Botanical Journal of the Linnean Society, 2022, **200**, 524–540. With 5 figures.

The evolution of chromosome number during the diversification of the tribe Vernonieae (Asteraceae)

MARÍA BETIANA ANGULO[1,2,*,†], LAURA CHALUP[1,3,†], JAVIER ELÍAS FLORENTÍN[1,2] and MASSIMILIANO DEMATTEIS[1,2]

[1]*Instituto de Botánica del Nordeste (CONICET-UNNE, Fac. Cs. Agrarias), Sargento Cabral 2131, C.C. 209, 3400 Corrientes, Argentina*
[2]*Facultad de Ciencias Exactas y Naturales y Agrimensura, UNNE, Av. Libertad 5460, 3400 Corrientes, Argentina*
[3]*Universidad Nacional del Chaco Austral, UNCAUS, Comandante Fernández 755, 3700 Pcia. Roque Sáenz Peña, Chaco, Argentina*

Received 2 September 2021; revised 21 February 2022; accepted for publication 6 March 2022

Changes in chromosome number have played an important role in the diversification and evolution of angiosperms. In Asteraceae, tribe Vernonieae are one of the most variable groups with regard to chromosome number. Previously, chromosome numbers $n = 9$ and 10 were thought to characterize the Old World members of the tribe, and $n = 14, 16, 17$ and 18 the New World members. This scenario was revised as a result of reports of new chromosome numbers, but the events leading to this wide variation remain unknown. Here we carried out a phylogenetic analysis of Vernonieae in a temporal framework, assessing patterns of diversification and establishing possible relationships with chromosome events. Chromosomal evolution was analysed with ChromEvol, from a phylogenetic tree dated in BEAST. Shifts in diversification rates using Bayesian analysis of macroevolutionary mixtures were inferred. Vernonieae originated ~46 Mya and the diversification rate increased sharply ~11 Mya after the Mid-Miocene Climatic Optimum. The ancestral chromosome number for the tribe was $n = 10$, which remained stable for Old World taxa, whereas $n = 9$ was the ancestral number for New World species. The tribe has undergone 32 chromosome rearrangements throughout its evolutionary history, with dysploidy and polyploidy possibly explaining the observed diversification pattern.

ADDITIONAL KEYWORDS: chromosome number – diversification rate shifts – dysploidy – New World – Old World – polyploidy.

INTRODUCTION

Changes in chromosome number have been widely recognized as important evolutionary forces that have profound effects on diversification rates and speciation in angiosperms (Soltis & Soltis, 2000; Soltis *et al.*, 2009). The remarkable diversity of flowering plants (angiosperms) has been attributed, in part, to the tremendous variation in their chromosome number (Stebbins, 1971). Variations in chromosome numbers can occur by whole genome duplication (WGD; polyploidy) or by decreases or increases through structural chromosome rearrangements leading to little or negligible change in the DNA content, such as chromosome fusion, i.e. descending dysploidy, or chromosome fission, i.e. ascending dysploidy (Mayrose, Barker & Otto, 2010; Schubert & Lysak, 2011; Winterfeld *et al.*, 2020; Mayrose & Lysak, 2021).

Polyploidy is frequent in plants and it is considered a fundamental process in the evolution of many lineages, with evidence of several rounds of ancient and recent polyploidization (Leitch & Bennett, 2004; Van de Peer, 2011; Escudero *et al.*, 2014). WGDs provide genetic material for evolutionary processes, such as chromosome rearrangement (Pontes *et al.*, 2004; Madlung *et al.*, 2005), neofunctionalization (Blanc & Wolfe, 2004), subfunctionalization (Cusack & Wolfe, 2007) and gene conservation due to dosage effects (Bekaert *et al.*, 2011; Hudson *et al.*, 2011). Such changes can result in organisms taking advantage

*Corresponding author. E-mail: angulobetiana@gmail.com.
†These authors are co-first authors and contributed equally to this work.

of new ecological opportunities or facing new environmental challenges (Ohno, 1970; Maere & Van de Peer, 2010; Schranz, Mohammadin & Edger, 2012; Fawcett, Van de Peer & Maere, 2013). However, other authors have questioned the evolutionary success of recent polyploids due to their reduced diversification rates compared to their diploid relatives (Guerra, 2008; Mayrose *et al.*, 2011). On the other hand, the role of dysploidy in plant diversification has begun to receive more attention. Although karyotype changes due to dysploidy persist over evolutionary time, some analyses have shown it to be a neutral process with respect to long-term diversification processes, neither substantially increasing nor decreasing the processes of speciation and extinction (Escudero *et al.*, 2014).

Recent and constant advances in molecular phylogeny have shown the influence of chromosome changes (polyploidy/dysploidy) on the timing and mode of diversification during the evolution of various groups of plants (Escudero *et al.*, 2012; Pimentel *et al.*, 2017; Sader *et al.*, 2019; Chiavegatto *et al.*, 2020). Here, we use this approach to assess the impact of chromosomal changes on diversification over time during the evolutionary history of Asteraceae tribe Vernonieae.

Vernonieae currently include 21 subtribes and 118 genera with ~1500 species (Keeley & Robinson, 2009). They show an extensive distribution in the New World, from Canada to Argentina, and Old World in Africa, South and Southeast Asia and Australia, and they also occur on island chains in both hemispheres (Keeley, Forsman & Chan, 2007). A dated phylogenetic tree for Vernonieae has placed their diversification in the Eocene at *c.* 50 Mya. Africa was the first centre of diversity, from which a single dispersal event established the monophyletic New World lineage. Long-distance dispersal from Africa and Brazil established the tribe on five continents and Oceania (Keeley, Cantley & Gallaher, 2021).

Vernonieae are known as the 'evil tribe' because of their complex morphology, including the presence of a large number of overlapping characters in many groups of the tribe. Despite the difficulties in understanding their evolutionary relationships, there was never any doubt that Vernonieae comprise two lineages, one from the Old World and one from the New World. This divergence was initially proposed by Gleason (1906) and was then confirmed by several phylogenetic studies (Keeley & Turner, 1990; Keeley & Jansen, 1994; Keeley & Robinson, 2009; Mandel *et al.*, 2019; Keeley *et al.*, 2021) conducted in an effort to understand the evolutionary relationships not only among species, but also between the subtribes (Keeley *et al.*, 2007, 2021; Keeley & Robinson, 2009; Loeuille, Keeley & Pirani, 2015; Siniscalchi *et al.*, 2019). The division into two lineages was also observed in the chromosome numbers between the Old and New World Vernonieae (Jones, 1977). Old World taxa routinely had $n = 9$ or 10, whereas New World species had $n = 14$, 16, 17 or 18, the haploid number of $n = 17$ being considered the basic chromosome number of the New World species due to its high frequency (Jones, 1977, 1979). Nevertheless, this scenario was revised with the incorporation of additional counts of haploid numbers of $n = 7$, 10, 11, 12, 13, 15 and 19 (Olsen, 1980; Turner, 1981; Sundberg, Cowan & Turner, 1986; Galiano & Hunziker, 1987; Rabakonandrianina & Carr, 1987; Keil, Luckow & Pinkava, 1988; Grupta & Gill, 1989; Dematteis, 1998, 2002; Mansanares, Forni-Martins & Semir, 2002; Dematteis *et al.*, 2007; Angulo & Dematteis, 2009a, b, 2010; Via do Pico & Dematteis, 2012; Marinho *et al.*, 2017).

From the beginning, this high variability in the number of chromosomes in the tribe caught the attention of researchers and different proposals were put forward to clarify the possible evolutionary trends of the tribe. The work of Jones (1979) on chromosome counts in several species of Vernonieae led to plausible hypotheses for both Old and New World lineages. For the Old World species, based on two most frequent basic numbers, $x = 9$ and 10, he proposed: (1) a reduction in an initial chromosome number of $x = 10$; (2) an increase from an initial number of $x = 9$; or (3) a series of possible hybridizations that gave rise to $x = 9$ and $x = 10$. On the other hand, for the New World species, Jones suggested that Vernonieae with $x = 17$ could represent an ancient polyploid lineage derived from the number $x = 9$ doubled to 18, and then followed by chromosome loss to 17. Later, Turner (1981) proposed that $x = 17$ could have arisen from an $x = 10$ ancestor doubled to 20, followed by a reduction to 17 or by an amphiploid origin of $x = 9 + 10$, followed by chromosome loss. Recently, Mota, Torices & Loureiro (2016) proposed $n = 10$ as the ancestral chromosome number for the tribe, based on a phylogenetic tree of Asteraceae by using a probabilistic model of chromosome evolution. However, their study was Asteraceae-wide and only included some Vernonieae.

Here, we analyse phylogenetic data and diversity and cytogenetic information to: (1) investigate the chromosome events that may have given rise to the high variability in the numbers of chromosomes in Vernonieae; and (b) assess the possible correlation between chromosome variation and diversification rates to provide a better understanding of the role of chromosome changes in the evolutionary history of the tribe.

MATERIAL AND METHODS

Taxon sampling

We sampled 136 species including 43 genera and 16 subtribes, with available DNA sequences and chromosome numbers. Sequences from nuclear ITS and plastid *ndhF* and *trnL-F* used in the phylogenetic analysis were extracted from the GenBank database. A complete list of taxa and GenBank accession numbers are given in Supporting Information, Data S1. Chromosome data were collected from our own records and from public databases, including the Index to Chromosome Numbers in the Compositae (http://www.lib.kobe-u.ac.jp/infolib/meta_pub/engG0000003asteraceae), the Index to Plant Chromosome Numbers (http://www.tropicos.org/Project/IPCN) and the Chromosome Counts Database (http://ccdb.tau.ac.il/) (Data S2). In addition, three species of tribe Liabeae [*Cacosmia rugosa* Kunth, *Liabum selleanum* Urb. and *Munnozia gigantea* (Rusby) Rusby] were used as outgroups (Keeley *et al.*, 2007, 2021; Loeuille *et al.*, 2015). The nomenclature of Robinson (1999a, b), Keeley & Robinson (2009), Loeuille, Semir & Pirani (2019) and Keeley *et al.* (2021) was used in this study.

Phylogenetic analyses and divergence time estimation

Sequences were automatically aligned by the ClustalW method using MEGA v.7.0 (Kumar, Stecher & Tamura, 2016) with manual adjustments as needed. The phylogenetic tree was built using BEAST v.2.4.0 (Drummond & Rambaut, 2007). Optimal nucleotide substitution models were chosen following the Akaike information criterion (AIC) (Akaike, 1974) implemented in MrModeltest v.2.1.10 (Darriba *et al.*, 2012). In the case of ITS, the substitution model was GTR+G+I, and the substitution model for *ndhF* and *trnL* was GTR+G (Rodriguez *et al.*, 1990). Initially, the three regions (ITS, *ndhF* and *trnL-F*) were analysed separately, and three independent trees were reconstructed. The results were compared visually to find possible inconsistencies between the regions, and after this inspection they were treated as a single partition. Although the phylogenetic resolution of the plastid DNA sequences is poor when taken alone, making it difficult to make a direct comparison with the nuclear ITS, the addition of data for plastid DNA improves node support in the phylogenetic tree from the concatenated data (as previously demonstrated in Vernonieae; Keeley *et al.*, 2007, 2021).

Two independent series of 1×10^8 Markov chain Monte Carlo (MCMC) iterations were performed, taking samples every 1000 generations, using a random start tree. The sampled trees were saved every 1000 generations. In addition, the age of the nodes and the substitution rates were estimated simultaneously using the Bayesian MCMC approach.

The use of secondary calibrations is the only source of calibration information for many groups, particularly for those in which the fossil record is scarce or non-existent (Forest, 2009). Although no fossils have been recorded so far in Vernonieae, the latest study to determine the age of the tribe was conducted by Keeley *et al.* (2021). This last study incorporated six fossils belonging to Asteraceae, including a secondary calibration and island ages for the crown radiation of the Hawaiian endemic *Hesperomannia* A.Gray. Therefore, we estimated the divergence rates in our tree according to these authors using a normal distribution. Specifically, we constrained the crown node of Vernonieae at 51.00 Mya, standard deviation (SD) of 5.0 Mya, and that of the New World clade at 36.75 Mya and SD of 3.5 Mya.

The clock was set using an uncorrelated relaxed logarithmic model, and the Yule calibration process was selected as a prior for the distribution of divergence dates. The convergence and stationarity of the parameters were inspected using Tracer v.1.7 (Rambaut *et al.*, 2018), aiming at minimum effective sample sizes (ESS) of at least 200. The initial 25% of the trees were discarded as burn-in, and the results were combined using LogCombiner as implemented in the BEAST package. The phylogenetic relationships were summarized in a maximum clade credibility (MCC) tree, and TreeAnotator v.2.4.7 was used to calculate the mean ages, 95% maximum post-density intervals (HPD), post-probabilities and replacement rates for each node. The trees were visualized in FigTree v.1.4.3 (Rambaut, 2014).

Diversification rate shifts

Shifts in the diversification rates in Vernonieae were estimated using Bayesian Analysis of Macroevolutionary Mixtures v.2.5.0 (BAMM; Rabosky *et al.*, 2014; Rabosky, Mitchell & Chang, 2017; latest version available at http://bamm-project.org). BAMM detects rate shifts in speciation and extinction, without a priori assumptions regarding the number and location of these events based on a birth–death process. The MCC tree from the BEAST analyses with secondary calibration was used as an input file. For this analysis, outgroup taxa were pruned using the 'APE' package (Paradis, Claude & Strimmer, 2004) implemented in R software. The priors for the diversification rate analyses were set using the 'setBAMMPriors' command in the 'BAMMtools' package v.2.1.7 (Rabosky *et al.*, 2019) in R v.1.1.419 (R Core Team, 2019). Diversification rates may be biased by incomplete sampling of the taxa (Shi & Rabosky, 2015).

Therefore, we specified the fraction of missing species in each genus of Vernonieae under the assumption of random sampling of taxa (FitzJohn, Maddison & Otto, 2009). The sampling fraction was calculated as a ratio of the number of species included divided by the total number of species currently accepted (Supporting Information, Data S3); these proportions were used as inputs for the 'SamplesProbsFilename' argument in the Control File. We ran four parallel MCMCs for 100 million generations and sampled the results every 5000 generations. The output files were analysed in R, using the 'BAMMtools' package.

Convergence was assessed in R using the 'CODA' package (Plummer *et al.*, 2006) by checking the ESS values for likelihood and the number of shift events; the first 10% of the sampled generations were discarded as burn-in. Values > 200 were considered indicative of convergence. Bayes factors were computed to compare the evidence for models with at least one rate shift to the evidence for the null model using the 'computeBayesFactors' function. The event output files were analysed by discarding 10% burn-in samples and assessing the distinct rate shift configurations in the 95% credible set using the 'credibleShiftSet' function. Subsequently, the position(s) of the significant rate shift was/were inferred by observing the nodes with the highest posterior probability (PP) values (up to 95%) using the 'distinctShiftConfigurations' function. To complement our analyses, we estimated rate shifts over time using the 'credibleShiftSet' function. A burn-in of 10% was applied and a diversification rate plot over time was obtained using the 'plotRateThroughTime' function. This analysis was initially carried out for the New World clade and, to visualize the diversification process for this clade separately, we plotted two datasets: subclade 2 and subclade 3.

Patterns of chromosome number evolution

Chromosome evolution in Vernonieae was modelled on the MCC tree produced with BEAST. For this analysis, the input tree was also pruned (see 'Diversification rate shifts' above) to eliminate those species with unknown chromosome numbers (*Liabum selleanum* and *Munnozia gigantea* of Liabeae) using the ChromEvol v.2.0 software (Glick & Mayrose, 2014). This program uses a likelihood-based method for inferring the probable direction of chromosome number change and the number and order of chromosome rearrangements across a phylogenetic tree (Glick & Mayrose, 2014). It also reconstructs the ancestral haploid chromosome numbers (Mayrose *et al.*, 2010; Cusimano, Sousa & Renner, 2012). In this analysis, ten models of chromosome evolution were tested, and the fit of each of them was studied using likelihood and AIC values.

All the chromosome numbers of each taxon were converted into haploid chromosome numbers (Supporting Information, Data S2). For species with different chromosome counts, i.e. n = 16 and 17, the frequency of each chromosome number (i.e. probability) was obtained, and the sum of all possible frequencies should equal 1.0 (Mayrose, 2014). In addition, the phylogenetic signal (the contribution of phylogeny to the covariance between species in a given trait) on chromosome number was assessed using Pagel's lambda (λ) (Pagel, 1999) implemented in the 'GEIGER' v.2.0 package (Pennell *et al.*, 2014) using the 'fitDiscrete' function in R.

RESULTS

Phylogenetic relationships, divergence time estimates and diversification analyses

Bayesian phylogenetic trees from the different nuclear (ITS) and plastid (*ndhF* and *trnL-F*) datasets were congruent in topology. The topology of the MCC tree obtained from our phylogenetic analyses is shown in Figure 1. Vernonieae were supported (PP = 1.00) as monophyletic and emerge as a sister to Liabeae (Fig. 1).

The analysed species of the Old World are organized in successive splits and form a series of successive sister groups to the New World clade. On the other hand, New World species constitute a large, strongly supported clade (PP = 1.00). In this large clade, a group emerged here named subclade 1 (PP = 0.76), which included a mixture of taxa from different non-monophyletic subtribes (Fig. 1). This subclade is the sister group of a clade that, in turn, is divided into two groups, here named subclades 2 and 3. Subclade 2 (PP = 0.90) contained various successive, well-supported splits. This subclade also had a mixture of taxa from different subtribes, of which Elephantopinae were the only one to be monophyletic. In addition, it grouped almost all the analysed species of Lepidaploinae with the exception of *Struchium* P.Browne and *Stenocephalum* Sch.Bip. Finally, subclade 3 (PP = 0.80) included all taxa of Lychnophorinae and, although this subtribe was non-monophyletic, the subclade contained most of the taxa attributed to the subtribe. Most of the taxa of Vernoniinae were grouped in this subclade.

The divergence of Vernonieae was in the Eocene onwards (46.00 Mya; HPD 49.16–52.73 Mya). The Old World lineages were the first to arise from the Mid- to Late Eocene onwards. The New World clade branched off from the Early Oligocene onwards (33.09 Mya; HPD 27.49–38.61 Mya), and the following subclades emerged in the Mid-Oligocene: subclade 1 (29.51 Mya;

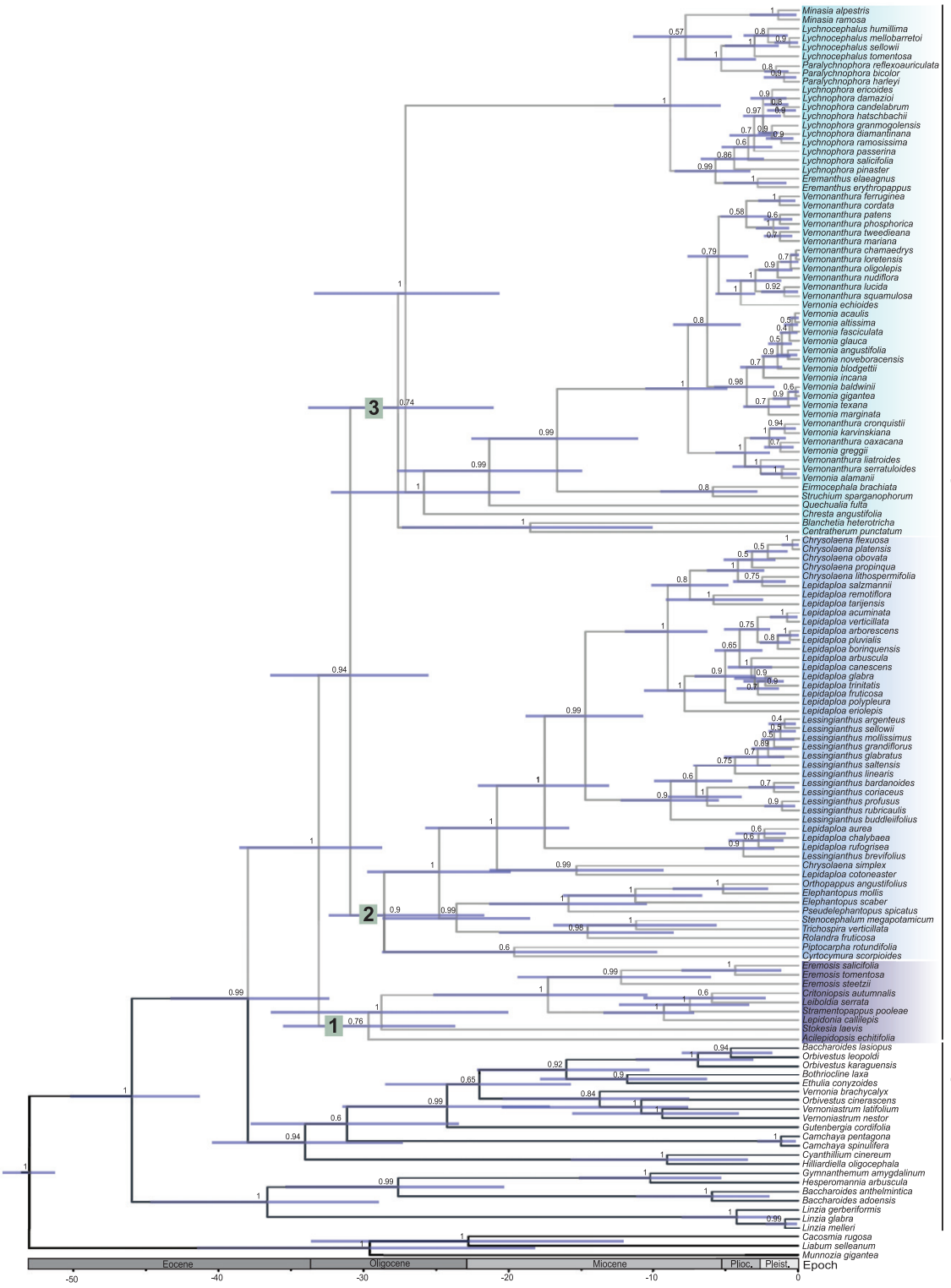

Figure 1. Maximum clade credibility tree obtained by combining nuclear and plastid markers (ITS, *ndhF* and *trnL-F*). Numbers in front of the nodes are posterior probabilities (PP). Horizontal bars indicate 95% credibility intervals for divergence times. The time-calibrated tree is scaled to the geological time scale with absolute time given in millions of years (Mya). Names of subclades are indicated in grey squares and the tips of each subclade are represented by different colours.

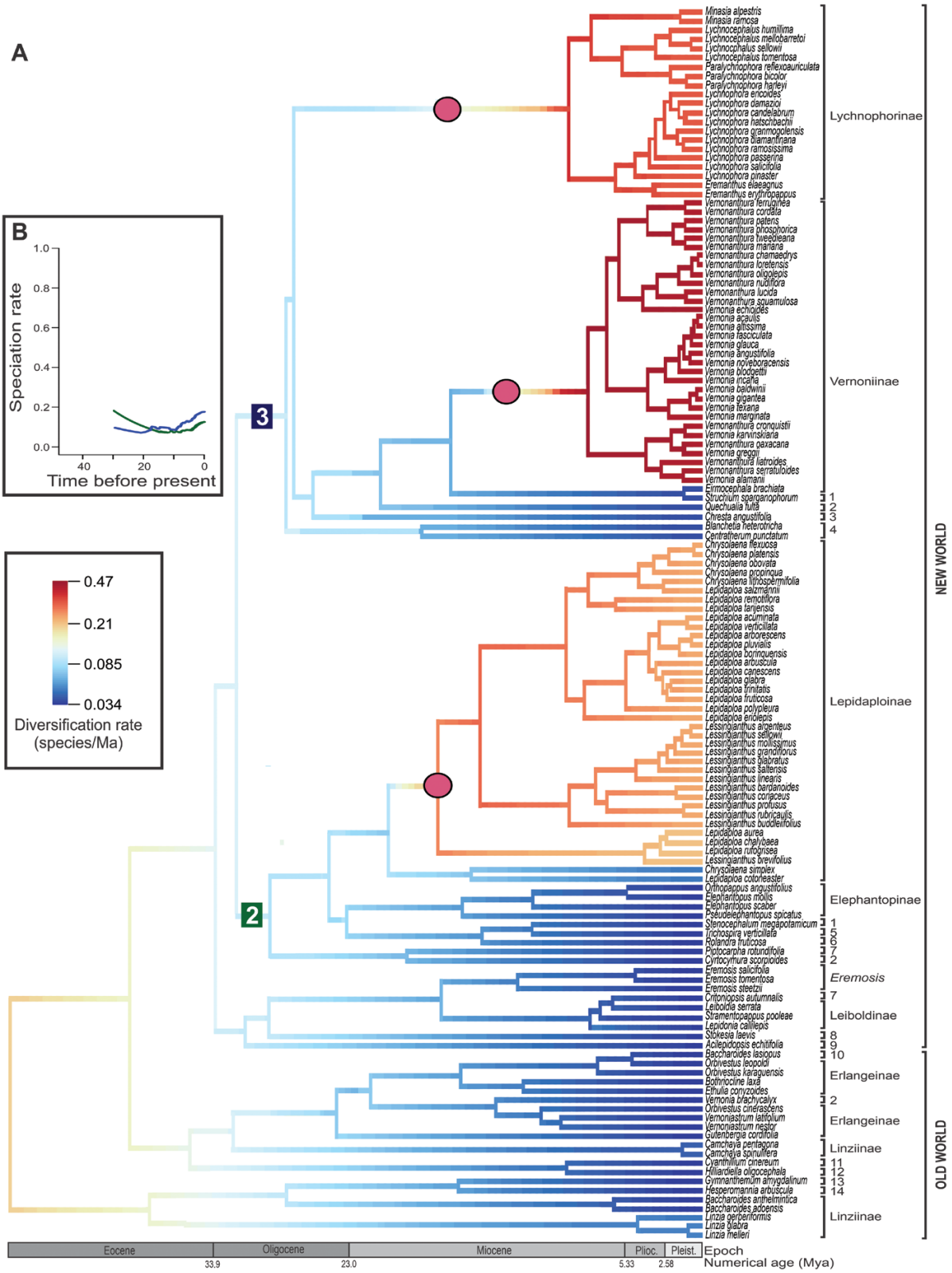

Figure 2. Rates of diversification of Vernonieae using BAMM based on the MCC BEAST tree. A, MCC BEAST tree with edges coloured in red and blue exhibiting high and low rates of net diversification, respectively. Three red circles indicate the

HPD 20.01–36.46 Mya); subclade 2 (28.76 Mya; HPD 24.04–36.64 Mya); and subclade 3 (27.63 Mya; HPD 26.73–37.86 Mya). In subclade 2, Lepidaploinae arose 20.81 Mya (HPD 15.82–25.74 Mya), whereas in subclade 3, Lychnophorinae arose 8.81 Mya (HPD 5.33–12.66 Mya) and Vernoniinae showed a slightly more recent divergence at 7.58 Mya (HPD 4.87–10.51 Mya) (Fig. 1).

The 95% credible set of diversification rate shift configurations sampled with BAMM included three distinct shift configurations, of which that with highest probability included three shifts. The best configuration (Fig. 2A) reveals three core shifts detected in the New World clade. Core shift 1 occurred on the branch prior to the divergence of a clade of Lepidaploinae (subclade 2), core shift 2 was located on the branch prior to the divergence of a clade of Vernoniinae (subclade 3), and core shift 3 was located on the branch prior to the divergence of a clade of Lychnophorinae (subclade 3).

Increases in diversification rates were observed in both subclades (Fig. 2B). A recent strong increase in diversification was observed in subclade 2 (~5 Mya, Pliocene; green line), whereas the diversification process in subclade 3 showed slight increases and decreases between ~20 and 10 Mya (blue line) and a strong increase was observed at ~9 Mya continuing to the present.

Pattern of chromosome number evolution

The analysis of chromosome number evolution in species of Vernonieae revealed that $n = 10$ was the ancestral chromosome number (Fig. 3). The selected model was model 1 [CONSTANT_RATE (AIC = 270.1); Supporting Information, Data S4] that considers three parameters (chromosome loss, chromosome gain and chromosome duplication). Based on this model, 32 events of chromosome rearrangement (with an expectation > 0.5) were inferred over the evolutionary history of Vernonieae. In general, dysploidy events were estimated to be more frequent than duplications. Sixteen chromosome losses (descending dysploidy, expectation ranging from 0.55 to 1.91), seven chromosome gains (ascending dysploidy, expectation ranging from 0.72 and 1.85) and nine duplications (WGD) events (expectation ranging from 0.55 and 1.10) were detected across the phylogenetic tree (Fig. 3).

The ancestral chromosome number $n = 10$ was stable in the deepest part of the phylogenetic tree and was observed at the base of all lineages from the Old World (Fig. 3), whereas $n = 9$ was revealed as the ancestral chromosome number of the New World taxa, which was a result of descending dysploidy ($n = 10 \longrightarrow n = 9$). This chromosome loss also occurred on three other occasions in Old World species, in *Cyanthillium cinereum* (L.) H.Rob., *Vernoniastrum nestor* (S.Moore) H.Rob. and *Orbivestus karaguensis* (Oliv. & Hiern) H. Rob.

In the New World clade, descending dysploidies were the most frequent events; ascending dysploidies were also observed, but less frequently. Both chromosome rearrangements were important in the origin of ancestral chromosome numbers of several genera or subtribes in Vernonieae. Thus, single events of dysploidy (descending and ascending) were inferred at the origin of the ancestral chromosome numbers $n = 10$ ($n = 9 \longrightarrow n = 10$), 11 ($n = 9 \longrightarrow n = 11$) and 18 ($n = 17 \longrightarrow n = 18$) and at the origin of $n = 16$ ($n = 17 \longrightarrow n = 16$) and 17 ($n = 16 \longrightarrow n = 17$) observed in small groups of species in subclades 2 and 3 (Fig. 3).

WGDs occurred in both the Old and New World lineages; six of these events were observed towards the terminals of the tree, at the origin of *Gymnanthemum amygdalinum* (Delile) Sch.Bip. and *Bothriocline laxa* N.E.Br. in the Old World and at the origin of *Acilepidopsis echitifolia* (Mart. ex DC.) H.Rob., *Chrysolaena simplex* (Less.) Dematt., *Lessingianthus argenteus* (Less.) H.Rob. and *Lessingianthus profusus* (Dematt. & Cabrera) M.B.Angulo in the New World, whereas another three events were observed deep in the tree (Fig. 3). These three duplications were followed immediately by dysploidy and gave rise to the ancestral chromosome numbers $n = 16$ (subclade 2), $n = 17$ (subclade 3) and $n = 19$ (subclade 1). That is, at the origin of $n = 16$ and $n = 17$, a duplication ($n = 9 \longrightarrow n = 18$) occurred first, followed by descending dysploidy ($n = 18 \longrightarrow n = 17$ and $n = 18 \longrightarrow n = 16$, respectively), whereas at the origin of $n = 19$, a duplication ($n = 9 \longrightarrow n = 18$) occurred first and then ascending dysploidy ($n = 18 \longrightarrow n = 19$) was inferred.

Both dysploidy and duplication promoted the large variation in the chromosome numbers observed in the New World species. This high variability occurred

most probable rate shift configurations found using BAMM. Numbers in green and blue squares indicate subclades 2 and 3, respectively. Numbers on the right margin of the figure indicate the name of the subtribes in which the species are included: 1, Lepidaploinae; 2, Vernoniinae; 3, Chrestinae; 4, Lychnophorinae; 5, Trichospirinae; 6, Rolandrinae; 7, Piptocarphinae; 8, Stokesiinae; 9, Mesanthophorinae; 10, Linziinae; 11, Erlangeinae; 12, Centrapalinae; 13, Gymnantheminae; 14, Hesperomanniinae. B, speciation rate through time (events/Mya per lineage) according to BAMM analysis. Green line corresponds to the speciation rate of subclade 2. Blue line corresponds to the speciation rate of subclade 3. Abbreviations: Plioc., Pliocene; Pleist., Pleistocene; Mya, million years ago.

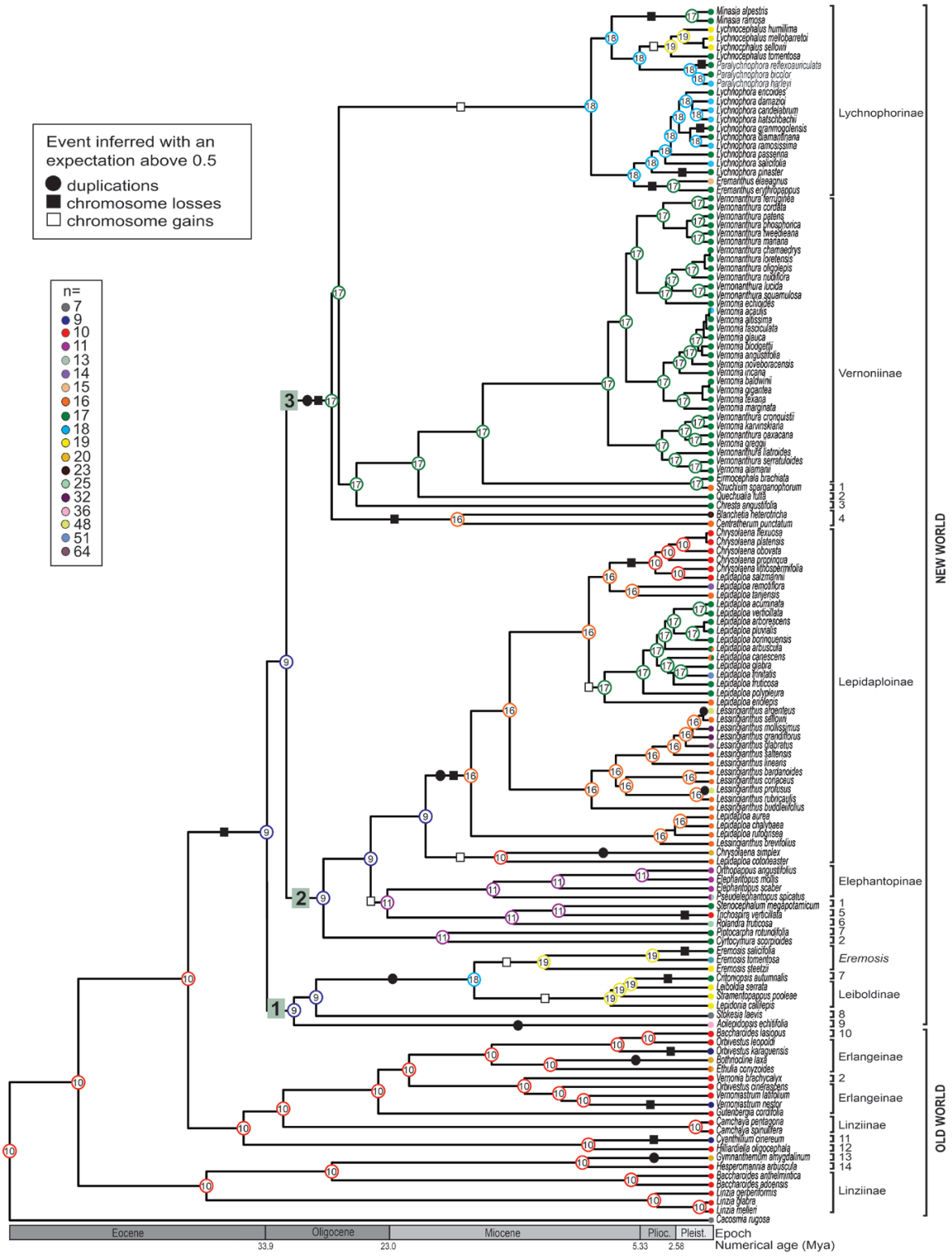

Figure 3. Reconstruction of ancestral chromosome number in tribe Vernonieae using ChromEvol. Circles at nodes represent the most probable ancestral chromosome number. Haploid chromosome numbers are represented by colour-coding explained in the inset. Names of subclades are indicated in grey squares. Black squares represent descending dysploidies,

in chromosome numbers of the most recent common ancestors (MRCAs) of Vernonieae (Fig. 4A) and of current taxa (Fig. 4B).

Chromosome mutation events affecting several taxa [*Ethulia conyzoides* L.f., *Lepidaploa arbuscula* (Less.) H.Rob., *Lepidaploa canescens* (Kunth) H.Robb., *Pseudelephantopus spicatus* (Juss. ex Aubl.) C.F.Baker and *Vernonia acaulis* Gleason] were not accounted for in the simulations (expectations < 0.5). Nevertheless, all of them were single decreases in the base chromosome number. On the other hand, chromosome number showed a strong phylogenetic signal, with a value very close to 1 ($\lambda = 0.98$; $P > 0.05$), i.e. the chromosome number of the species is highly correlated with the phylogenetic relationships that we found in our analysis.

Regarding the chromosome events and shift in diversification rates observed in the tribe, only one of the changes coincided with chromosome changes detected (Fig. 5). This was observed in the Lepidaploinae clade (in subclade 2), in which WGD occurred followed by descending dysploidy.

DISCUSSION

Phylogeny, divergence time and diversification in Vernonieae

In the present study, we reconstructed the phylogenetic relationships of Vernonieae, combining nuclear (ITS) and plastid (*ndhF* and *trnL-F*) markers from all the species with available chromosome numbers previously analysed by Keeley *et al.* (2021). The tree obtained was used to estimate the ancestral chromosome number and to test the role of chromosome changes in the diversification of Vernonieae.

The relationships among species are in general agreement with previous analyses (Keeley *et al.*, 2007, 2021; Keeley & Robinson, 2009; Loeuille *et al.*, 2015; Siniscalchi *et al.*, 2019). The main groups containing representatives from the Old World and the New World were mainly recovered with similar relationships to those previously proposed, although some discrepancies were observed. These differences are probably due to the smaller number of taxa analysed in this study because only species with known chromosome numbers were considered.

The dates inferred in our analysis for the onset of the differentiation of Vernonieae and for the divergences of its main lineages were roughly consistent with previous results by other authors (Mandel *et al.*, 2019; Keeley *et al.*, 2021), although some periods of diversification for the tribe were slightly different. The differences may be due to the calibration points and the species included in the sample. Nevertheless, both results are congruent and agree with the same confidence intervals.

Diversification studies in Asteraceae show an acceleration in the rate of diversification in stems leading to subfamily Cichoroideae, to which Vernonieae belongs, and in the rate of diversification of Vernonieae (Panero & Crozier, 2016). In addition, more recent studies in the family showed an acceleration in the rate of diversification of Vernonieae more specifically (Mandel *et al.*, 2019). The present study indicates that the diversification history of Vernoniae is a complex one characterized by three specific changes in diversification rate in the large New World clade. The three main changes consisted of accelerations in the rate of diversification in two of the three subclades identified for the New World (subclades 2 and 3).

The diversification process of New World clade began in the Early Miocene and an acceleration in diversification rates was observed from the Mid-Miocene to the present. This transitional period was critical in geological history when long-term global climatic cooling was interrupted by the Miocene Climatic Optimum (MMCO, 14.7–17.3 Mya) as modern ecosystems were being established worldwide (Zachos *et al.*, 2001). This scenario reflects the changes in the diversification rates of subclades 2 and 3. Increments in the diversification rates occurred in the Late Miocene for subclade 3 and in the Pliocene for subclade 2, probably due to favourable climatic conditions (drier and colder than in earlier times) (Zachos *et al.*, 2001; Zachos, Dickens & Zeebe, 2008), in which the shrubby and herbaceous vegetation replaced pre-existing woodlands, dramatically expanding their climatic and geographical ranges (Retallack, 2001; Strömberg, 2011). In addition, this period was characterized by strong climatic instability and rapid cycles of glaciations, a strong decline in temperatures interposed with periods of mild and warm climates, and changes in precipitation regimes (Lomolino, Riddle & Whittaker, 2017; Colli-Silva & Pirani, 2019).

white squares indicate ascending dysploidies and black circles represent whole genome duplications. Numbers on the right margin of the figure indicate the name of the subtribes in which the species are included: 1, Lepidaploinae; 2, Vernoniinae; 3, Chrestinae; 4, Lychnophorinae; 5, Trichospirinae; 6, Rolandrinae; 7, Piptocarphinae; 8, Stokesiinae; 9, Mesanthophorinae; 10, Linziinae; 11, Erlangeinae; 12, Centrapalinae; 13, Gymnantheminae; 14, Hesperomanniinae. Abbreviations: Plioc., Pliocene; Pleist., Pleistocene; Mya, million years ago.

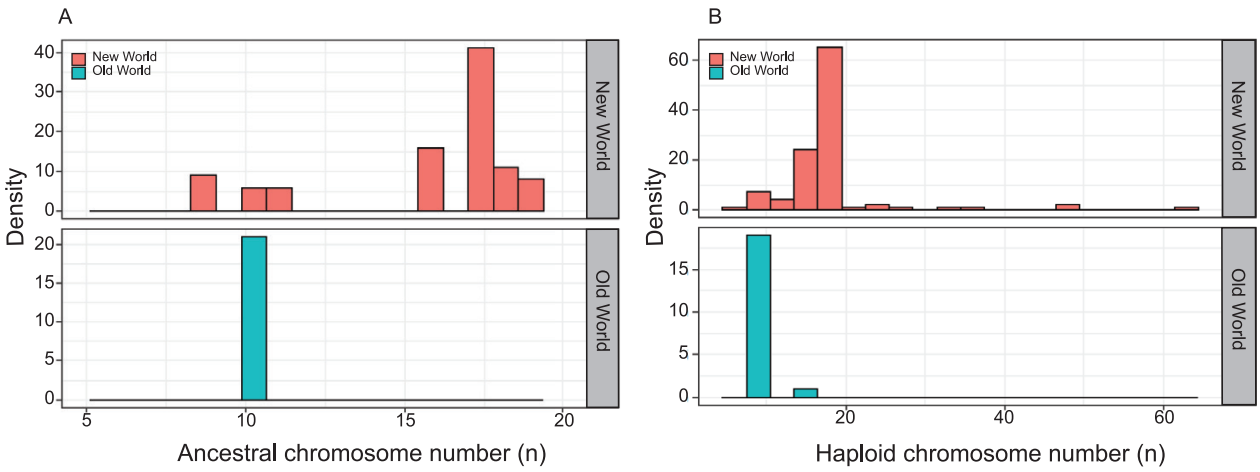

Figure 4. A, density plots of inferred ancestral haploid chromosome number (n) for New and Old World lineages. B, density plots of haploid chromosome numbers (n) for New and Old World lineages.

It is widely accepted that any change in the rate of diversification is strongly affected by many factors (Ricklefs, 2007; Vasconcelos *et al.*, 2020), and thus these climatic conditions, with an increase in the area of open habitats worldwide, could have stimulated the diversification rate of these clades (in particular, that of the New World which presents the highest diversification rates) and consequently of the tribe.

Dispersal is one of the most well-studied factors that can accelerate diversification rates, as it involves arrival in previously unoccupied habitats (Schurr *et al.*, 2012; Linder *et al.*, 2014). Keeley *et al.* (2021) proposed a series of geographically localized adaptive radiations for the New World, mainly through dispersals, and distributional expansions, but also long-distance dispersals starting in the Late Miocene. Changes in the diversification rates in the New World clade found in our studies strongly support the inferences made by these authors.

Evolution of chromosome numbers in Vernonieae

Our analyses using ChromEvol allowed the inference of the most probable ancestral chromosome numbers for the tribe through an evolutionary model and a robust and statistically well-understood approach. Several hypotheses regarding the origin of the base chromosome numbers for the Old and New World have been proposed for the tribe (Jones, 1979; Turner, 1981); however, these assumptions were carried out when the chromosome numbers were only known for a few species. Nevertheless, some of the evolutionary trends proposed by Jones (1979) were observed in our analyses, such as the origin of chromosome number $x = 17$ (discussed below), although Jones considered that this number was the most likely ancestral number for the New World species.

According to our results, the most likely common ancestor of Vernonieae had $n = 10$ chromosomes. The most probable ancestral chromosome number for the tribe confirms previous studies by Mota *et al.* (2016). They conducted a study with a more global approach, the main objective being to infer ancestral numbers for Asteraceae, and from these analyses they recovered the ancestral chromosome number for the Vernonieae clade.

On the other hand, our results showed that the ancestors of all the Old World species had $n = 10$. This homogeneity is reflected in the low variation in chromosome number observed in this lineage. In contrast, the New World species showed a large variation in ancestral chromosome numbers. This variation is also reflected in the current chromosome numbers (Jones, 1973; Jones, 1979; Olsen, 1980; Turner *et al.*, 1981; Sundberg *et al.*, 1986; Galiano & Hunziker, 1987; Rabakonandrianina & Carr, 1987; Keil *et al.*, 1988; Grupta & Gill, 1989; Dematteis, 1998, 2002; Mansanares, Forni-Martins & Semir, 2002, 2007; Dematteis *et al.*, 2007; Oliveira, Forni-Martins & Semir, 2007a, b; Angulo & Dematteis, 2009a, b, 2010, 2015; Salles-de-Melo *et al.*, 2010; Angulo & Dematteis, 2012; Via do Pico & Dematteis, 2012; Marinho *et al.*, 2017) resulting from different chromosome changes. Furthermore, with our model of chromosome number evolution we were able to infer that the most likely ancestral chromosome number of the New World clade is $n = 9$ and to demonstrate the important role of chromosomal events (polyploidy and dysploidy) in the high variability of chromosome number in the New World clade.

There is no doubt that the evolution of chromosome numbers in Vernonieae was a dynamic process. The tribe underwent polyploidization, ascending dysploidy and descending dysploidy events, the last

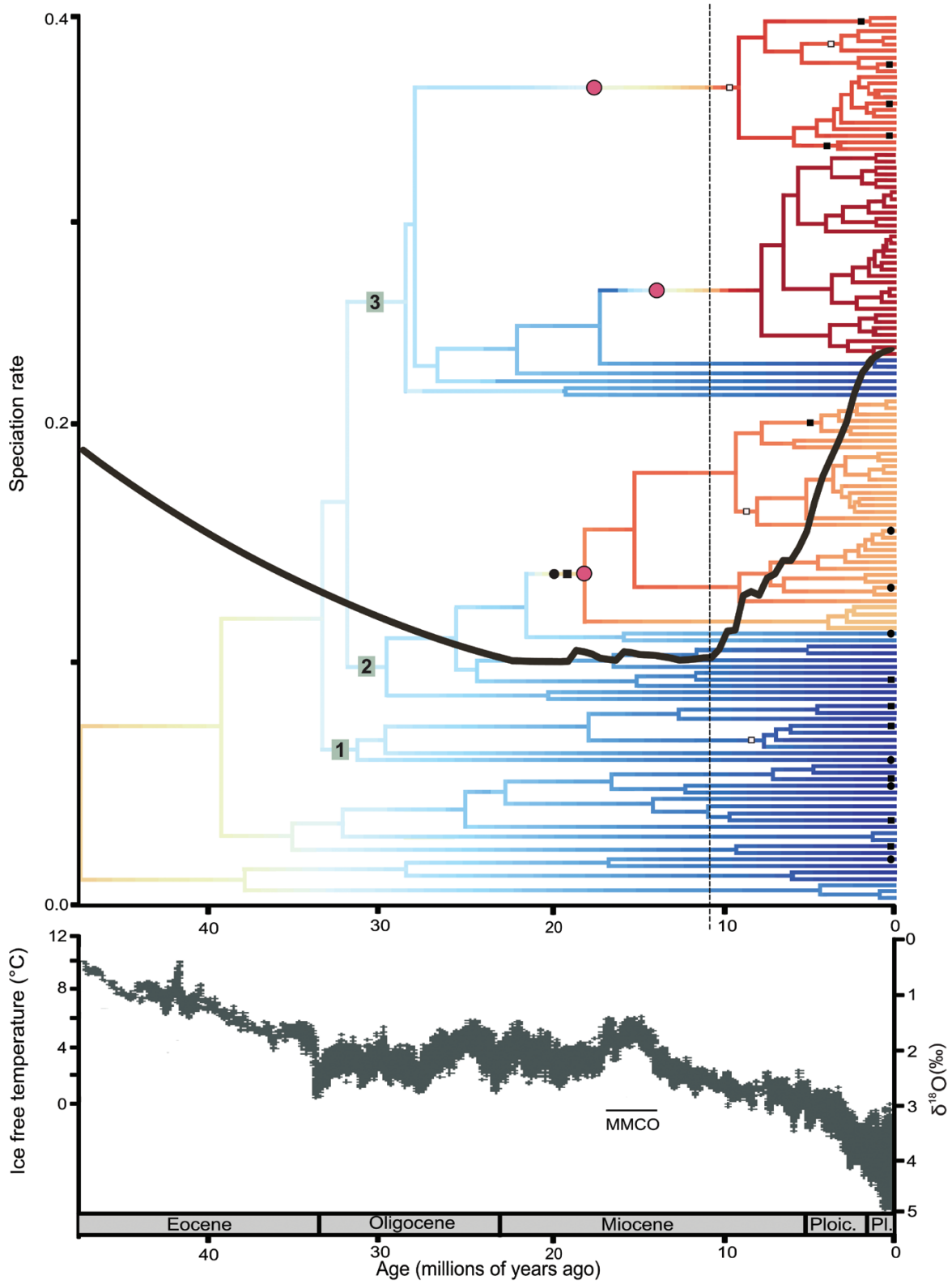

Figure 5. Diversification and chromosome evolution analyses in Vernonieae. Speciation rate through time (events/Mya per lineage) according to BAMM analysis. MCC tree with three shifts of net diversification rate showing chromosome events in subclade 2 and at ~11 Mya onwards (dashed line). The lower part of this figure shows evolution of atmospheric CO_2 levels

being the most frequent, which occurred at the family level in Asteraceae (Mota *et al.*, 2016) and in other groups, such as Melanthiaceae (Pellicer *et al.*, 2014), Colchicaceae (Chacón, Cusimano & Renner, 2014), Poaceae (Pimentel *et al.*, 2017; Chiavegatto *et al.*, 2020) and Cyperaceae (Márquez-Corro *et al.*, 2019). All these previous studies and our analyses identified dysploidy as a process that generates gains and losses of single chromosomes leading to little or negligible change in DNA content (Escudero *et al.*, 2014), it being a common cytoevolutionary pattern among angiosperm families. We cannot, however, rule out the fact that other variations (losses or gains) in chromosome number may occur due to aneuploidy, although this process involves changes in the genetic material, which are rarely tolerated by plants (De Storme & Mason, 2014; Mayrose & Lysak, 2021), and is usually transient, thus playing a minor role in evolutionary terms (Weiss-Schneeweiss & Schneeweiss, 2013).

Descending dysploidy, which occurred in both Old and New World lineages in Vernonieae, is one of the causes that could explain the variability in the chromosome numbers of both lineages. Usually, this chromosome rearrangement is equally distributed throughout phylogenetic trees (Escudero *et al.*, 2014; Carta, Bedini & Peruzzi, 2020); however, the difference between the two lineages was that this chromosome event only occurred towards the tips of the tree in the Old World lineage, whereas in the New World lineage it occurred both deeper in the tree and towards the tips.

Ascending dysploidy was present at lower frequency during the evolution of Vernonieae, as reported for many other plant groups (Moraes *et al.*, 2017; Sader *et al.*, 2019; Chiavegatto *et al.*, 2020). This chromosome change gave rise to the ancestral chromosome numbers n = 11, 17 [(*Lepidaploa* (Cass.) Cass.], 18 and 19, also demonstrating an important role in the chromosome evolution of the tribe. However, several authors proposed that descending dysploidy played a more important role than ascending dysploidy during the process of chromosome number evolution in plants (Guerra, 2012; Carta *et al.*, 2020; Mayrose & Lysak, 2021). The present study showed that although descending dysploidy was the most frequent event, both mechanisms operated together in the tribe during the evolution of ancestral chromosome numbers, demonstrating that unidirectional progressive dysploidy did not occur in Vernonieae.

Most of the polyploidization events are located towards the tips of the tree (seven of nine) and are related to the origin of species of *Acilepidopsis* H.Rob. (Mesantophorinae), *Bothriocline* Oliv. ex Benth. (Erlangeinae), *Gymnanthemum* Cass. (Gymnantheminae), *Chrysolaena* H.Rob. and *Lessingianthus* H.Rob. (Lepidaploinae), whereas ancient polyploidy events are poorly represented, being only found in the New World clade. Polyploidization events that were mainly inferred towards the tips of the tree led to a high number of haploid chromosomes, as detected in other studies involving other plant families (Cusimano *et al.*, 2012; Escudero *et al.*, 2014).

On the other hand, ancient polyploidization events were followed by descending dysploidies. In Asteraceae, post-polyploid diploidization by descending dysploidy is a frequent event (Huang *et al.*, 2016) and it also occurs in other plant groups (Escudero *et al.*, 2014; Sader *et al.*, 2019; Chiavegatto *et al.*, 2020), generating diploidizations that convert polyploids into functional diploids, resulting in evolutionary success (Dodsworth, Chase & Leitch, 2016; Mandáková & Lysak, 2018). Probably for this reason, in the present study they were related to the origin of the ancestral chromosome numbers n = 16 and n = 17 of the clades grouping several genera and subtribes.

We should not rule out that the evolution of polyploidy in Vernonieae is probably much more complex and does not involve only WGD, or WGD followed by dysploidy, as detected in this study. In the Lepidaploinae subclade, consisting of *Chrysolaena*, *Lessigianthus* and *Lepidaploa*, both events have been observed. Recently, Marques *et al.* (2020) suggested that these three genera deserve more attention as their separation is doubtful and that they should be synonymized as a single genus. In our analyses, this subclade has been found to be polyphyletic. Although there are no studies on hybridization in these genera, it is known that hybridization is possible in Vernonieae (Jones, 1977; Stutts, 1988), especially as these genera live in sympatry. Therefore, allopolyploidy events could have occurred and contributed to the different haploid numbers in this clade. Allopolyploidy is an important driver of diversification, both in speciation and in triggering a cascade of processes operating at the genomic and genetic levels (Weiss-Schneeweiss & Schneeweiss, 2013), and it should be considered in the evolution of the tribe. Furthermore, a combination of

and global climate from figure 2 in Zachos *et al.* (2008) adapted to the divergence time of the tribe. Edges coloured in red and blue exhibit high and low rates of net diversification, respectively. Names of subclades are indicated in grey squares. Black squares represent descending dysploidies, white squares indicate ascending dysploidies and black circles represent whole genome duplications. Abbreviations: MMCO: Mid-Miocene Climatic Optimum; Plioc., Pliocene; Pleist., Pleistocene.

events could also occur, i.e. hybridization followed by diploidization events, as suggested by Salles-de-Melo *et al.* (2010).

Impact of chromosome events in the shift in diversification rates of Vernonieae

Many authors researching polyploidy claim that WGD events probably resulted in the evolution of a large number of new gene functions (Stephens, 1951; Wendel, 2000; Otto, 2007; Te Beest *et al.*, 2012; Soltis, Visger & Soltis, 2014). Genome reduction processes following polyploidy (diploidization) (Ohno, 1970; Leitch & Bennett, 2004; Edger & Pires, 2009; Mandáková & Lysak, 2018) could be highly selective and help to establish successful sustainable populations following dramatic changes in stressful environments. Asteraceae are one of the largest angiosperm families, and, according to Huang *et al.* (2015) and Tank *et al.* (2015), their success has been associated with multiple WGD events. However, other authors consider that the WGD events in Asteraceae are not perfectly correlated with the origin or diversification of the family (Barker *et al.*, 2016). Our results showed that nine WGD events occurred during the diversification of Vernonieae. However, of the three shifts in diversification rate only the Lepidaploinae clade was related to a WGD event followed by dysploidy; the other two were not associated with a chromosome number change. The dysploid changes may have led to reproductive isolation and contributed to speciation in this clade. The onset of diversification of Lepidaploinae occurred almost at the beginning of the MMCO which possibly also contributed to an increase in the diversification rate.

Although the analyses carried out in this study do not establish direct causality between chromosome number and diversification, we can infer from the results obtained that at least 32 chromosome events occurred during the ~46 Mya that the tribe has been diverging. The diversification rate of Vernonieae began to increase exponentially ~11 Mya, shortly after the MMCO, and more than half of the chromosome events have occurred since that time, suggesting that polyploidy and dysploidy have greatly influenced the diversification of Vernonieae. Finally, the high value obtained in the phylogenetic signal analysis shows that the phylogenetic tree presented explains the observed patterns of chromosome numbers.

CONCLUSIONS

In general terms, our results show that the study of chromosome number in combination with phylogenetic and statistical studies are useful in understanding, in part, the evolutionary history of Vernonieae. We have established a time-calibrated phylogenetic tree that is highly compatible with the phylogenetic trees of Vernonieae of other authors, including species with available chromosome numbers, which supported $n = 10$ as the ancestral chromosome number of the tribe. This number is also the ancestral chromosome number characteristic of the Old World species, whereas $n = 9$ is the ancestral chromosome number for the New World species. Most of the colonization that occurred in the New World was accompanied by distinct chromosome changes that resulted in the high variability of chromosome numbers observed. Undoubtedly, the large number of chromosome events that occurred have played an important role during the diversification of the tribe. The diversification rate was affected sharply from the Mid-Miocene to the present, with more than half of the chromosome events occurring from ~11 Mya to the present. Certainly, the chromosome events observed in the tribe occurred in a context of both rapid and gradual climatic changes during the ~46 Mya since the origin of tribe. It is likely that this combination of chromosome events and climatic factors, with the long-distance dispersals proposed by other authors, have promoted the great diversification and evolution of Vernonieae.

ACKNOWLEDGEMENTS

We are deeply indebted to M. Sc. Federico Agostini for technical support with bioinformatics. Three anonymous reviewers are thanked for critically reading the manuscript and suggesting substantial improvements. The authors declare that they have no competing interests.

FUNDING

This research has been supported by Secretaría General de Ciencia y Técnica de la Universidad Nacional del Nordeste (PI No. 19-P005), Consejo Nacional de Investigaciones Científicas y Técnicas (PIP No. 11220150100125) and Agencia Nacional de Promoción Científica y Tecnológica (PICT No. 2016-2686, PICT No 2015-2804).

REFERENCES

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19:** 716–723.

Angulo MB, **Dematteis M. 2009a.** Karyological analysis of South American species of *Vernonia* (Vernonieae, Asteraceae). *Plant Biosystems* **143:** 20–24.

Angulo MB, **Dematteis M. 2009b.** Karyotype analysis in eight species of *Vernonia* (Vernonieae, Asteraceae) from South America. *Caryologia* **62:** 81–88.

Angulo MB, **Dematteis M. 2010.** La identidad de *Vernonia setososquamosa* (Asteraceae, Vernonieae): evidencias cromosómicas y palinológicas. *Darwiniana* **48:** 17–24.

Angulo MB, **Dematteis M. 2012.** Cytotaxonomy of some species of the South American genus *Lessingianthus* (Asteraceae, Vernonieae). *Plant Systematics and Evolution* **298:** 277–285.

Angulo MB, **Dematteis M. 2015.** Karyotypes of some species of the genus *Lessingianthus* (Vernonieae, Asteraceae) and taxonomic implications. *Nordic Journal of Botany* **33:** 239–248.

Barker MS, **Li Z**, **Kidder TI**, **Reardon CR**, **Lai Z**, **Oliveira LO**, **Scasciatelli M**, **Rieseberg LH. 2016.** Most Compositae are descendants of a paleohexaploid and all share a paleotetraploid ancestor with the Calyceraceae. *American Journal of Botany* **103:** 1203–1211.

Bekaert M, **Edger PP**, **Pires JC**, **Conant GC. 2011.** Two-phase resolution of polyploidy in the *Arabidopsis* metabolic network gives rise to relative and absolute dosage constraints. *Plant Cell* **23:** 1719–1728.

Blanc G, **Wolfe KH. 2004.** Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* evolution. *Plant Cell* **16:** 1679–1691.

Carta A, **Bedini G**, **Peruzzi L. 2020.** A deep dive into the ancestral chromosome number and genome size of flowering plants. *New Phytologist* **228:** 1097–1106.

Chacón J, **Cusimano N**, **Renner SS. 2014.** The evolution of Colchicaceae, with a focus on chromosome numbers. *Systematic Botany* **39:** 415–427.

Chiavegatto RB, **Carta A**, **Pereira DG**, **Benites FR**, **Techio VH**, **Peruzzi L. 2020.** Reconstructing ancestral chromosome numbers and inflorescence features in Eleusininae (Poaceae: Chloridoideae: Cynodonteae). *Botanical Journal of the Linnean Society* **193:** 402–418.

Colli-Silva M, **Pirani JR. 2019.** Biogeographic patterns of Galipeinae (Galipeeae, Rutaceae) in Brazil: species richness and endemism at different latitudes of the Atlantic Forest "hotspot". *Flora* **251:** 77–87.

Cusack BP, **Wolfe KH. 2007.** When gene marriages don't work out: divorce by subfunctionalization. *Trends Genetics* **23:** 270–272.

Cusimano N, **Sousa A**, **Renner SS. 2012.** Maximum likelihood inference implies a high, not a low, ancestral haploid chromosome number in Araceae, with a critique of the bias introduced by '*x*'. *Annals of Botany* **109:** 681–692.

Darriba D, **Taboada GL**, **Doallo R**, **Posada D. 2012.** jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9:** 772.

Dematteis M. 1998. Karyotype analysis in some *Vernonia* species (Asteraceae) from South America. *Caryologia* **51:** 279–288.

Dematteis M. 2002. Cytotaxonomic analysis of South American species of *Vernonia* (Vernonieae: Asteraceae). *Botanical Journal of the Linnean Society* **139:** 401–408.

Dematteis M, **Molero J**, **Angulo MB**, **Rovira AM. 2007.** Chromosome studies on some Asteraceae from South America. *Botanical Journal of the Linnean Society* **153:** 221–230.

De Storme N, **Mason A. 2014.** Plant speciation through chromosome instability and ploidy change: cellular mechanisms, molecular factors and evolutionary relevance. *Current Plant Biology* **1:** 10–33.

Dodsworth S, **Chase MW**, **Leitch AR. 2016.** Is post-polyploidization diploidization the key to the evolutionary success of angiosperms? *Botanical Journal of the Linnean Society* **180:** 1–5.

Drummond AJ, **Rambaut A. 2007.** BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 2141–8.

Edger PP, **Pires JC. 2009.** Gene and genome duplications: the impact of dosage-sensitivity on the fate of nuclear genes. *Chromosome Research* **17:** 699–717.

Escudero M, **Hipp AL**, **Waterway MJ**, **Valente LM. 2012.** Diversification rates and chromosome evolution in the most diverse angiosperm genus of the temperate zone (*Carex*, Cyperaceae). *Molecular Phylogenetics and Evolution* **63:** 650–655.

Escudero M, **Martín-Bravo S**, **Mayrose I**, **Fernández-Mazuecos M**, **Fiz-Palacios O**, **Hipp AL**, **Pimentel M**, **Jiménez-Mejıás V**, **Valcárcel V**, **Vargas P**, **Luceño M. 2014.** Karyotypic changes through dysploidy persist longer over evolutionary time than polyploid changes. *PLoS One* **9:** e85266.

Fawcett JA, **Van de Peer Y**, **Maere S. 2013.** Significance and biological consequences of polyploidization in land plant evolution. In: Leitch IJ, Greilhuber J, Doležel J, Wendel JF, eds. *Plant genome diversity*. Vienna: Springer, 277–294.

FitzJohn RG, **Maddison WP**, **Otto SP. 2009.** Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. *Systematic Biology* **58:** 595–611.

Forest F. 2009. Calibrating the Tree of Life: fossils, molecules and evolutionary timescales. *Annals of Botany* **104:** 789–794.

Galiano NG, **Hunziker JH. 1987.** Estudios cariológicos en Compositae. IV. Vernonieae y Eupatorieae. *Darwiniana* **28:** 1–8.

Gleason HA. 1906. A revision of the North American Vernonieae. *Bulletin of the New York Botanical Garden* **4:** 144–243.

Glick L, **Mayrose I. 2014.** ChromEvol: assessing the pattern of chromosome number evolution and the inference of polyploidy along a phylogeny. *Molecular Biology and Evolution* **31:** 1914–1922.

Grupta RC, **Gill BS. 1989.** Cytopalynology of north and central Indian Compositae. *The Journal of Cytology and Genetics* **24:** 96–105.

Guerra M. 2008. Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenetic and Genome Research* **120:** 339–350.

Guerra M. 2012. Cytotaxonomy: the end of childhood. *Plant Biosystems* **146:** 703–710.

Huang C-H, **Sun R**, **Hu Y**, **Zeng L**, **Zhang N**, **Cai L**, **Zhang Q**, **Koch MA**, **Al- Shehbaz I**, **Edger PP**, **Pires JC**, **Tan D-Y**,

Zhong Y, **Ma H. 2015.** Resolution of Brassicaceae phylogeny using nuclear genes uncovers nested radiations and supports convergent morphological evolution. *Molecular Biology and Evolution* **33:** 394–412.

Huang C-H, **Zhang C**, **Liu M**, **Hu Y**, **Gao T**, **Qi J**, **Ma H. 2016.** Multiple polyploidization events across Asteraceae with two nested events in the history revealed by nuclear phylogenomics. *Molecular Biology and Evolution* **33:** 2820–2835.

Hudson CM, **Puckett EE**, **Bekaert M**, **Pires JC**, **Conant GC. 2011.** Selection for higher gene copy number after different types of plant gene duplications. *Genome Biology and Evolution* **3:** 1369–1380.

Jones SB. 1973. Revision of *Vernonia* section *Eremosis* (Compositae) in North America. *Brittonia* **25:** 86–115.

Jones SB. 1977. Vernonieae systematic review. In: Heywood VH, Harborne JB, Turner BL, eds. *The biology and chemistry of the Compositae*. London: Academic Press, 503–521.

Jones SB. 1979. Chromosome numbers of Vernonieae (Compositae). *Bulletin of the Torrey Botanical Club* **106:** 79–84.

Keeley SC, **Cantley JT**, **Gallaher TJ. 2021.** The "evil tribe" spreads across the land: a dated molecular phylogeny provides insight into dispersal, expansion, and biogeographic relationships within one of the largest tribes of the sunflower family (Vernonieae: Compositae). *American Journal of Botany* **108:** 505–519.

Keeley SC, **Forsman ZH**, **Chan R. 2007.** A phylogeny of the "evil tribe" (Vernonieae: Compositae) reveals Old/New World long distance dispersal: support from separate and combined congruent datasets (*trnL-F*, *ndhF*, ITS). *Molecular Phylogenetics and Evolution* **44:** 89–103.

Keeley SC, **Jansen RK. 1994.** Chloroplast DNA restriction site variation in the Vernonieae (Asteraceae), an initial appraisal of the relationship of New and Old World taxa and the monophyly of *Vernonia*. *Plant Systematics and Evolution* **193:** 249–265.

Keeley SC, **Robinson H. 2009.** Vernonieae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ, eds. *Systematics, evolution and biogeography of Compositae*. Vienna: International Association for Plant Taxonomists, 439–469.

Keeley SC, **Turner BL. 1990.** A preliminary cladistic analysis of the genus *Vernonia* (Vernonieae: Asteraceae). In: Mabry TJ, Wagenitz G, eds. *Research advances in the Compositae*. Vienna: Springer, 45–66.

Keil DJ, **Luckow MA**, **Pinkava DJ. 1988.** Chromosome studies in Asteraceae from the United States, Mexico, the West Indies, and South America. *American Journal of Botany* **75:** 652–668.

Kumar S, **Stecher G**, **Tamura K. 2016.** MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33:** 1870–1874.

Leitch IJ, **Bennett MD. 2004.** Genome downsizing in polyploid plants. *Botanical Journal of the Linnean Society* **82:** 651–663.

Linder HP, **Rabosky DL**, **Antonelli A**, **Wüest R**, **Ohlemüller R. 2014.** Disentangling the influence of climatic and geological changes on species radiations. *Journal of Biogeography* **41:** 1313–1325.

Loeuille B, **Keeley SC**, **Pirani JR. 2015.** Systematics and evolution of syncephaly in American Vernonieae (Asteraceae) with emphasis on the Brazilian subtribe Lychnophorinae. *Systematic Botany* **40:** 286–298.

Loeuille B, **Semir J**, **Pirani JR. 2019.** A synopsis of Lychnophorinae (Asteraceae: Vernonieae). *Phytotaxa* **398:** 1–139.

Lomolino MV, **Riddle BR**, **Whittaker RJ. 2017.** *Biogeography*. Oxford: Oxford University Press.

Madlung A, **Tyagi AP**, **Watson B**, **Jiang H**, **Kagochi T**, **Doerge RW**, **Martienssen R**, **Comai L. 2005.** Genomic changes in synthetic *Arabidopsis* polyploids. *The Plant Journal* **41:** 221–230.

Maere S, **Van de Peer Y. 2010.** Duplicate retention after small- and large-scale duplications. In: Dittmar K, Liberles D, eds. *Evolution after gene duplication*. Hoboken: Wiley, 31–56.

Mandáková T, **Lysak MA. 2018.** Post-polyploid diploidization and diversification through dysploid changes. *Current Opinion in Plant Biology* **42:** 55–65.

Mandel JR, **Dikow RB**, **Siniscalchi CM**, **Thapa R**, **Watson LE**, **Funk VA. 2019.** A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. *Proceedings of the National Academy of Sciences, USA* **116:** 14083–14088.

Mansanares ME, **Forni-Martins ER**, **Semir J. 2002.** Chromosome numbers in the genus *Lychnophora* Mart. (Lychnophorinae, Vernonieae, Asteraceae). *Caryologia* **55:** 367–374.

Mansanares ME, **Forni-Martins ER**, **Semir J. 2007.** Cytotaxonomy of *Lychnophoriopsis* Sch. Bip. and *Paralychnophora* MacLeish species (Asteraceae: Vernonieae: Lychnophorinae). *Botanical Journal of the Linnean Society* **154:** 109–114.

Marinho MAO, **Sales-Melo MRC**, **Melo de Oliveira MB**, **Maciel VDO**, **de Carvalho R. 2017.** Karyotype variation in 11 species of the Vernonieae Cass. tribe (Asteraceae Bercht. & J. Presl). *Plant Biosystems* **151:** 291–303.

Marques D, **de Oliveira Franca R**, **Angulo MB**, **Via do Pico GM**, **Dematteis M**, **Marzinek J. 2020.** Comparative anatomy of cypselae in the complex group *Chrysolaena*, *Echynocoryne*, *Lepidaploa*, and *Lessingianthus*: contributions to the systematic of Vernonieae (Compositae). *Systematic Botany* **45:** 668–680.

Márquez-Corro JI, **Martín-Bravo S**, **Spalink D**, **Luceño M**, **Escudero M. 2019.** Inferring hypothesis-based transitions in clade-specific models of chromosome number evolution in sedges (Cyperaceae). *Molecular Phylogenetics and Evolution* **135:** 203–209.

Mayrose I. 2014. *ChromEvol version. 2.0 Manual*. Tel Aviv: Tel Aviv University.

Mayrose I, **Barker MS**, **Otto SP. 2010.** Probabilistic models of chromosome number evolution and the inference of polyploidy. *Systematic Biology* **59:** 132–144.

Mayrose I, **Lysak MA. 2021.** The evolution of chromosome numbers: mechanistic models and experimental approaches. *Genome Biology and Evolution* **13:** evaa220.

Mayrose I, **Zhan SH**, **Rothfels CJ**, **Magnuson-Ford K**, **Barker MS**, **Rieseberg LH**, **Otto SP. 2011.** Recently formed polyploid plants diversify at lower rates. *Science* **333:** 1257.

Moraes AP, **Koehler S**, **Cabral JS**, **Gomes SS**, **Viccini LF**, **Barros F**, **Felix LP**, **Guerra M**, **Forni-Martins ER. 2017.** Karyotype diversity and genome size variation in Neotropical Maxillariinae orchids. *Plant Biology* **19:** 298–308.

Mota L, **Torices R**, **Loureiro J. 2016.** The evolution of haploid chromosome numbers in the sunflower family. *Genome Biology and Evolution* **8:** 3516–3528.

Ohno S. 1970. *Evolution by gene duplication*. Berlin: Springer-Verlag.

Oliveira VM, **Forni-Martins ER**, **Semir J. 2007a.** Cytotaxonomic studies in six species of *Vernonia* (Asteraceae: Vernonieae). *Caryologia* **60:** 37–47.

Oliveira VM, **Forni-Martins ER**, **Semir J. 2007b.** Cytotaxonomy of species of *Vernonia*, section *Lepidaploa*, group Axilliflorae (Asteraceae, Vernonieae). *Botanical Journal of the Linnean Society* **154:** 99–108.

Olsen J. 1980. In IOPB chromosome number reports. *Taxon* **29:** 366–367.

Otto SP. 2007. The evolutionary consequences of polyploidy. *Cell* **131:** 452–462.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401:** 877–884.

Panero JL, **Crozier BS. 2016.** Macroevolutionary dynamics in the diversification of Asteraceae. *Molecular Phylogenetics and Evolution* **99:** 116–132.

Paradis E, **Claude J**, **Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20:** 289–290.

Pellicer J, **Kelly LJ**, **Leitch IJ**, **Zomlefer WB**, **Fay MF. 2014.** A universe of dwarfs and giants: genome size and chromosome evolution in the monocot family Melanthiaceae. *New Phytologist* **201:** 1484–1497.

Pennell MW, **Eastman JM**, **Slater GJ**, **Brown JW**, **Uyeda JC**, **FitzJohn RG**, **Alfaro ME**, **Harmon LJ. 2014.** Geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* **30:** 2216–2218.

Pimentel M, **Escudero M**, **Sahuquillo E**, **Minaya MA**, **Catalán P. 2017.** Are diversification rates and chromosome evolution in the temperate grasses (Pooideae) associated with major environmental changes in the Oligocene–Miocene? *PeerJ* **5:** e3815.

Plummer M, **Best N**, **Cowles K**, **Vines K. 2006.** CODA: convergence diagnosis and output analysis for MCMC. *R News* **6:** 7–11.

Pontes O, **Neves N**, **Silva M**, **Lewis MS**, **Madlung A**, **Comai L**, **Viegas W**, **Pikaard CS. 2004.** Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid *Arabidopsis suecica* genome. *Proceedings of the National Academy of Sciences, USA* **101:** 18240–18245.

R Core Team. **2019.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: http://www.r-project.org/

Rabakonandrianina E, **Carr GD. 1987.** Chromosome numbers of Madagascar plants. *Annals of the Missouri Botanical Garden* **74:** 123–125.

Rabosky D, **Grundler M**, **Title P**, **Anderson C**, **Shi J**, **Brown J**, **Huang H**, **Mitchell J. 2019.** *Package BAMMtools*. Available at: https://cran.r-project.org/web/packages/BAMMtools/index.html

Rabosky DL, **Grundler M**, **Anderson C**, **Title P**, **Shi J**, **Huang H**, **Brown JW**, **Larson J. 2014.** BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution* **5:** 701–707.

Rabosky DL, **Mitchell JS**, **Chang J. 2017.** Is BAMM flawed? Theoretical and practical concerns in the analysis of multi-rate diversification models. *Systematic Biology* **66:** 477–498.

Rambaut A. 2014. *Figtree, a graphical viewer of phylogenetic trees, version v.1.4.3*. Available at: http://tree.bio.ed.ac.uk/software/figtree.

Rambaut A, **Drummond AJ**, **Xie D**, **Baele G**, **Suchard MA. 2018.** Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67:** 901–904.

Retallack GJ. 2001. Cenozoic expansion of grasslands and climatic cooling. *The Journal of Geology* **109:** 407–426.

Ricklefs RE. 2007. Estimating diversification rates from phylogenetic information. *Trends in Ecology & Evolution* **22:** 601–610.

Robinson H. 1999a. Revisions in paleotropical Vernonieae (Asteraceae). *Proceedings of the Biological Society of Washington* **112:** 220–247.

Robinson H. 1999b. Generic and subtribal classification of American Vernonieae. *Smithsonian Contributions to Botany* **89:** 1–116.

Rodriguez FJ, **Oliver JL**, **Marin A**, **Medina JR. 1990.** The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142:** 485–501.

Sader MA, **Amorim BS**, **Costa L**, **Souza G**, **Pedrosa-Harand A. 2019.** The role of chromosome changes in the diversification of *Passiflora* L. (Passifloraceae). *Systematics and Biodiversity* **17:** 7–21.

Salles-de-Melo MRC, **de Lucena RM**, **Semir J**, **de Carvalho R**, **Pereira RDCA**, **Benko-Iseppon AM. 2010.** Karyological features and cytotaxonomy of the tribe Vernonieae (Asteraceae). *Plant Systematics and Evolution* **285:** 189–199.

Schranz EM, **Mohammadin S**, **Edger PP. 2012.** Ancient whole genome duplications, novelty and diversification: the WGD radiation lag-time model. *Current Opinion in Plant Biology* **15:** 147–153.

Schubert I, **Lysak MA. 2011.** Interpretation of karyotype evolution should consider chromosome structural constraints. *Trends in Genetics* **27:** 207–216.

Schurr FM, **Pagel J**, **Sarmento Cabral J**, **Bykova O**, **O'Hara RB**, **Hartig F**, **Kissling WD**, **Lindr HP**, **Midgley GF**, **Schröder B**, **Singer A**, **Zimmermann NE. 2012.** How to understand species' niches and range dynamics: a demographic research agenda for biogeography. *Journal of Biogeography* **39:** 2146–2162.

Shi JJ, **Rabosky DL. 2015.** Speciation dynamics during the global radiation of extant bats. *Evolution* **69:** 1528–1545.

Siniscalchi C, **Loeuille B**, **Funk VA**, **Mandel JR**, **Pirani JR. 2019.** Phylogenomics yields new insight into relationships within Vernonieae (Asteraceae). *Frontiers in Plant Science* **10:** 1224.

Soltis DE, **Albert VA**, **Leebens-Mack J**, **Bell CD**, **Paterson AH**, **Chunfang Zheng C**, **Sankoff D**, **de Pamphilis C**, **Wall K**, **Soltis PS. 2009.** Polyploidy and angiosperm diversification. *American Journal of Botany* **96:** 336–348.

Soltis PS, **Soltis DE. 2000.** The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences, USA* **97:** 7051–7057.

Soltis DE, **Visger CJ**, **Soltis PS. 2014.** The polyploidy revolution then… and now: Stebbins revisited. *American Journal of Botany* **101:** 1057–1078.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold.

Stephens SG. 1951. Possible significance of duplication in evolution. *Advances in Genetics* **4:** 247–265.

Strömberg CAE. 2011. Evolution of grasses and grassland ecosystems. *Annual Review of Earth and Planetary Sciences* **39:** 517–544.

Stutts JG. 1988. Taxonomic revision of *Vernonia* subsect. *Chamaedrys* (Compositae: Vernonieae). *Rhodora* **90:** 37–99.

Sundberg S, **Cowan CP**, **Turner BL. 1986.** Chromosome counts of Latin American Compositae. *American Journal of Botany* **73:** 33–38.

Tank DC, **Eastman JM**, **Pennell MW**, **Soltis PS**, **Soltis DE**, **Hinchliff CE**, **Brown JW**, **Sessa EB**, **Harmon LJ. 2015.** Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications. *New Phytologist* **207:** 454–467.

Te Beest M, **Le Roux JJ**, **Richardson DM**, **Brysting AK**, **Suda J**, **Kubešová M**, **Pyšek P. 2012.** The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* **109:** 19–45.

Turner BL. 1981. New species and combinations in *Vernonia* sections *Leiboldia* and *Lepidonia* (Asteraceae), with a revisional conspectus of the groups. *Brittonia* **33:** 401–412.

Van de Peer Y. 2011. A mystery unveiled. *Genome Biology* **12:** 113.

Vasconcelos TN, **Alcantara S**, **Andrino CO**, **Forest F**, **Reginato M**, **Simon MF**, **Pirani JR. 2020.** Fast diversification through a mosaic of evolutionary histories characterizes the endemic flora of ancient Neotropical mountains. *Proceedings of the Royal Society B: Biological Sciences* **287:** 20192933.

Via do Pico GM, **Dematteis M. 2012.** Chromosome number, meiotic behavior and pollen fertility of six species of *Chrysolaena* (Vernonieae, Asteraceae). *Caryologia* **65:** 176–181.

Weiss-Schneeweiss H, **Schneeweiss GM. 2013.** Karyotype diversity and evolutionary trends in angiosperms. In: Leitch IJ, Greilhuber J, Doležel J, Wendel J, eds. *Plant genome diversity, Vol. 2.* Vienna: Springer, 209–230.

Wendel JF. 2000. Genome evolution in polyploids. In: Doyle JJ, Gaut BS, eds. *Plant molecular evolution*. Dordrecht: Springer, 225–249.

Winterfeld G, **Ley A**, **Hoffmann MH**, **Paule J**, **Röser M. 2020.** Dysploidy and polyploidy trigger strong variation of chromosome numbers in the prayer-plant family (Marantaceae). *Plant Systematics and Evolution* **306:** 1–17.

Zachos JC, **Dickens GR**, **Zeebe RE. 2008.** An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* **451:** 279–283.

Zachos J, **Pagani M**, **Sloan L**, **Thomas E**, **Billups K. 2001.** Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292:** 686–693.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Data S1. GenBank accession numbers for all samples used in analyses.
Data S2. Haploid chromosome number of Vernonieae species included in the study.
Data S3. Number of species analysed in relation to the total number of species per genus of tribe Vernonieae.