysis (HiSSE; Beaulieu and O'Meara, 2016) on the binary traits. The hidden or unmeasured state may drive differences in diversification rates among taxa in addition to (or instead of) the observed state. We tested five models (model details in Beaulieu and O'Meara, 2016) using the R package *hisse*

v1.9.18: the full BiSSE model; the character-independent diversification (CID) model, assuming that diversification is trait-independent but not constant across the tree; and three HiSSE models (the full HiSSE model, the HiSSE model for state 0, and the HiSSE model for state 1). The best-fit model was identified by AIC analysis.

Geographic state-dependent diversification analysis

We tested whether American species of *Atriplex* (state 1) have higher diversification rates than species absent from the Americas (collectively called non-American, state 2). We used the geographic hidden state speciation and extinction (GeoHiSSE) model (Caetano *et al.*, 2018) implemented in the R package *hisse*. First, we pruned our 107-taxon tree to a 90-taxon tree, leaving only the genus *Atriplex* (the Atriplex s.str. clade). Then, we ran the same four models presented in the GeoHiSSE vignette: two models with a range-independent diversification process, and two other models in which the range has an effect on the diversification rate of the lineages (each with either one or two rate classes), including or not hidden states: (1) dispersal parameters vary only, no range-dependent diversification; (2) canonical GeoSSE model, range effect on diversification; (3) GeoHiSSE model with one hidden trait, no range-dependent diversification; and (4) GeoHiSSE model with one hidden trait, range-dependent diversification. To evaluate the relative importance of each of the models in explaining the variation observed in the data, we computed Akaike weights (ω_i), and selected the model with the highest value. Finally, we average the models, and plotted the results onto the tree.

RESULTS

Phylogenetic analyses and estimated clade ages

The phylogenetic consensus tree based on the concatenated nuclear + chloroplast matrix (Supplementary Data Material S3, Fig. A) yielded a well-supported phylogenetic hypothesis, with the same topology as Brignone *et al.* (2019, see Fig. S2) but with some main branches better supported (e.g. C_4-*Atriplex* clade not supported in the nuclear tree, but with maximum support here). Atripliceae was divided into two major clades: Archiatriplex and Atriplex. Within the Atriplex clade, genus *Halimione* splits from genus *Atriplex* (Atriplex s.str. clade); C_3-*Atriplex* species were arranged in successive clades sister to the C_4 lineage. The C_4-*Atriplex* clade was recovered with maximum support, with internal geographically structured clades: Eurasia + Australia 2 clade, Eurasia clade, Australia 1 clade and American clade. The American clade included three subclades with South American species (SA1, SA2 and SA3) and a grade with North American species. The Eurasian, Australian, North American and South American species were not recovered as monophyletic groups.

The BEAST MCC tree (Supplementary Data Material S3, Fig. B) had the same topology for major groups as the 50 % majority-rule consensus tree obtained from MrBayes; minor differences are related to C_3-*Atriplex* species (grouped into two successive sister clades and two isolated species in the Bayesian tree, i.e. *Atriplex cana*, *A. lanfrancoi*, *Atriplex* sect. *Teutliopsis*,

and *Atriplex* sect. *Atriplex*; and two sister clades in the MCC tree, i.e. C_3-*Atriplex* 1 and C_3-*Atriplex* 2). Besides the expected ages of the calibration points, we found that Archiatriplex and Atriplex clades split 28.87 Mya [95 % highest posterior density (HPD) = 30.65–27.02 Mya], in the Middle Oligocene; genus *Halimione* diverged from Atriplex s.str. 24.72 Mya (95 % HPD = 27.7–21.46 Mya), and C_4 photosynthesis in *Atriplex* originated ~14 Mya (95 % HPD = 16.93–11.23 Mya), in the Middle Miocene. Divergence of the American clade started ~9 Mya (95 % HPD = 11.66–6.5 Mya), in the Later Miocene. All these node ages are similar to those in Brignone *et al.* (2019).

Ancestral-state reconstructions

The ER transition model best fitted the photosynthesis pathway trait, while the ARD model was best for the plant sex trait (Supplementary Data Material S4). The number of changes between states is also shown in Supplementary Data Material S4. Main node PP values of Figs 3 and 4 are presented in Supplementary Data Material S6.

The ancestral reconstruction of the life form within Atripliceae was ambiguous at the earliest nodes corresponding to the Atripliceae, Archiatriplex and Atriplex clades, genus *Halimione*, Atriplex s.str and C_3-*Atriplex* 1. For Atriplex core, C_4 *Atriplex* and Eurasia + Australia 2, reconstruction was ambiguous, but the ancestor could have been a therophyte (PP > 0.5). For the SA3 clade, ambiguity included mainly therophyte and chamaephyte states (both with PP < 0.5). The ancestor of the C_3-*Atriplex* 2 clade probably would have been a therophyte (PP = 0.74; Supplementary Data Material S5). The putative most recent common ancestor (MRCA) for the Australia 1 clade, American clade, SA1, SA2 and North-SA3 clades was reconstructed as nanophanerophyte (PP > 0.7, Supplementary Data Material S5).

The reconstruction of the photosynthesis pathway for the Atripliceae revealed only one change from the C_3 state to the C_4 in the ancestor of the C_4-*Atriplex* clade (Fig. 3). The putative MRCA for the Eurasia + Australia 2, Eurasia 2, Australia 1 and American clades was inferred to have a C_4 photosynthetic pathway. There were no reversions to C_3 photosynthesis in any of these groups.

For plant sex (Fig. 3), the MRCA for Atripliceae and Archiatriplex clades were inferred to be ambiguous, but probably monoecious (PP = 0.61 and 0.68, respectively). The MRCA of the Atriplex clade and *Halimione*, Atriplex s.str., Atriplex core, C_3-*Atriplex* 1, C_3-*Atriplex* 2, C_4-*Atriplex*, and Eurasia + Australia 2 clades were reconstructed as monoecious (PP = 0.72, 0.89, 0.78, 0.74, 0.85, 0.89, 0.71 and 0.87, respectively). Dioecy arose two times within the Archiatriplex clade (in *Exomis* and *Grayia*), and once in the Eurasia + Australia 2 clade (in *Atriplex vesicaria*). On the contrary, the MRCA of the American clade + Australian 1 clade was reconstructed as dioecious (PP = 0.96, not shown in Supplementary Data Material S5), and multiple shifts between monoecious and dioecious occurred within the group; MRCAs of the SA1 and SA3 clades were monoecious (PP = 0.78 and 1, respectively), contrary to the ancestral state of the SA2 clade, inferred to be dioecious (PP = 0.99). The absence of dioecy in the SA3 clade could be considered a reversion to an ancestral state.

Time-dependent diversification analyses

Our BAMM and MEDUSA analyses are presented together in Fig. 5A. Both analyses indicated heterogeneity in the evolutionary rate dynamics across the phylogeny. BAMM revealed two independent macroevolutionary rate dynamics (macroevolutionary cohort analysis, Supplementary Data Material S6) corresponding to the Archiatriplex + *Halimione* + C_3-*Atriplex* 1 clades, and the Atriplex core (including the C_3-*Atriplex* 2 and C_4-*Atriplex* clades), but both regimens showed internal homogeneity. According to BAMM documentation (http://bamm-project.org/postprocess.html#bayesfactors), the overall best model from a BAMM analysis is the model with the highest Bayes factor relative to the null model (the one with 0 shifts). In our analysis, the null model was not sampled by the BAMM algorithm, so the Bayes factor model comparisons could be inaccurate for our data (Supplementary Data Material S7), but other evidence was analysed. In our BAMM analysis, the one-shift model had the highest PP (0.63; Supplementary Data Material S7), with the shift placed in the branch of the MRCA of the Atriplex core, sampled with a frequency of $f = 0.91$ (Fig. 5A, grey circle). This shift could have occurred 19–15 Mya, during the Early–Middle Miocene. MEDUSA analysis showed strong support for two independent shifts (Fig. 5A, green circles; Supplementary Data Material S8): one in the branch leading to the Atriplex core, as the overall best configuration from BAMM, and a second shift near the MRCA of the SA2 + North-SA3 clades, ~9 Mya. The described shift in the Atriplex core indicated acceleration in the speciation rates, while the backbone of the tree, the Archiatriplex clade, the genus *Halimione* and the C_3-*Atriplex* 1 clade underwent slower rates.

The speciation-through-time plot and lineages-through-time plots (Fig. 5B and C, respectively) indicated that Atripliceae increased its speciation rate ~15 Mya to the present. This could be caused by the Atriplex clade, which experienced an abrupt increase in the speciation rate ~17 Mya, while the diversification of the Archiatriplex clade constantly declined through time; the American clade continuously increased its speciation rate through time since its origin ~10 Mya (Fig. 5D).

Mean speciation, extinction and net diversification rates for Atripliceae and main clades obtained from the BAMM analysis are shown in Table 1. The mean net diversification rate recovered for the whole Atripliceae was 0.215 lineages Myr^{-1}, and the highest values of net diversification and speciation rates were in the American clade (mean r rate = 0.335, mean λ rate = 0.427 lineages Myr^{-1}). The C_4-*Atriplex* clade also showed high estimated values of speciation and net diversification (mean λ rate = 0.420 lineages Myr^{-1}, mean r rate = 0.329 lineages Myr^{-1}). The Archiatriplex clade showed the lowest speciation and net diversification rates (mean λ rate = 0.103, mean r rate = 0.057 lineages Myr^{-1}), but also the lowest extinction rate (mean μ rate = 0.046 lineages Myr^{-1}). We are cautious with speciation and extinction rate results because, although net diversification can be reasonably well assessed, its components are more difficult to estimate (Revell, 2018). Also, extinction rates may be inaccurate without fossil data (Rabosky, 2010).

A Spearman rank correlation test for a relationship between crown ages and species richness of target clades was not significant, revealing no correlation (Spearman's $\rho = -0.15$, $P < 0.68$, $R^2 = 0.02$), indicating that species richness across Atripliceae
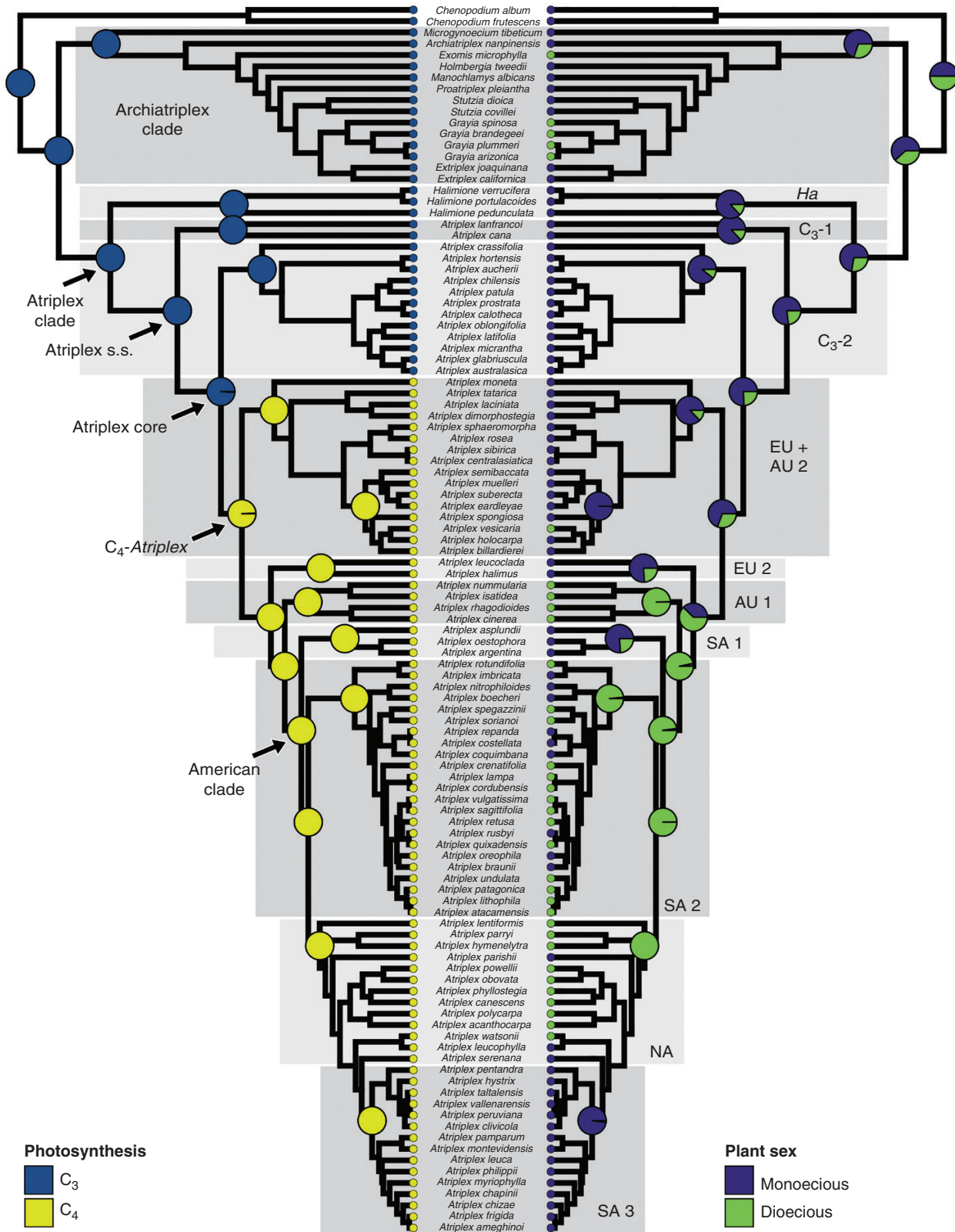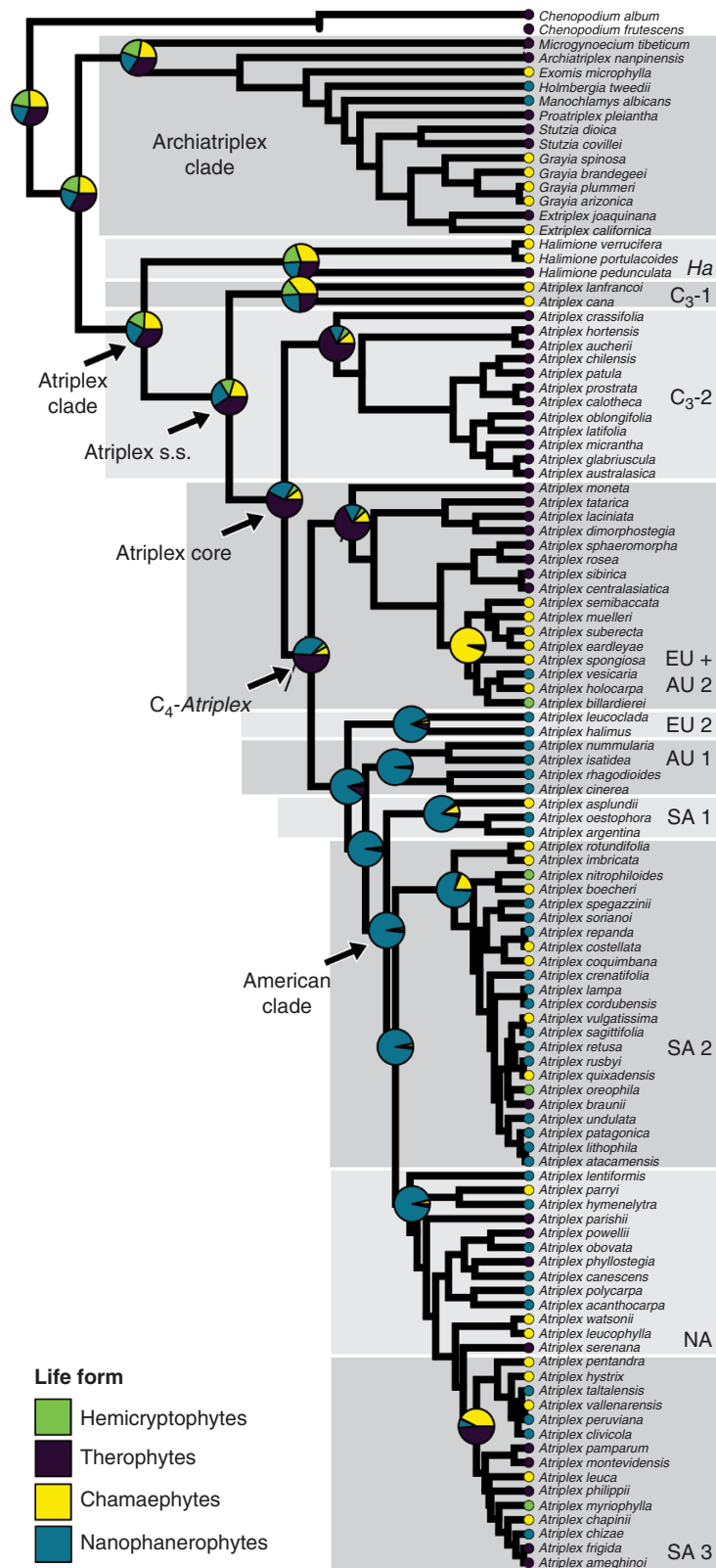
FIG. 3. Ancestral-state reconstructions for the photosynthesis pathway and plant sex in Atripliceae using SCM. Pie charts at nodes indicate the PP of each trait state. *Ha*, *Halimione*; C$_3$-1, C$_3$ *Atriplex* 1; C$_3$-2, C$_3$ *Atriplex* 2; EU + AU 2, Eurasia + Australia 2; EU 2, Eurasia 2; AU 1, Australia 1; SA 1, South America 1; SA 2, South America 2; SA 3, South America 3; NA, North American grade.

Fig. 4. Ancestral-state reconstructions for life form in Atripliceae using SCM. Pie charts at nodes indicate the PP of each trait state. *Ha*, *Halimione*; C$_3$-1, C$_3$-*Atriplex* 1; C$_3$-2, C$_3$-*Atriplex* 2; EU + AU 2, Eurasia + Australia 2; EU 2, Eurasia 2; AU 1, Australia 1; SA 1, South America 1; SA 2, South America 2; SA 3, South America 3; NA, North American grade.
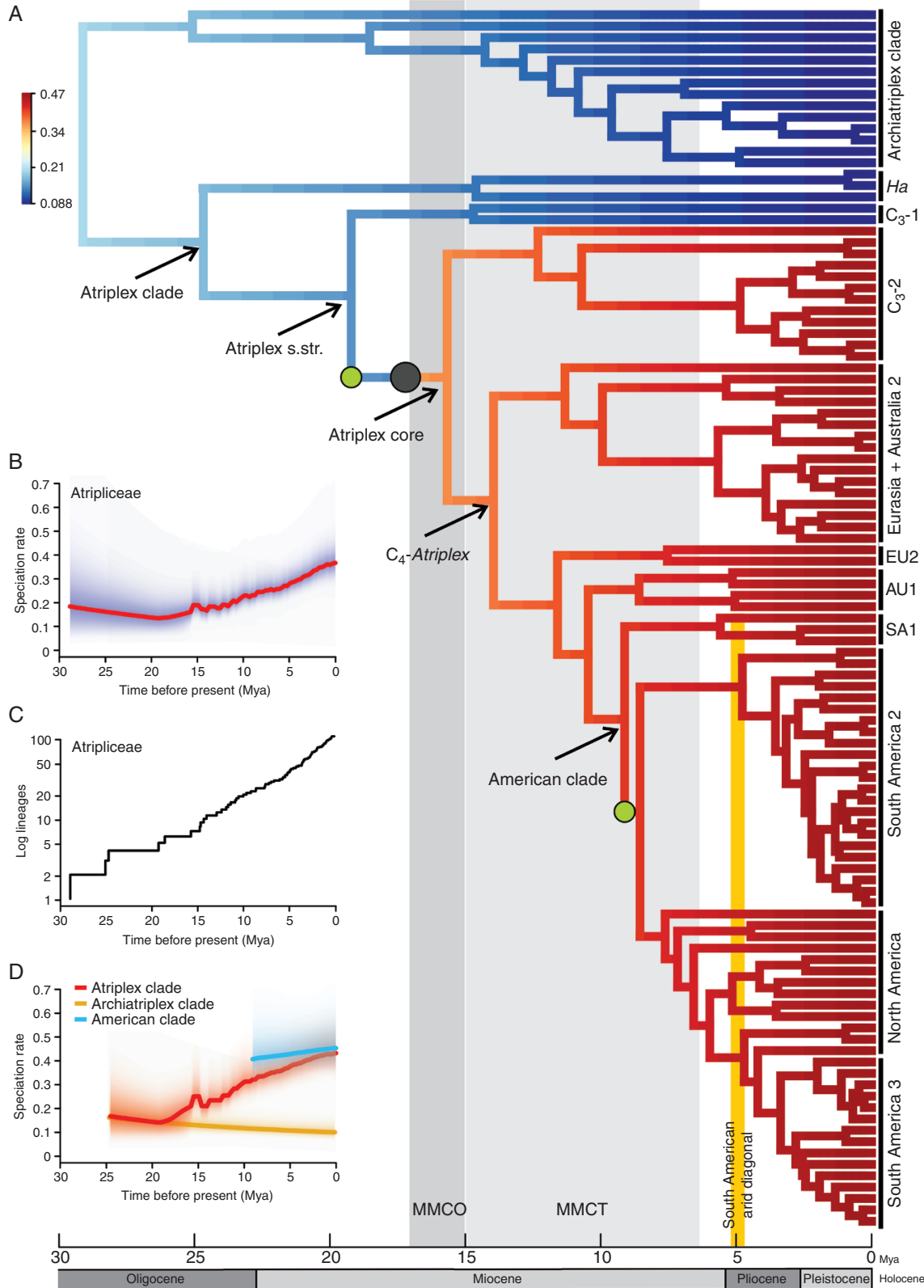
FIG. 5. Diversification history of Atripliceae. (A) Best shift configuration estimated in BAMM over the BEAST phylogeny showing speciation rates (Myr^{-1}). Colours of branches denote directionality and strength of rate change: cooler and warmer colours designate slower and faster rates, respectively. Circles indicate significant shifts in speciation as inferred by BAMM (grey) and MEDUSA (green). (B) Speciation-through-time plot for Atripliceae. (C) Lineages-through-time plot for Atripliceae. (D) Speciation-through-time plots for the Atriplex, Archiatriplex and American clades. Shaded areas represent the 95 % CI. *Ha*, *Halimione*; EU 2, Eurasia 2; AU 1, Australia 1; SA 1, South America 1.

TABLE 1. *Speciation, extinction and net diversification rates (lineages Myr⁻¹) for Atripliceae of main clades obtained from the BAMM analysis. Values in parentheses correspond to the 95 % CI.*

Clade	Mean speciation rate	Mean extinction rate	Mean net diversification rate
Atripliceae	0.287 (0.233–0.359)	0.072 (0.013–0.169)	0.215 (0.167–0.260)
Archiatriplex clade	0.103 (0.061–0.166)	0.046 (0.003–0.135)	0.057 (0.007–0.101)
Atriplex clade	0.356 (0.288–0.450)	0.081 (0.012–0.206)	0.274 (0.212–0.331)
C_3-*Atriplex* 2	0.404 (0.309–0.528)	0.089 (0.005–0.251)	0.314 (0.227–0.389)
C_4-*Atriplex*	0.420 (0.335–0.536)	0.090 (0.006–0.248)	0.329 (0.250–0.399)
Eurasia + Australia 2	0.417 (0.324–0.548)	0.091 (0.005–0.257)	0.326 (0.244–0.402)
Australia 1	0.406 (0.311–0.529)	0.087 (0.005–0.249)	0.318 (0.228–0.394)
American clade	0.427 (0.337–0.550)	0.091 (0.006–0.251)	0.335 (0.250–0.415)

clades is not associated with the amount of time available for diversity to accumulate.

Trait-dependent and geographic state-dependent diversification analyses

The best-fit BiSSE model for the photosynthesis pathway and plant sex and the best-fit MuSSE model for life form are highlighted in bold in Supplementary Data Material S9 (Tables A and B, respectively). The results of the Bayesian BiSSE/MuSSE analyses were also visualized as PP distribution plots (Supplementary Data Material S9, Fig. S9). We found evidence for trait-dependent diversification in Atripliceae associated with three traits: photosynthesis pathway (*lambda.free* model), plant sex (*mu.q.free* model) and life form (*lambda.q.fre*e model). Regarding the photosynthesis pathway, the C_4 species had higher speciation rates than the C_3 species ($\lambda1 = 0.45$ versus $\lambda0 = 0.17$), and net diversification rates for C_4 species were also higher. Related to plant sex, the BiSSE analysis and Markov chain Monte Carlo (MCMC) estimates indicated that monoecious species have higher extinction rates than dioecious species ($\mu0 = 0.34$ versus $\mu1 = 0.13$), and transition rates were higher from dioecious to monoecious ($q_{10} = 0.18$ versus $q_{01} = 0.01$); lastly, net diversification rate was higher for dioecious species (all rates' values are in events Myr⁻¹). The best-supported model for the photosynthesis pathway was the HiSSE model for state 1 (C_4), while for plant sex the best-supported model was the full HiSSE model (Supplementary Data Material S9, Table C). The HiSSE analyses suggested that the focal traits explain some, but not all, of the diversification heterogeneity. Finally, nanophanerophytes had higher speciation rates ($\lambda3 = 0.74$), and transition rates were higher from nanophanerophytes towards chamaephytes ($q_{32} = 0.34$) and from hemicryptophytes to nanophanerophytes, with the same rate ($q_{03} = 0.34$). MCMC estimates showed higher net diversification rates for nanophanerophytes (Supplementary Data Material S9, Table C).

The best-fit GeoHiSSE model was the canonical GeoSSE model, suggesting a range effect on diversification ($\omega_i = 0.8885$, Supplementary Data Material S10). Thus, the American species showed higher net diversification rates than the non-American species.

DISCUSSION

Diversification dynamics in Atripliceae: a general framework

Our results demonstrate opposite evolutionary dynamics between diverging lineages in Atripliceae: while the Atriplex core clade accelerates its speciation and net diversification rates to the present, the Archiatriplex, *Halimione* and C_3-*Atriplex* 1 clades experience a slowdown in rates (Table 1, Fig. 5D). Kadereit *et al.* (2010) considered that the ancient origin, the long branches within the clade, the reduced number of species, their disjunct distribution and the morphologically heterogeneous genera may be considered as signals to interpret the Archiatriplex taxa as relictual, and probably the remnants of a formerly widely distributed lineage, with extinction playing a crucial role within the Archiatriplex clade. Our results showed similar extinction rates in the Atriplex and Archiatriplex clades, being a little higher in the Atriplex clade (Table 1), but this result may be inaccurate since extinction rates may be difficult to infer in the absence of fossil data (Rabosky, 2010). Indeed, the low mean speciation rate in the Archiatriplex clade seemed to be the cause of the low number of species compared with its sister clade. The Archiatriplex clade showed a constant deceleration in speciation rate along its internal branches, while the Atriplex clade displayed a shift in diversification dynamics in an early branch and a posterior constant acceleration of speciation rate.

Additionally, our analyses identified a primary shift in speciation rates placed in a branch near the MRCA of the Atriplex s.str. clade – the Atriplex core. This shift could be estimated to have happened ~16–15 Mya; therefore, the acceleration in Atripliceae diversification seems to have started before the transition between C_3 and C_4 metabolism (~14 Mya), and before the arrival of *Atriplex* in the Americas (~9 Mya). The Atripliceae shift in diversification dynamics matches the Mid-Miocene Climatic Optimum (MMCO) at ~17–15 Mya (Zachos *et al.*, 2008), a period of major global climatic change (Fig. 5A). It was the warmest period in the last 24 Mya (Zachos *et al.*, 2001), with high global mean annual temperature accompanied by peaks of high CO_2 concentration as hypothesized by Tripati *et al.* (2009). The MMCO was followed by a global gradual cooling until the early Pliocene (6 Mya) and a substantial decline in atmospheric CO_2 (~15–8 Mya) (Zachos *et al.*, 2008). This Middle Miocene Climate Transition (MMCT, Fig.

5A) of polar cooling and changing global carbon cycling had large effects on the terrestrial biosphere, including aridification of mid-latitude continental lands (Flower and Kennett, 1994). Consequently, the early Atripliceae shift in speciation rate could be related to the global climate scenario in MMCO, previous to the C_3–C_4 transition or to the arrival of *Atriplex* in the Americas. The subsequent acceleration of diversification dynamics occurring in the Atriplex core in different regions of the world (i.e. Eurasia, Australia and America) seemed to have occurred during the MMCT global conditions, within the global expansion of arid environments. This agrees with other evidence for the Late Miocene–Pliocene as the period for the establishment of many succulent linages, like cacti, agaves and ice plants, in arid environments worldwide (Arakaki *et al.*, 2011). Besides, the decline in atmospheric CO_2 levels at the MMCT would have favoured the posterior evolution and expansion of C_4 plants; the Late Miocene has long been recognized as the moment of global emergence of grasslands dominated by C_4 photosynthesis grasses (Cerling *et al.*, 1997).

We also found some evidence supporting the idea that *Atriplex* ancestors accelerated speciation after their arrival in the Americas. In BAMM analysis, the highest values for net diversification and speciation rates were found in the American clade, and MEDUSA analysis suggested a second shift to accelerated speciation rates near the MRCA of the American clade (but excluding the SA1 clade), ~9 Mya. The GeoHiSSE analysis showed that the American species of *Atriplex* exhibit slightly higher net diversification rates than the Australian and Eurasian species. We could infer that the formation of the South American Arid Diagonal (~5 Mya, Fig. 5A), associated with the last phase of the Andean uplift, and glacial and interglacial periods in the Pleistocene could have promote an increase in the speciation rate in American species of *Atriplex* by offering new habitats for colonization (Brignone *et al.*, 2019, and literature cited there). Apart from these geological and climatic events, trait-dependence phenomena (see below) could also have driven evolution dynamics in America.

Finally, we found that the species richness across Atripliceae clades is probably not associated with the amount of time available for diversity to accumulate, but with other drivers.

Trait evolution in Atripliceae

Life form. The MRCA of all main clades in Atripliceae, i.e. deeper nodes in the phylogeny, were suggested to be ambiguous or therophytes. The most frequent trend described for plants is the change from woody to herbaceous habit (Takhtajan, 1980; Del Pero Martínez *et al.*, 2002; Zacharias, 2007), and our results did not confirm that for Atripliceae. Except for the SA3 clade, the nanophanerophyte state was reconstructed in more recent nodes. This could be related to *Atriplex* adaptation to dry environments and cavitation theory (Lens *et al.*, 2013). But because of the ambiguous reconstructions of early Atripliceae nodes, we cannot state a secondary woodiness progression. Also, BiSSE results showed that life form would be related to speciation and diversification within Atripliceae, and nanophanerophytes showed higher net diversification rates. There are few species with herbaceous habit, and most are therophytes or annuals; this trait state defined the C_3-*Atriplex* 2 clade and some C_4 subclades

within Eurasia + Australia 2, but is less frequent in American species. Most American herbaceous (hemicryptophytes and therophytes) species correspond to the SA3 clade, and species of this lineage have occupied lowlands more frequently than species from the SA1 or SA2 clades. The *Atriplex* colonization of lowland areas in South America may have occurred by a herbaceous ancestor during the recolonization of South America from the North American lineage (Brignone *et al.*, 2019). Also, expansion from mountainous to lowland areas associated with arid conditions and fluctuation in water availability have been related to perennial to annual life-cycle switches (Lo Presti and Oberprieler, 2009; Özüdoğru *et al.*, 2015), but in this context, the probably SA3 therophyte ancestor could have arrived in South America via the Caribbean Islands, which have served as stepping stones (Brignone *et al.*, 2019).

Photosynthesis pathway. The C_4 photosynthetic pathway is inferred to have originated >60 times independently in angiosperms (Christin *et al.*, 2011; Sage *et al.*, 2011), and it has evolved at least in nine lineages within Chenopodiaceae s.str. (Sage, 2016). The presence of C_4 chenopods in extremely arid environments suggests that the C_4 species in Atripliceae expanded their niches into even more arid climates than that occupied by their closest C_3 relatives (Bena *et al.*, 2017). According to our BiSSE and HiSSE results, we found that net diversification rates in C_4 species are higher than in *Atriplex* with C_3 metabolism, although part of this variance is explained by an associated hidden state; we also concluded that a shift in diversification dynamics in Atripliceae occurred before the transition C_3–C_4 ~14 Mya. The dating of the C_4 origin in *Atriplex* supports the current view that the increased aridity and seasonality of the Miocene climate enhanced the origin of C_4 lineages (Kadereit *et al.*, 2010). The idea that shifts in diversification seem to entail both intrinsic (morphological, physiological) and extrinsic factors (such as the occupation of new territories or climate changes) was crystalized by Donoghue and Sanderson (2015), referring this to a key confluence. We can illustrate a key confluence in Atripliceae related to the C_4 photosynthesis pathway: our results also contribute to the idea that in Chenopodiaceae s.str. drought tolerance, achieved by physiological and morphological adaptations, was a pre-adaptation that promoted the evolution of the C_4 photosynthesis pathway (Kadereit *et al.*, 2012). This is opposite to what was found with grasses, where the C_4 photosynthesis pathway would be a pre-adaptation to arid environments (Bena *et al.*, 2017, and literature cited there). There is evidence that in some amaranthoid groups there was a shift from C_4 to C_3 photosynthesis, e.g. in the Salsoloideae/ Camphorosmoideae lineage (Kadereit *et al.*, 2012), but we find that reversals to C_3 did not occur within Atripliceae, despite the limited sampling.

Plant sex. Dioecy is a relatively rare sexual system occurring in ~7–10 % of angiosperms, yet it occurs in almost half of all flowering plant families (Renner and Ricklefs, 1995; Heilbuth, 2000; Vamosi *et al.*, 2003; Sabath *et al.*, 2016). Within Atripliceae sampled in this work, 30 % of the species are dioecious, 36 % of the species clustered in the Archiatriplex clade are dioecious (*Exomis microphylla* and species of *Grayia*), the Australia 1 clade is fully dioecious, 65 % of the *Atriplex* species belonging to the SA2 clade are dioecious, and North American

species are mainly dioecious, while the genus *Halimione*, C$_3$-*Atriplex* species and the Eurasia + Australia 2, Eurasia 2, SA1 and SA3 clades are largely monoecious. We found that dioecy evolved multiple times in the tribe (Fig. 3), as in the subfamily Chenopodioideae (Kadereit *et al.*, 2010). The ancestor of the whole tribe Atripliceae, and also deeper branches, seems to be monoecious, and shifts to dioecy occurred in some species of the Archiatriplex clade (in the South African *Exomis* and the North American *Grayia*), and in fewer Australian ones. At the moment of acceleration of diversification in Atripliceae, i.e. in the shift in the Atriplex core, ancestors were monoecious. Later, the ancestor of the Australia 1 + American clades probably turned to dioecious, and this condition was prevalent during diversification in these two groups. Considering the South American lineages of *Atriplex*, the reproductive system could have mediated the differentiation of the SA1 and SA2 clades: SA1 species shifted from dioecious to monoecious, while most SA2 species retained the ancestral dioecious condition. Closely related species can face environmental fluctuations in sympatric conditions by adopting different ecological strategies of survival (e.g. Verhulst *et al.*, 2008). Nevertheless, it is not clear yet how dioecy/monoecy could have influenced in the sympatric evolution of those two clades. Besides, our results suggested higher extinction rates for monoecious species and higher net diversification rates for dioecious species, which could explain the differences in species richness in the SA1 and SA2 clades, but inferences using extinction rates are not trustworthy. On the other hand, the recolonization of South America from North America in the Pliocene was probably triggered by a monoecious ancestor.

According to Thomson and Brunet (1990) and Schwander and Crespi (2009), dioecy is a rare feature in angiosperms because it slows down speciation, accelerates extinction, and is lost faster than gained, but we found different trends in Atripliceae. Our results also lead us to propose an opposite trend to the one described by Heilbuth (2000) for many groups; he reported that species richness is generally far lower in dioecious clades when compared with their hermaphroditic or monoecious sister clades, and probably must have higher rates of extinction and/or lower rates of speciation. On the other hand, Sabath *et al.* (2016) found that dioecious lineages exhibited higher diversification rates in certain genera but lower diversification rates in others; they used *Atriplex* (among other genera) as a model to evaluate dioecy as a driver of diversification in angiosperms, but they obtained higher net diversification rates for monoecious species than for dioecious ones. Finally, we found that the transition rates are higher from dioecious to monoecious species, as suggested by Richards (1997). In dryer environmental conditions, where Atripliceae species prevail, dioecy can be an advantage (Case and Barret, 2004), by promoting precocious reproductive maturity (Ohya *et al.*, 2017). Case and Barret (2004) suggested that dioecy is favoured in arid and semi-arid environments through the increase in seed fitness of females relative to monoecious individuals. Most studies on dioecy evolution were focused on tropical and subtropical species (Case and Jesson, 2018, and literature cited there). Particularly, Vamosi *et al.* (2003) and Vamosi and Vamosi (2004) suggested that dioecy is correlated with several traits, such as tropical distribution, woody habit, abiotic pollination, inconspicuous flowers/inflorescences and fleshy fruits. This hypothesis does not strictly apply to

the Atripliceae, with most species having dry fruits (except *Holmbergia*) and a geographical distribution in temperate, dry habitats.

Conclusions

Our study constitutes a first approach to a future and more comprehensive inquiry about mechanisms involved in the diversification process and evolution of Atripliceae. We concluded that the traits life form, photosynthesis pathway and plant sex played a key role in the diversification of Atripliceae, and we can speculate that climate changes could probably have triggered speciation. The extreme arid or saline environments where Atripliceae species prevail may explain the differences in evolutionary trends or trait correlations of the tribe compared with other angiosperms, and this highlights the importance of conservation efforts needed to preserve them as genetic resources to deal with climatic changes. Results from this study are being integrated into a wider phylogenetic study as part of current collaborative efforts on a worldwide phylogeny of *Atriplex* and allied genera.

SUPPLEMENTARY DATA

Supplementary data are available online and consist of the following. Material S1: sampling fractions. Material S2: trait coding for the three traits used in ancestral-state reconstructions and BiSSE, HiSSE and MuSSE analyses. Material S3: trees obtained from the phylogenetic analyses. Material S4: ancestral-state reconstruction results. Material S5: posterior probabilities of main nodes in ancestral-state reconstructions. Material S6: macroevolutionary cohort matrix of Atripliceae estimated by BAMM. Material S7: pairwise Bayes factor matrix and marginal shift probability tree obtained in BAMM for Atripliceae. Material S8: MEDUSA results. Material S9: trait-dependent diversification analysis results. Material S10: geographic state-dependent diversification analysis.

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