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Spatio-temporal history of the disjunct family Tecophilaeaceae: a tale involving the colonization of three Mediterranean-type ecosystems

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• *Background and Aims* Tecophilaeaceae (27 species distributed in eight genera) have a disjunct distribution in California, Chile and southern and tropical mainland Africa. Moreover, although the family mainly occurs in arid ecosystems, it has colonized three Mediterranean-type ecosystems. In this study, the spatio-temporal history of the family is examined using DNA sequence data from six plastid regions.
• *Methods* Modern methods in divergence time estimation (BEAST), diversification (LTT and GeoSSE) and biogeography (LAGRANGE) are applied to infer the evolutionary history of Tecophilaeaceae. To take into account dating and phylogenetic uncertainty, the biogeographical inferences were run over a set of dated Bayesian trees and the analyses were constrained according to palaeogeographical evidence.
• *Key Results* The analyses showed that the current distribution and diversification of the family were influenced primarily by the break up of Gondwana, separating the family into two main clades, and the establishment of a Mediterranean climate in Chile, coinciding with the radiation of *Conanthera*. Finally, unlike many other groups, no shifts in diversification rates were observed associated with the dispersals in the Cape region of South Africa.
• *Conclusions* Although modest in size, Tecophilaeaceae have a complex spatio-temporal history. The family is now most diverse in arid ecosystems in southern Africa, but is expected to have originated in sub-tropical Africa. It has subsequently colonized Mediterranean-type ecosystems in both the Northern and Southern Hemispheres, but well before the onset of the Mediterranean climate in these regions. Only one lineage, genus *Conanthera*, has apparently diversified to any extent under the impetus of a Mediterranean climate.

Key words: Biogeography, California, Chile, disjunct distribution, Greater Cape region, Mediterranean climate, Tecophilaeaceae.

INTRODUCTION

Tecophilaeaceae are a small family of eight genera and 27 species of perennial herbs with a cormous, usually tunicated rootstock, mostly basal or rarely cauline leaves and long-lasting, often colourful flowers, typically in racemose or paniculate, cymose inflorescences, but sometime solitary and axillary. The flowers are actinomorphic or zygomorphic, with 3 + 3 petaloid tepals fused into a short tube adnate to the ovary, and six stamens, all fertile or some reduced to staminodes, with more or less porose dehiscence. The ovary is inferior or semi-inferior and tricarpellate, and matures into a loculicidal capsule (Ravenna, 1988; Simpson and Rudall, 1998; Manning and Goldblatt, 2012).

The family has an unusual, highly disjunct distribution in California, Chile and southern and tropical mainland Africa (Simpson and Rudall, 1998). Furthermore, the absence of the family in south-western Australia is noteworthy given its presence in other Southern Hemisphere Mediterranean-climate regions. The reported occurrence of the family in Madagascar is based on *Walleria paniculata* Fritsch, a synonym of *Dianella ensifolia* (L.) DC. (Xanthorrhoeaceae *sensu* Chase *et al.*, 2009; Manning and Goldblatt, 2012). Tecophilaeaceae are best represented in Africa, where almost two-thirds of the species are found (Manning and Goldblatt, 2012). *Cyanastrum* Oliv. (three species) and *Kabuyea* Brummit (one species) are strictly tropical in wooded and forested habitats, but *Walleria* J.Kirk (three species), *Eremiolirion* J.C.Manning & F.Forest (one species) and *Cyanella* Royen ex L. (nine species) are primarily distributed in sub-tropical and temperate southern African grasslands and open shrublands. The New World taxa comprise *Odontostomum* Torr. (one species) from north-central California, and *Conanthera* Ruíz & Pav. (five species) and *Zephyra* D.Don (=*Tecophilaea* Bertero & Colla) (four species) from Chile. In addition to their highly disjunct distribution, Tecophilaeaceae are found in three of the five major Mediterranean-type ecosystems: California, Chile and the Cape of South Africa.

With the small size, unusual distribution and occurrence in three major Mediterranean-type climate regions, as well as in tropical, sub-tropical and arid ecosystems, an understanding of the spatio-temporal history of Tecophilaeaceae will provide additional information about the evolutionary mechanisms that shaped the establishment of the current Mediterranean floras. We apply modern methods in divergence time, biogeography and diversification to investigate the spatio-temporal history of

Tecophilaeaceae based on plastid DNA sequences for 21 of the 27 species in the family. We accommodated uncertainty in our estimates of phylogenetic and divergence times while inferring the biogeographical scenario of the family, following recommendations by Buerki *et al.* (2011) and Espindola *et al.* (2012). In addition, ancestral area reconstructions were constrained by applying a stratified palaeogeographical model adapted from Buerki *et al.* (2011). Because we have covered almost all described taxa, shifts in diversification rates were investigated by inspecting lineage-through-time curves (LTT curves). The influence of ecology (arid, tropical to sub-tropical and Mediterranean-type ecosystems) on the diversification of the family was examined based on the Geographic State Speciation and Extinction model (GeoSSE; Goldberg *et al.*, 2011).

MATERIALS AND METHODS

Taxon sampling

One hundred and thirty-six (124 for the ingroup) sequences were generated for the present study. See Appendix 1 for voucher information and GenBank accession numbers. Nineteen species of Tecophilaeaceae were sampled, representing all eight genera (see Appendix 2). We also include *Conanthera sabulosa* and *C. variegata*, which are considered as synonyms of *C. campanulata* by some authors; preliminary analyses showed that these taxa did not form a monophyletic group with *C. campanulata*, thus casting some doubts on the validity of their synonymization. Outgroup taxa were selected based on previous phylogenetic studies of Asparagales (e.g. Fay *et al.*, 2000; Pires *et al.*, 2006; Seberg *et al.*, 2012) and include representatives of Doryanthaceae, Iridaceae and Ixioliriaceae (see Appendix 1).

DNA sequencing and alignment

Total genomic DNA was extracted from 0·03–0·3 g of silica gel-dried plant material or 0·25–1·25 g of fresh material using a modified version of the 2× cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). The total DNA was further purified for long-term storage in the DNA Bank at RBG Kew using a caesium chloride/ethidium bromide gradient (1·55 g mL^{-1}) followed by a dialysis procedure.

To infer phylogenetic relationships in Tecophilaeaceae, six plastid regions were sequenced: two coding regions (*matK*; *rbcL*), two introns (*rpl16*; *trnL*) and two intergenic spacers (*atpB–rbcL*; *trnL–trnF*). The *trnL* intron and *trnL–F* spacer were generally amplified in one reaction using primers c and f, but in some cases the intron (primers c and d) and the intergenic spacer (primers e and f) were amplified separately (all primers are from Taberlet *et al.*, 1991). Amplification of the *rbcL* coding region was performed using a combination of primers, usually in one PCR using primers 1F and 1360R, but also using internal primers in some cases (Fay *et al.*, 1998; Reeves *et al.*, 2001). A portion of the *matK* coding region (about 850 bp in length) was amplified using primers XF and 5R (see www.kew.org/barcoding). Primers atpB-2R and rbcL-1R (reverse complement of primers from Savolainen *et al.*, 2000) were used to amplify the *atpB–rbcL* intergenic spacer. Amplification of the *rpl16* intron was performed using primers rpl16F71 and rpl16R1516 from Shaw *et al.* (2005).

For the *trnL* intron, *trnL–F* intergenic spacer, and *rbcL* and *matK* coding regions, 50 μL PCRs were prepared using the ReddyMix PCR Master Mix (2·5 mM $MgCl_2$; ABgene, Epsom, Surrey, UK) with the addition of 1 μL of bovine serum albumin (BSA; 0·4 %) and 50 ng of each primer. For the *atpB–rbcL* spacer, a 50 μL PCR was prepared using 2 U of *Taq* polymerase (Bioline, London, UK), NH_4 reaction buffer (supplied by the manufacturer), 1·5 mM $MgCl_2$, 50 ng of each primer, 10 nM of dNTPs and 1 μL of 0·4 % BSA. The 20 μL PCR mix used to amplify *rpl16* contained 2 U of *Taq* polymerase (Bioline), NH_4 reaction buffer (supplied by the manufacturer), 2·5 mM $MgCl_2$, 50 ng of each primer, 10 nM of dNTPs and 4 μL of 0·4 % BSA. PCR for the *trnL* intron, *trnL–F* and *atpB–rbcL* intergenic spacers and *rbcL* was performed using the following program: 2 min at 94 °C, 32 cycles of 1 min at 94 °C, annealing at 50 °C for 1 min, 1·5 min at 72 °C, and a final extension of 3 min at 72 °C. PCR conditions for *matK* were as follows: initial denaturation at 94 °C for 2 min, followed by 32 cycles of 1 min at 94 °C, 1 min at 53 °C, 1·5 min at 72 °C, and a final elongation of 4 min at 72 °C. Amplification of the *rpl16* intron was performed as follows: 3 min at 94 °C, 32 cycles of 1 min at 94 °C, annealing at 52 °C for 1 min, 3 min at 72 °C, and a final extension of 7 min at 72 °C. All PCR amplifications were performed on a 9700 GeneAmp thermocycler (ABI; Warrington, Cheshire, UK).

All PCR products were purified with either the QIAquick PCR kit (Qiagen) or the Nucleospin Extract II kit (Machery-Nagel, Germany) and following the manufacturers' protocols. Cycle sequencing reactions were performed in 10 μL reactions using 1 μL of BigDye® Terminator cycle sequencing chemistry (v3·1; ABI; Warrington, Cheshire, UK) and the same primers as for PCR. Complementary strands were sequenced on an ABI 377, ABI 3100 or ABI 3730 automated sequencer.

Phylogenetic and divergence time estimation

A temporal framework for the evolution of Tecophilaeaceae was provided using the Bayesian inference approach implemented in the package BEAST v.1·5·4 (Drummond and Rambaut, 2007). Because the six DNA regions are all part of the plastid genome, we considered them as one partition in the BEAST analysis. The best-fit evolutionary model for the partition was determined using the Akaike information criterion (AIC) as implemented in MrModeltest (Nylander, 2004). The best-fit evolutionary model was the GTR model + gamma + invariable sites. An uncorrelated relaxed molecular clock assuming a log-normal distribution of rates and a Yule speciation model were applied. Four runs of 20×10^6 generations were performed, sampling one tree every 1000th generation. Parameter convergence was confirmed by examining their posterior distributions in TRACER 1·4 (Drummond and Rambaut, 2007). In addition, we have considered the Monte Carlo Markov Chain (MCMC) sampling sufficient when the effective sampling size (ESS) was >200, as assessed using TRACER v1·4 (Rambaut and

Drummond, 2007). A maximum clade credibility tree with median branch lengths and a 95 % highest posterior density (HPD) interval on nodes was built using TreeAnnotator 1·5·4 (Drummond and Rambaut, 2007) based on the set of trees after burn-in (for each run, a burn-in period of 2 million generations was applied).

A second phylogenetic algorithm was applied to confirm the results obtained by the Bayesian MCMC inference. Phylogenetic relationships were inferred using maximum likelihood (ML) as implemented in GARLI (version 1·0; Zwickl, 2006). The analysis was conducted on the CIPRES portal (http://www.phylo.org/) using the default options.

Calibration

Calibration of molecular phylogenetic trees is generally better performed using the fossil record (Forest, 2009), but monocots rarely fossilize well and there are currently no unequivocal fossils assignable to Tecophilaeaceae. Thus, two secondary calibration points were used following the same approach as in a recent study conducted by the authors on Asparagaceae subfamily Scilloideae (or Hyacinthaceae; see Buerki *et al.*, 2012; Fig. 1). In the first, the most recent common ancestor of Tecophilaeaceae and the outgroup taxa was constrained using a normal distribution with a mean of 110 Ma and a standard deviation of 5. For the second, the crown node of Tecophilaeaceae was constrained with a normal distribution, a mean of 80 Ma and a standard deviation of 5 (Fig. 1). These values were obtained from a previous molecular estimate of a monocot-wide analysis (Anderson and Janssen, 2009) and were used to calibrate the BEAST analysis.

Biogeographical inferences

Four geographical areas were defined based on palaeogeographical evidence and the current distribution of taxa (Fig. 2): (A) Chile (South America); (B) California (North America); (C) tropical and sub-tropical Africa; and (D) the Greater Cape region (winter-rainfall parts of South Africa and southern Namibia; Born *et al.*, 2007). Because this study included approx. 80 % of the species richness of the family, the distribution of terminals was scored at the species level

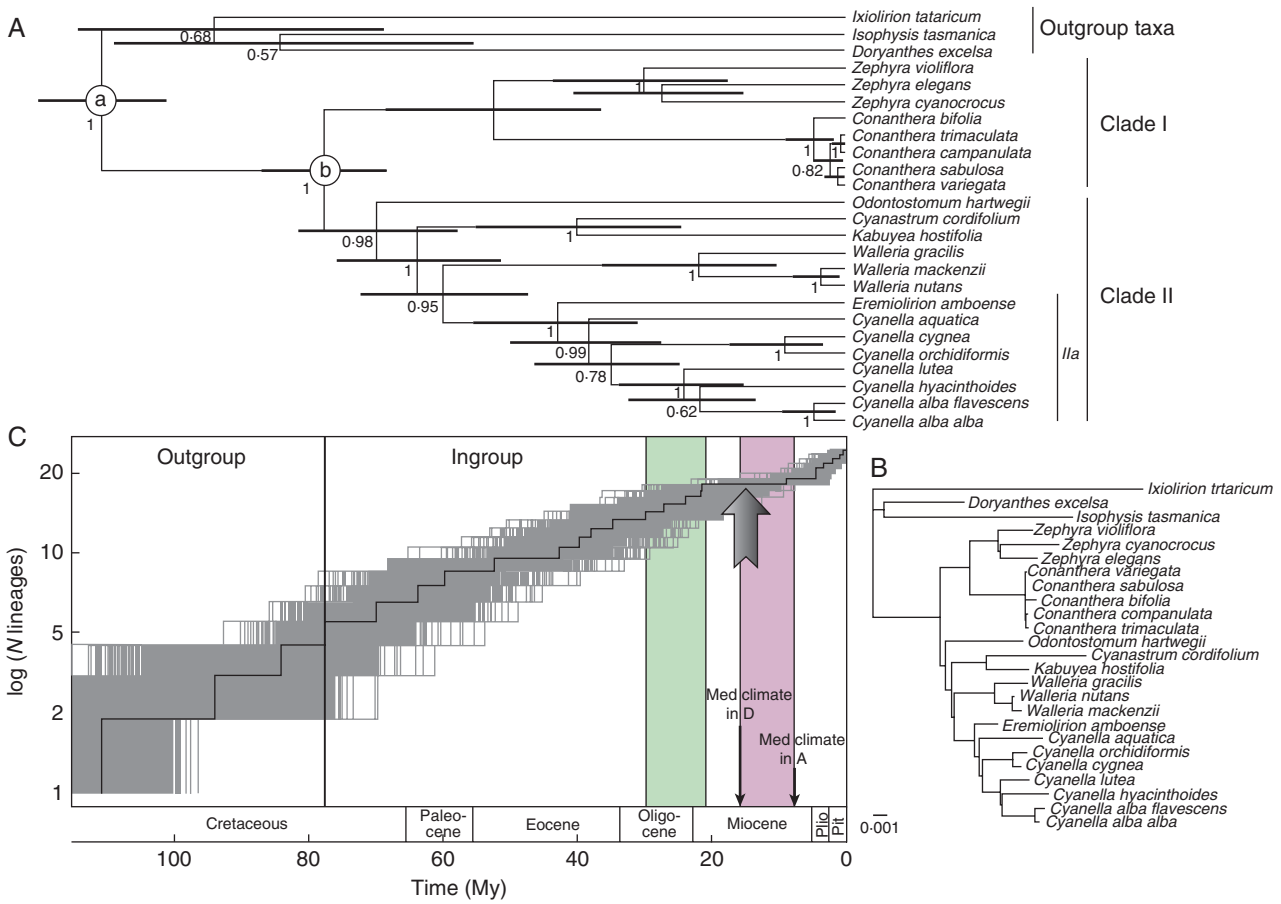

FIG. 1. (A) BEAST maximum clade credibility tree for Tecophilaeaceae; 95 % highest posterior density intervals on time divergence estimates and posterior probabilities assigned to each node are indicated. The position of the two calibration points used for the BEAST inference are indicated on the nodes (a and b; see text for more details). (B) Maximum likelihood phylogenetic tree inferred using GARLI. (C) Multiple lineage-through-time plot of the 1000 randomly selected trees from the BEAST analysis (after burn-in) used for the LAGRANGE analyses (in grey) and the maximum clade credibility tree (in black). The green interval represents the timing of the first Andean uplift, and the pink interval corresponds to the timing of the second Andean uplift and the establishment of the Mediterranean climates and Atacama desert. Abbreviations: Med, Mediterranean; My, million years; A, Chile, D, Greater Cape region (see Fig. 2).

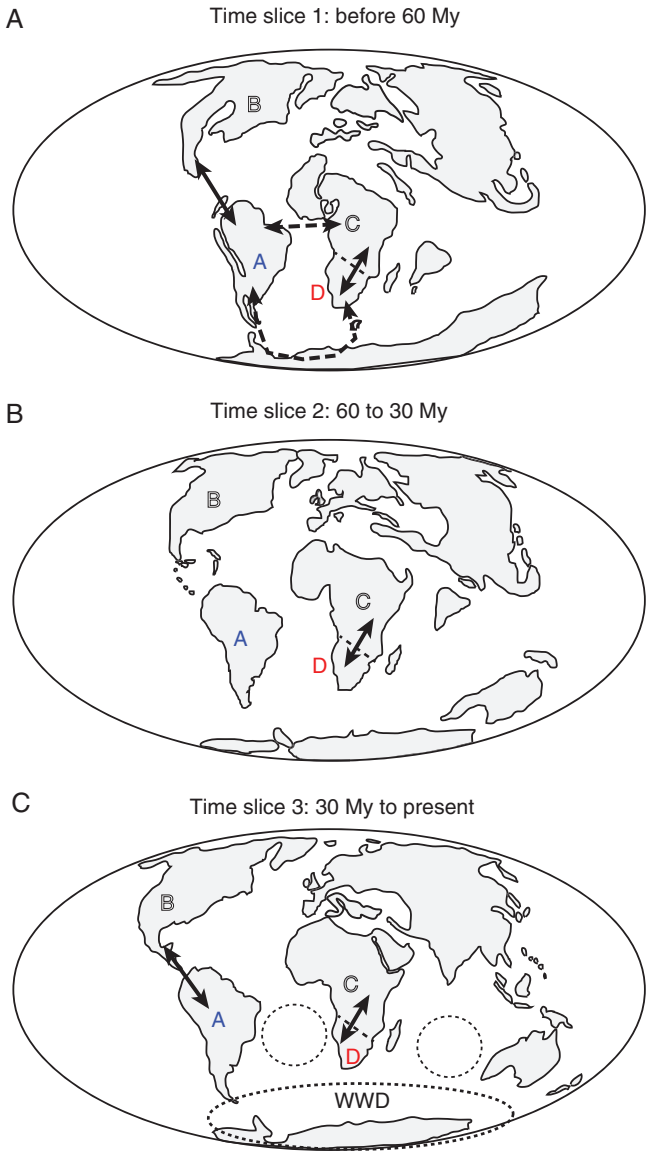

FIG. 2. Stratified palaeogeographical model used to constrain the LAGRANGE biogeographical analyses of Tecophilaeaceae (adapted from Buerki *et al.*, 2011). A dispersal probability of 1·0 was applied for solid lines, whereas a probability of 0·5 was set for dashed lines. For all the other dispersal routes, a probability of 0·01 was applied (see text for more details). Biogeographical areas: (A) Chile (South America); (B) California (North America); (C) tropical and sub-tropical Africa; (D) the Greater Cape region (winter-rainfall parts of South Africa and southern Namibia). Abbreviation: My, million years.

(see Appendix 2 for more details on the distribution of the missing taxa).

The dispersal–extinction–cladogenesis (DEC) likelihood model implemented in LAGRANGE v.2·0·1 (Ree *et al.*, 2005; Ree and Smith, 2008) was used to investigate the biogeographical history of Tecophilaeaceae (outgroup taxa were not included in the biogeographical analyses). Because most taxa are confined to a single area, the LAGRANGE analyses were run with a maximum area set to two. However, we had to include additional widespread areas to be able to calculate the likelihood of the tree and estimate extinction (see Ree *et al.*, 2005). One of the advantages of the DEC model is its ability to adapt a transition matrix (i.e. Q-matrix) to reflect the changing palaeogeography, connections between areas (e.g. land bridges) through time or dispersal capabilities of the group of interest (Buerki *et al.*, 2011). To reflect this ability, the stratified palaeogeographical model of Buerki *et al.* (2011) was adapted to reflect geological connections between areas through time (see Fig. 2). The palaeogeographical model spanned the last 80 million years (My) and was divided into three time slices: (1) before 60 My; (2) 60 My to 30 My; and (3) 30 My to the present (see Fig. 2 and Buerki *et al.*, 2011). In this model, direct connections between areas were assigned a probability of 1; connections mediated by abiotic factors (e.g. sub-equatorial currents) had a probability of 0·5 and all the other dispersal probabilities were assigned a probability of 0·01 (Fig. 2 and see Buerki *et al.*, 2011, for more details). Finally, to take phylogenetic and dating uncertainty into account while inferring the biogeographical scenario, we applied the same approach as used by Espindola *et al.* (2012) and ran the constrained LAGRANGE analyses over a set of 1000 randomly selected BEAST trees, after burn-in. The biogeographical scenario, which encompasses the output of the 1000 LAGRANGE analyses, was subsequently summarized on the maximum credibility clade tree of BEAST using pie charts as implemented in Bayes-DIVA (Nylander *et al.*, 2008) and subsequently accommodated for LAGRANGE outputs in Espindola *et al.* (2012) using R scripts (R Development Core Team, 2010). All the ancestral areas with probabilities $<0{\cdot}1$ were pulled together and depicted in black in the pie charts (referred to as 'Trash'). Finally, the ancestral area with the highest probability per node was extracted using an R script (R Development Core Team, 2010) and a contingency table depicting dispersals and extinctions per time slice constructed based on the specificities of the DEC model (see Buerki *et al.*, 2011, for more details). This table was used to represent the biogeographical scenario on palaeogeographical maps.

Diversification analyses

To support the biogeographical inference and investigate further the evolution of Tecophilaeaceae, we performed two sets of diversification analyses. First, the temporal accumulation of lineages in the family was assessed based on LTT curves as implemented in the R package *ape* (Paradis *et al.*, 2004). LTT curves were inferred for the 1000 randomly selected BEAST trees and for the maximum clade credibility tree. To investigate further temporal accumulation of lineages through time in light of taxa divergence, the maximum clade credibility tree and LTT curves were plotted at the same scale using an adapted version of the ltt.coplot function of the R package *ape* (Paradis *et al.*, 2004).

The effect of ecology on the diversification of the family was investigated by applying the GeoSSE model (Goldberg *et al.*, 2011) to our data set following Buerki *et al.* (2012). The occurrence of each taxon in the following ecosystems has been recorded based on taxonomic revisions (Carter, 1962; Brummitt *et al.*, 1998; Simpson and Rudall, 1998; Manning and Goldblatt, 2012): (1) Mediterranean; (2) arid (non-Mediterranean); and (3) sub-tropical to tropical. These

analyses are crucial to unravel diversification patterns in the family and are complementary to the biogeographical inferences because most of the ecosystems are shared between areas. For instance, Mediterranean ecosystems are found in Chile (area A), California (B) and the Greater Cape (in the Cape region in D). The GeoSSE method simultaneously features the characteristics of the constant-rates birth–death model with a three-state Markov model similar to the DEC model (Goldberg *et al.*, 2011). This likelihood-based approach estimates ecosystem-dependent rates of speciation, extinction and range evolution based on a fully resolved maximum clade credibility tree of BEAST. Seven parameters can be estimated by the model [speciation within regions A (sA) and B (sB), between-region speciation (sAB), extinction from regions A (xA) and B (xB), dispersal from A to B (dA) and dispersal from B to A (dB)] (see fig. 1 in Goldberg *et al.*, 2011). For each ecosystem, an ML parameter estimation and model comparison was conducted followed by Bayesian parameter estimation through MCMC as implemented in the R package *diversitree* (FitzJohn, 2010). To reduce the complexity of the analysis, two GeoSSE models, the full model and the model without between-region speciation (sAB) as suggested by Goldberg *et al.* (2011), were estimated under an ML framework and compared using a likelihood ratio test as implemented in *diversitree* (FitzJohn, 2010). Subsequently an MCMC approach was used to perform a Bayesian analysis based on the best GeoSSE model (incomplete taxon sampling was also considered, but had limited effect since only five species were not sampled; see Appendix 2). Maximum likelihood rate estimates were used as priors to seed the MCMC analysis. The MCMC was run for 5000 generations with a burn-in period of 1000 generations. Finally, posterior probability distributions for the GeoSSE parameters were summarized using functions implemented in *diversitree* (Fitzjohn, 2010). We could not perform the GeoSSE analyses based on the biogeographical areas because all taxa (with the exception of *Walleria nutans*) are restricted to a single area (see Goldberg *et al.*, 2011 for more details).

RESULTS

Divergence time estimates

The various statistics related to the six plastid DNA regions are summarized in Table 1. With the exception of *rbcL* (7·6 % variable characters), all the DNA regions yielded a similar percentage of variable characters (12 % for *rpl16* to 17·4 % for *matK*) and the combined matrix had 12·1 % variable characters (Table 1).

The ML and BEAST phylogenetic trees had identical topologies and are displayed in Fig. 1. Despite the relatively low level of variability observed in the plastid DNA regions used here (Table 1), phylogenetic relationships in Tecophilaeaceae are well resolved, with only one node with a Bayesian posterior probability (BPP) below 0·5 (i.e. the split between *Zephyra elegans* and *Z. cyanocrocus*; Fig. 1). The monophyly of the family is strongly supported, with a BPP of 1·0, and two highly supported clades are retrieved: clade I (BPP: 1·0) which comprises the genera *Conanthera* and *Zephyra*; and clade II (BPP: 0·98) containing genera *Odontostomum*, *Cyanastrum*, *Kabuyea*, *Walleria*, *Eremiolirion* and *Cyanella* (Fig. 1). In clade II, sub-clade IIa corresponds to the southern African *Eremiolirion* and *Cyanella* (Fig. 1). All genera are confirmed as monophyletic in all analyses (Fig. 1).

Although the stem age leading to the two genera in clade I is estimated to be Palaeocene and Eocene with a large 95 % HPD interval, their crown ages are strikingly different; *Zephyra* originated sometime during the Oligocene, whereas the diversification of *Conanthera* was delayed until the Pliocene (Fig. 1). In clade II, the split of the earliest lineage (*Ondontostomum hartwegii*) from the rest of the clade is estimated to be in the Late Cretaceous. Most of the species in clade II seemed to have diversified gradually through time (see LTT curves, Fig. 1), with the origin of the genera taking place between the Eocene and Oligocene (Fig. 1).

Biogeographical inference

The biogeographical scenario inferred from the 1000 randomly selected BEAST trees and constrained according to palaeogeographical evidence (Fig. 2) is displayed on the maximum credibility clade tree using pie charts (Fig. 3). In addition, dispersal and extinction events per time slice were plotted on palaeogeographical maps (Fig. 4). The biogeographical scenario reported here is based on the highest ancestral area probability per node (as done in Buerki *et al.*, 2011). The most recent common ancestor (MRCA) of Tecophilaeaceae is assessed as widespread between South America and tropical Africa, although this result has to be considered with caution because analyses were performed without outgroup taxa for technical reasons (Figs 3 and 4A). The DEC model estimates that a peripheral-isolate speciation took place from the root (AC) of the family to the nodes, leading to clades I and II (Fig. 3). In this context, the MRCA of clade II inherited the whole ancestral area (AC), whereas the MRCA of clade I became extinct in area C and persisted only in A (Figs 3 and 4). All taxa belonging to clade I are currently restricted to area A (Chile and South

TABLE 1. *Statistics summarizing the plastid DNA regions used to infer phylogenetic relationships in Tecophilaeaceae*

	atpB–rbcL	*matK*	*rbcL*	*rpl16*	*trnL*	*trnL–trnF*	Supermatrix
No. of sequences (ingroup/outgroup)	22/0	20/3	22/3	16/0	22/3	22/3	22/3
Alignment length (bp)	736	882	1298	1081	473	406	4876
No. of variable characters: ingroup/all (%)	90/NA (12·2/NA)	153/213 (17·4/24·1)	98/146 (7·6/11·2)	130/NA (12·0/NA)	63/96 (13·3/20·3)	57/91 (14·0/22·4)	591/766 (12·1/15·7)
No. of potentially pasimony-informative characters: ingroup/all (%)	37/NA (5·0/NA)	85/104 (9·6/11·8)	43/66 (3·3/5·0)	52/NA (4·8/NA)	26/38 (5·5/8·0)	24/32 (5·9/7·9)	267/329 (5·5/6·7)

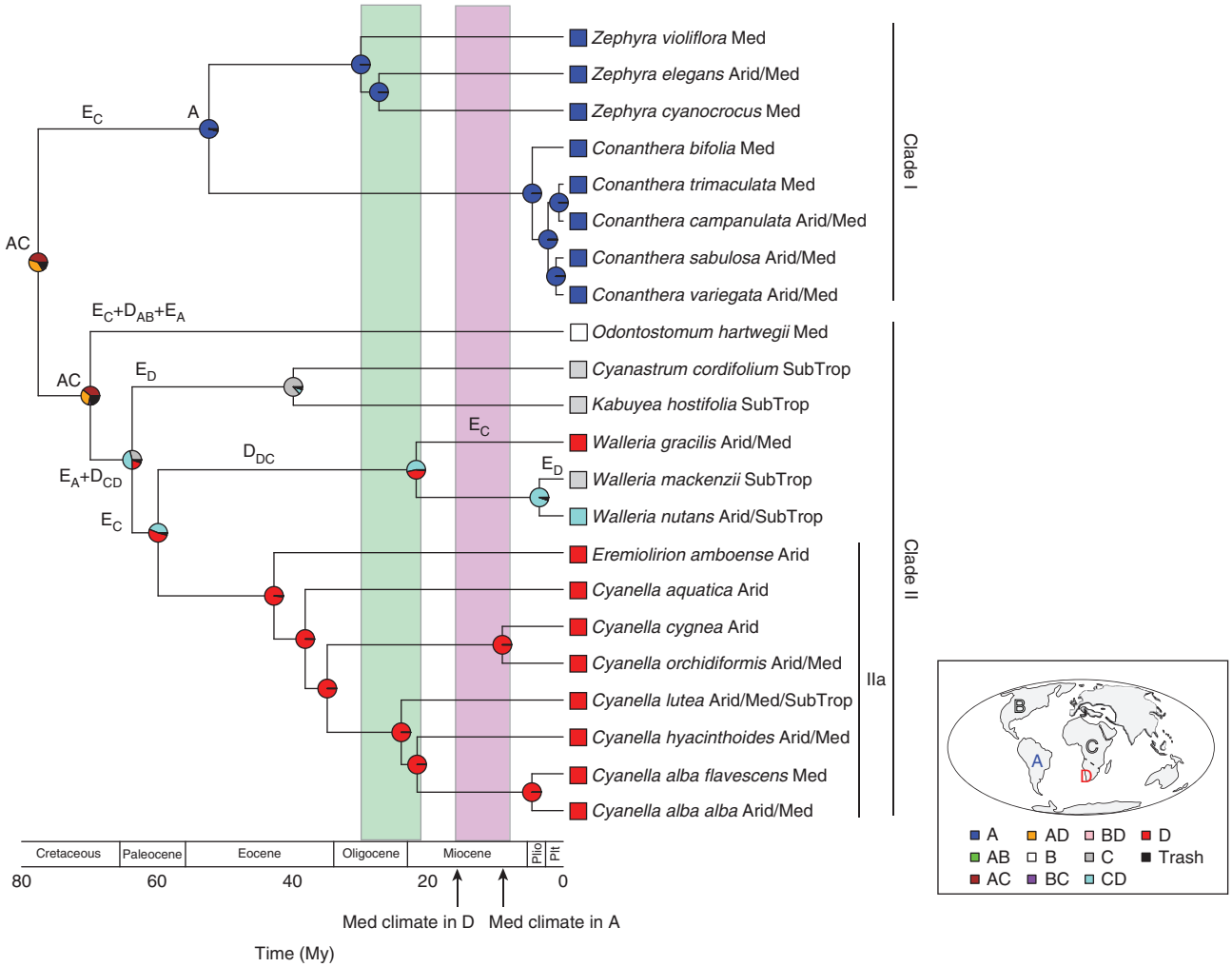

FIG. 3. Biogeographical scenario for Tecophilaeaceae inferred from the 1000 randomly selected trees from the BEAST analysis and constrained using the palaeogeographical model (Fig. 2). The biogeographical ancestral reconstructions are displayed on the maximum clade credibility tree using pie charts. The abbreviations of biogeographical areas are provided in Fig. 2. The green interval represents the timing of the first Andean uplift, whereas the pink interval corresponds to the timing of the second Andean uplift and the establishment of the Mediterranean climates and Atacama desert. Abbreviations: for the ecology, Med = Mediterranean, Arid = deserts and arid climates, SubTrop = sub-tropical; extinction (E) dispersal (D) events; My, million years.

America) and the biogeographical inferences supported the sympatric speciation of this clade in this area (Figs 3 and 4). Speciation in the two genera of clade I proceeded at very different tempos, with *Zephyra* spp. originating during time slice 2 (at the Eocene/Oligocene boundary) and *Conanthera* spp. originating later during time slice 3 (i.e. Pliocene; Figs 3 and 4). All taxa in clade II, with the exception of the monotypic genus *Odontostomum* endemic to California (B), are distributed between tropical Africa (C) and the Greater Cape region (D). To explain the current distribution of *Odontostomum* in B, the model invoked three biogeographical events (starting from AC): (1) an extinction in C followed by (2) dispersal from A to B and (3) a final extinction in A (Figs 2 and 3A). The first event corresponds to a vicariance speciation event between *Odontostomum* and the rest of clade II (Fig. 3). In the remainder of clade II, this vicariance event was followed by a dispersal event from C to D (during time slice 1; Figs 3 and 4A). A final vicariance event was inferred by the DEC model leading to the extinction of the MRCA of the pair *Cyanastrum–Kabuyea* in D and the extinction of the MRCA of the *Walleria/Eremiolirion/Cyanella* clade in C (Figs 3 and 4). A final dispersal event was inferred from D to C (during time slice 2) for the MRCA of *Walleria* (Figs 3 and 4B). Clade IIa is restricted to D and underwent sympatric speciation within this area mainly during the second time slice, with additional taxa emerging during the last time slice (Fig. 3).

Diversification analyses

The LTT curves show a relatively constant speciation rate from the origin of the group until the Miocene, followed by a plateau persisting until the Pliocene (Fig. 1B). Finally, LTT curves indicate an acceleration of speciation from the Pliocene onwards, mostly due to the diversification in *Conanthera* (clade I) and, to a lesser extent, to the appearance of new species in *Cyanella* (clade IIa; Fig. 1).

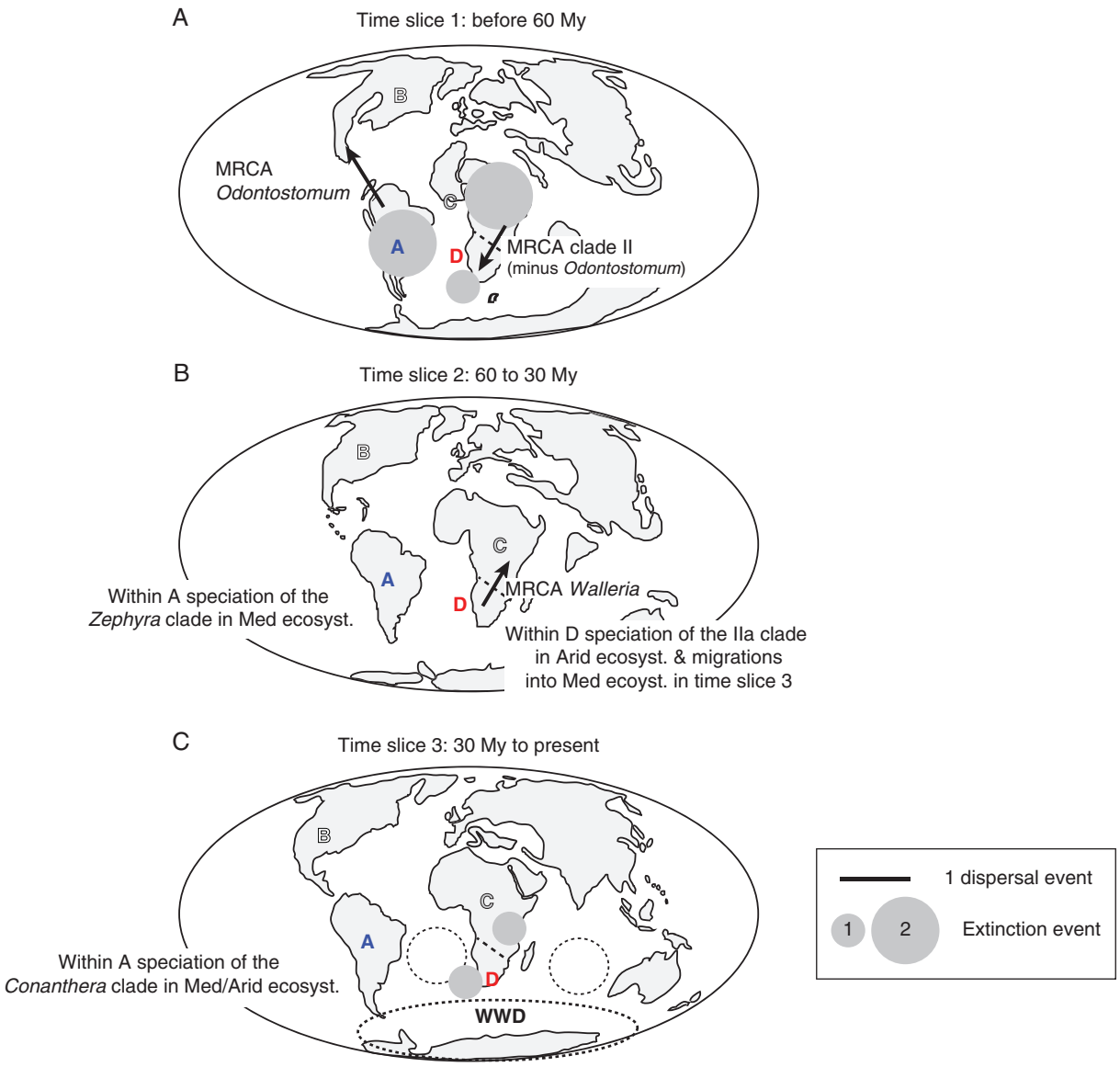

FIG. 4. Dispersal and extinction events occurring during the evolution of Tecophilaeaceae represented on palaeogeographical maps (adapted from Buerki *et al.*, 2011). See Figs 1 and 2 for abbreviations.

The ecology of each taxon is provided in Fig. 3 (and Appendix 2) and categorized as arid, Mediterranean and/or sub-tropical. Several taxa were restricted to the Mediterranean ecosystem (e.g. *Cyanella alba* subsp. *flavescens*), but usually they were shared between the former and arid ecosystems. The colonization of sub-tropical to tropical ecosystems is confined to clade II in two lineages (forming a grade at the base of the clade): the *Cyanastrum* and *Kabuyea* clade and the *Walleria* clade (Fig. 3). The occurrence or migrations into the Mediterranean ecosystem followed two contrasting patterns between clades A and B (Fig. 3). In clade I (especially in *Conanthera*), taxa seemed to have originated in the Mediterranean ecosystem and subsequently migrated into arid regions, whereas the opposite pattern is found in clade IIa (Fig. 3).

With the exception of the Mediterranean ecosystem (see below), the model without sAB in GeoSSE always fit the data better, suggesting that there are ecosystem differences in diversification. Results of the GeoSSE analyses are shown in Fig. 5. In all analyses, it appeared that the speciation and extinction rates are always higher outside the investigated ecosystem, although less noticeable for speciation rates in the sub-tropical and Mediterranean ecosystems. The most interesting pattern was found for the Mediterranean ecosystem, where sAB had to be invoked, implying co-diversification between ecosystems (Fig. 5C). The posterior distribution of sAB covered the whole range of distribution of sA and sB, indicating that between-region speciation is important in the diversification of species in this ecosystem.

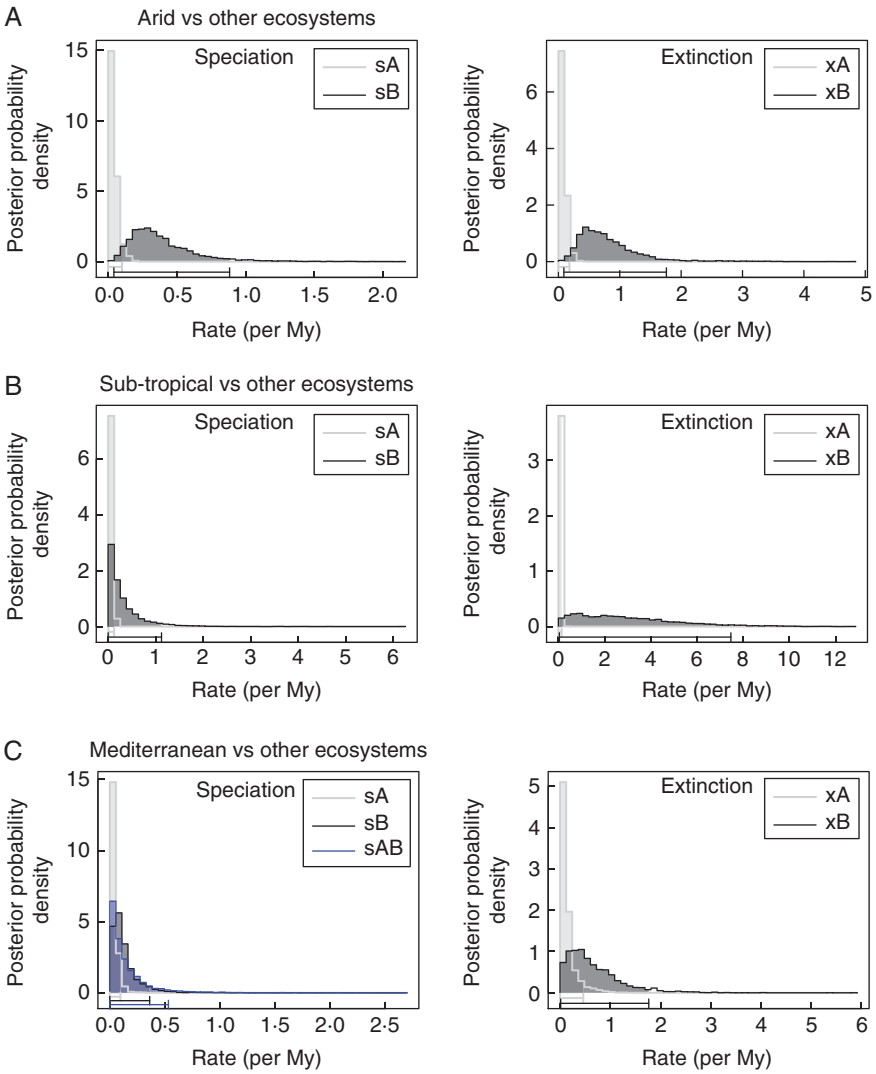

FIG. 5. Posterior probability distributions for the speciation and extinction parameters obtained with GeoSSE. Speciation and extinction shown in a given ecology/ecosystem (sA and xA, respectively) and the remainder of the distributional range of Tecophilaeaceae (sB and xB, respectively) for each area estimated by the GeoSSE analyses for the Tecophilaeaceae data set (see text for more details; speciation in the joint areas AB is identified in blue). My, million years.

DISCUSSION

Taxonomic implications and character evolution

The phylogenetic relationships uncovered here provide support for the monophyly of all genera of Tecophilaeaceae as recognized by Ravenna (1988) (i.e. the inclusion of *Tecophilaea* in *Zephyra*), Brummitt *et al.* (1998) and Manning and Goldblatt (2012). We confirm the sister relationship between the tropical African *Cyanastrum* and *Kabuyea* (Fig. 1) inferred from numerous morphological and anatomical synapomorphies identified by Brummitt *et al.* (1998). The evidently close relationship between these two genera led Brummitt *et al.* (1998) to unite them as sole members of subfamily Cyanastroideae Engl., although preliminary evidence from their phylogenetic trees based on *rbcL* of a small sample of taxa located the two genera among others in the family, suggesting that this classification was not phylogenetically sound. The cladistic topology of Brummitt *et al.* (1998) is strongly supported by our analysis, which nests Cyanastroideae deeply within Tecophilaeoideae. To retain Cyanastroideae would require the recognition of at least two additional subfamilies to render Tecophilaeoideae monophyletic. This is unwarranted due to the modest size of the family. Even the recognition of tribes is excessive, and we conclude that subfamily Cynastroideae should no longer be recognized.

Simpson and Rudall (1998) interpreted the presence of staminodes as a probable synapomorphy uniting the Chilean genera *Tecophilaea* and *Zephyra*, and retained the two genera as separate despite an earlier decision by Ravenna (1988) to treat them as congeneric. Our analysis nests *Zephyra* within *Tecophilaea*, providing no support for treating them as distinct genera. The two genera were distinguished on the degree of development of the inflorescence and by the number and position of the staminodes: *Tecophilaea* with one or two flowers per inflorescence and the posterior three stamens sterile; and *Zephyra* with a racemose or paniculate inflorescence and the postero-lateral two stamens

sterile. Modifications in androecial symmetry and form are a recurrent theme in the family. In *Cyanella*, species have either a 3 + 3 or a 5 + 1 arrangement of stamens and, although most species have racemose or paniculate inflorescences, *C. alba* produces just 1–9 flowers per stem. The variation in the inflorescence and androecium in *Tecophilaea* + *Zephyra* is thus no more extreme than in *Cyanella*. Molecular and morphological evidence is thus consistent with Ravenna's (1988) decision to include *Tecophilaea* in *Zephyra*.

Most genera of Tecophilaeaceae develop tunicated corms, but those of *Walleria* and *Kabuyea* + *Cyanastrum* are non-tunicated. The membranous tunics characteristic of the New World genera are evidently plesiomorphic, and the evolution of fibrous tunics in *Eremiolirion* and *Cyanella* represents a synapomorphy for these two genera, evidently a reversal from the non-tunicated condition in other African genera. The cauline foliage and solitary, axillary flowers of *Walleria* are both autapomorphies for the genus. The basic chromosome number in the family is probably $x = 12$, but *Odontostomum* has $x = 10$ (see Simpson and Rudall, 1998), thus an autapomorphy for the genus. The evolution of an asymmetric or zygomorphic androecium in *Odontostomum* (5 posterior + 1 anterior) and in *Cyanella* (5 + 1 and 3 +3) is most parsimoniously interpreted as homoplasious, and the evolution of a yellow or orange perianth in some *Cyanella* spp. is similarly interpreted as derived from the ancestral blue or white state characteristic of the other genera.

The spatio-temporal evolution of Tecophilaeaceae

This small family poses a challenge for biogeographical analyses. Tecophilaeaceae originated sometime during the Late Cretaceous and now present a disjunct distribution between Chile (A), California (B) and mainland Africa (areas C and D), having colonized three of the five regions of the world with a Mediterranean-type climate (the family is not found in the Mediterranean Basin or south-west Australia). Disjunct continental distributions are well known among angiosperm families [see, for example, Milne (2006) for a review] but the distribution of Tecophilaeaceae is peculiar and unmatched in other flowering plants. Three other characteristics hinder the inference of its biogeographical history, especially the cradle of the family: (1) low species richness (only 27 species); (2) many species with a narrow distribution; and (3) a relatively long evolutionary history and many old taxa. Most taxa in Tecophilaeaceae originated sometime during the Eocene/Oligocene, with the notable exception of *Conanthera*, which originated in the Pliocene. Most species are also restricted to a single ecogeographic region (Fig. 3). Species richness in the family is unevenly distributed, with the highest diversity in Chile (eight species) and the Greater Cape region (ten species), but only three species in tropical Africa and one in California (Fig. 3).

We infer the cradle of the family to be in tropical Africa and South America sometime during the Late Cretaceous before the break up of Gondwana (Figs 1–3; see the Results section for more details). Further analyses with an expanded outgroup selection is required to clarify the area optimization on this node. The LAGRANGE analyses strongly suggested vicariance resulting from the break up of Gondwana as an important factor in shaping the current distribution of taxa at deeper nodes, resulting in the establishment of the two main clades in Tecophilaeaceae (Figs 3 and 4). In clade II, a second Late Cretaceous vicariance event split the Californian *Odontostomum hartwegii* from the rest of the clade, currently restricted to mainland Africa (Figs 3 and 4). This biogeographical event was followed by the dispersal of the MRCA of *Odontostomum* from South America to North America (Fig. 3). North and South America were connected during the Cretaceous by the proto-Greater Antilles land bridge (Briggs, 1994), but this connection was disrupted in the Palaeocene/Eocene with the emergence of the Caribbean Plate, to reappear later during the Neogene, first through the Greater Antilles and the Aves Ridge and later through the uplift of the Panama Isthmus (3·5 Ma; Iturralde-Vinent, 2006). Although the MRCA of *Odontostomum* most probably utilized this dispersal route from South to North America, it is impossible to date the event more precisely at present because of the long branch leading to this taxon (Figs 1 and 3).

Species diversity in tropical Africa is markedly lower that of the Greater Cape region and South America (Fig. 3). *Cyanastrum* and *Kabuyea* appear to represent relictual lineages restricted to sub-tropical ecosystems in tropical Africa. The most likely biogeographical scenario infers a dispersal of the MRCA of *Walleria* and of *Eremiliorion*–*Cyanella* into the Greater Cape region from tropical Africa, followed by the diversification of *Cyanella* in southern Africa since the mid Eocene (Figs 3 and 4).

Our analyses highlight the importance of sympatric speciation in Chile (clade I) and the Greater Cape region (clade IIa), both characterized by Mediterranean-type climates (Fig. 3). The onset or intensification of a Mediterranean climate in southern Africa has been identified as a driver of diversification in several groups, including Aizoaceae (Klak *et al.*, 2004), Asparagaceae subfamily Scilloideae (Buerki *et al.*, 2012) and *Gladiolus* (Iridaceae; Valente *et al.*, 2012). Initial assumptions that the radiations in the Greater Cape flora were primarily initiated by climatic changes in the Late Miocene or Pliocene have been confounded by increasing evidence from phylogenetic dating that the radiation of typical Cape clades was spread over much of the Neogene, with an acceleration from the Late Miocene (Linder, 2008). Our diversification analyses do not support any shift in diversification associated with the Mediterranean ecosystem and suggest that co-speciation (sAB) processes between arid and Mediterranean ecosystems have been important in shaping the current species diversity in this group (Fig. 5).

Andean uplift and past climate change in South America

The uplift of the Andes is known to have greatly influenced the Amazonian biota (see Antonelli *et al.*, 2009; Hoorn *et al.*, 2010), but few studies (e.g. Heibl and Renner, 2012) have focused on the effects of this major geological event and associated collateral events, including the establishment of the Mediterranean climate in Chile and the Atacama desert, on the biogeography and diversification of western South American plant lineages. Our analyses show that the South American species of Tecophilaeaceae form a monophyletic group (clade I) that originated during the Eocene before the

first Andean uplift, as a consequence of the break up of the Gondwana (see above; Figs 1 and 3). The two genera recognized in clade I express strikingly different patterns of diversification. The origin of *Zephyra* coincides with the first Andean uplift (Oligocene), whereas *Conanthera* originated at the Miocene/Pliocene boundary after the second Andean uplift, which resulted in the establishment of the Mediterranean climate in this area (Figs 1 and 3). Although these two sister genera have different tempos of evolution, both are currently restricted to Mediterranean and arid ecosystems in Chile (Simpson and Rudall, 1998).

The Chilean Mediterranean-type ecosystem, established between 15 and 8 Ma (Armesto *et al.*, 2007), is highly heterogeneous, ranging from the Atacama desert, the driest desert in the world, in the north to mixed deciduous–evergreen temperate forests in the south. Although the first Andean uplift was not of the same magnitude as the second (during which the Andes rose to elevations ranging from 3000 to >6 000 m; Armesto *et al.*, 2007; Hoorn *et al.*, 2010; see Hughes *et al.*, 2013, and references therein), this geological event initiated the progressive aridity along the western margin of South America, also triggered by equatorial currents from the cold Antarctic waters in the South Pacific (Armesto *et al.*, 2007). We hypothesize that the MRCA of *Zephyra* was adapted to arid regions and subsequently dispersed to the Mediterranean-type ecosystem. In contrast, the origin of *Conanthera* coincides with the establishment of a Mediterranean climate in Chile and suggests subsequent dispersals into the arid parts of Chile during the Pliocene onwards. The long branch leading to the MRCA of *Conanthera* might be the signature of either a significant extinction event that occurred sometime between the Eocene and the end of the Miocene or a very low speciation rate in this lineage until only recently (see Crisp and Cook, 2009). A conceivable cause of the extinction event is the drastic ecological changes resulting from the first Andean uplift (see above). In any event, the *Conanthera* lineage diversified rapidly in the Pliocene during the establishment of a Mediterranean climate in the region.

Sympatric diversification in the Greater Cape region

The phylogenetic framework and ecological data support an origin of species in clade IIa in arid ecosystems during the Eocene, with subsequent diversification in south-western Africa well before the onset of aridification in the Mid Miocene. Although most southern African *Cyanella* spp. are endemic to the Mediterranean climate of the Greater Cape region, radiation in the genus does not appear to be closely linked to the establishment of the Mediterranean climate in the Mid Miocene. More recent minor diversification events from the Late Miocene and Pliocene, e.g. the species pair *C. cygnea/C. orchidiformis* and the subspecies of *C. alba*, are coincident with the establishment of a Mediterranean climate, but Tecophilaeaceae clearly belong to the more arid element of the Greater Cape flora and have only marginally colonized the core Cape region.

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LITERATURE CITED

Anderson CL, Janssen T. 2009. Monocots. In: Hedges SB, Kumar S. eds. *The timetree of life*. Oxford: Oxford University Press, 203–212.

Antonelli A, Nylander JAA, Persson C, Sanmartín I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution: evidence from the coffee family. *Proceedings of the National Academy of Sciences, USA* **106**: 9749–9754.

Armesto JJ, Arroyo MTK, Hinojosa LF, Veblen TT, Young KR, Orme AR. 2007. The Mediterranean environment of central Chile. In: Veblen TT, Orme AR, Young KG. eds. *The physical geography of South America*. Oxford: Oxford University Press, 184–199.

Born J, Linder HP, Desmet P. 2007. The Greater Cape floristic region. *Journal of Biogeography* **34**: 147–162.

Briggs JC. 1994. The genesis of Central America: biology versus geophysics. *Global Ecology and Biogeography* **4**: 169–172.

Brummitt RK, Banks H, Johnson MAT, *et al*. 1998. Taxonomy of Cyanastroideae (Tecophilaeaceae): a multidisciplinary approach. *Kew Bulletin* **53**: 769–803.

Buerki S, Forest F, Alvarez N, *et al*. 2011. An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. *Journal of Biogeography* **38**: 531–550.

Buerki S, Jose S, Yadav SR, Goldblatt P, Manning JC, Forest F. 2012. Contrasting biogeographic and diversification patterns in two Mediterranean-type ecosystems. *PLoS One* **7**: e39377. http://dx.doi.org/10.1371/journal.pone.0039377.

Carter S. 1962. Revision of *Walleria* and *Cyanastrum* (Tecophilaeaceae). *Kew Bulletin* **16**: 185–195.

Chase MW, Reveal JL, Fay MF. 2009. A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society* **161**: 132–136.

Crisp MD, Cook LG. 2009. Explosive radiation of cryptic mass extinction? Interpreting signatures in molecular phylogenies. *Evolution* **63**: 2257–2265.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, Botanical Society of America* **19**: 11–15.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**: 214. http://dx.doi.org/10.1186/1471-2148-7-214.

Espindola A, Buerki S, Alvarez N. 2012. Ecological and historical drivers of diversification in the fly genus *Chiastocheta* Pokorny. *Molecular Phylogenetics and Evolution* **63**: 466–474.

Fay MF, Bayer C, Alverson WS, de Bruijn AY, Chase MW. 1998. Plastid *rbcL* sequence data indicate a close affinity between *Diegodendron* and *Bixa*. *Taxon* **47**: 43–50.

Fay MF, Rudall PJ, Sullivan S, *et al*. 2000. Phylogenetic studies of Asparagales based on four plastid DNA regions. In: Wilson KL, Morrison DA. eds., *Monocots: systematics and evolution*. International Conference on the Comparative Biology of the Monocotyledons. Collingwood, Australia: CSIRO, 360–371.

FitzJohn RG. 2010. Quantitative traits and diversification. *Systematic Biology* **59**: 619–633.

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

Goldberg EE, Lancaster LT, Ree RH. 2011. Phylogenetic inference of reciprocal effects between geographic range evolution and diversification. *Systematic Biology* **60**: 451–465.

Heibl C, Renner SS. 2012. Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. *Systematic Biology* **61**: 823–834.

Hoorn C, Wesselingh P, ter Steege H, *et al*. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330**: 927–931.

Hughes CE, Pennington RT, Antonelli A. 2013. Neotropical plant evolution: assembling the big picture. *Botanical Journal of the Linnean Society* **171**: 1–18.

Iturralde-Vinent MA. 2006. Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *International Geology Review* **48**: 791–827.

Klak C, Reeves G, Hedderson TA. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature* **427**: 63–65.

Linder HP. 2008. Plant species radiations: where, when, why? *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**: 3097–3105.

Manning JC, Goldblatt P. 2012. A revision of Tecophilaeaceae subfam. Tecophilaeoideae in Africa. *Bothalia* **42**: 21–41.

Milne RI. 2006. Northern Hemisphere plant disjunctions: a window on Tertiary land bridges and climate change? *Annals of Botany* **98**: 465–472.

Nylander JAA, Olsson U, Alström P, Sanmartín I. 2008. Accounting for phylogenetic uncertainty in biogeography: a Bayesian approach to dispersal–vicariance analysis of the thrushes (Aves: *Turdus*). *Systematic Biology* **57**: 257–268.

Nylander JAA. 2004. *MrModeltest*, version 2·2. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University. http://www.abc.se/~nylander/.

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

Pires JC, Maureira IJ, Givnish TJ, *et al*. 2006. Phylogeny, genome size, and chromosome evolution of the Asparagales. *Aliso* **22**: 287–304.

R Development Core Team. 2010. *R: a language and environment for statistical computing*. http://www.r-project.org.

Rambaut A, Drummond AJ. 2007. Tracer *version 1·5*. Available from: http://beast.bio.ed.ac.uk/Tracer.

Ravenna P. 1998. New or noteworthy Tecophilaeaeceae. *Phytologia* **64**: 288–289.

Ree RH, Smith SA. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57**: 4–14.

Ree RH, Moore BR, Webb CO, Donoghue MJ. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* **59**: 2299–2311.

Reeves G, Chase MW, Goldblatt P, Rudall P, Fay MF, Cox AV, Lejeune B, Souza-Chies T. 2001. Molecular systematics of Iridaceae: Evidence from four plastid DNA regions. *American Journal of Botany* **88**: 2074–2087.

Savolainen V, Chase MW, Hoot SB, *et al*. 2000. Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. *Systematic Biology* **49**: 306–362.

Seberg O, Petersen G, Davis JI, *et al*. 2012. Phylogeny of the Asparagales based on three plastid and two mitochondrial genes. *American Journal of Botany* **99**: 875–889.

Shaw J, Lickey EB, Beck JT, *et al*. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**: 142–166.

Simpson MG, Rudall PJ. 1998. Tecophilaeaceae. In: Kubitzki K. ed. *The families and genera of vascular plants III. Flowering plants. Monocotyledons. Lilianae (except Orchidaceae)*. Heidelberg: Springer, 429–436.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.

Valente LM, Savolainen V, Manning JC, Goldblatt P, Vargas P. 2012. Explaining disparities in species richness between Mediterranean floristic regions: a case study in *Gladiolus* (Iridaceae). *Global Ecology and Biogeography* **20**: 881–892.

Zwickl DJ. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD dissertation, The University of Texas at Austin. Available from: http://garli.googlecode.com.

APPENDIX 1

List of species included in the phylogenetic analysis of Tecophilaeaceae, including voucher information and GenBank accession numbers.

Taxon	Voucher	*atpB–rbcL*	*matK*	*rbcL*	*rpl16*	*trnL* intron	*trnL–trnF*
Conanthera bifolia Ruiz & Pav.	Living Collection, University of California, Irvine 42; no voucher	KC161439	KC161460	KC161479	KC161500	KC161516	KC161534
Conanthera campanulata Lindl.	*Chase 523* (K)	KC161440	KC161461	KC161480	KC161501	KC161517	KC161535
Conanthera sabulosa Ravenna	*Chase 13820* (K)	KC161441	KC161462	KC161481	KC161502	KC161518	KC161536
Conanthera trimaculata (D.Don) F. Meigen	*Chase 1788* (K)	KC161442	KC161463	KC161482	KC161503	KC161519	KC161537
Conanthera variegata Fenzl. ex Reichardt	*Chase 2970* (K)	KC161443	–	KC161483	KC161504	KC161520	KC161538
Cyanastrum cordifolium Oliv.	*Chase 18736* (K)	AY147743	KC161464	Z73696–U41572	–	KC161521	KC161539
Cyanella alba L.f. subsp. *alba*	*Manning s.n.* (NBG)	KC161444	KC161465	KC161484	KC161505	KC161522	KC161540
Cyanella alba L.f. subsp. *flavescens* J.C. Manning	*Goldblatt & Manning 10741* (NBG)	KC161445	KC161466	KC161485	–	KC161523	KC161541
Cyanella aquatica Oberm ex G. Scott	*Goldblatt & Manning 10581A* (NBG)	KC161446	KC161467	KC161486	KC161506	KC161524	KC161542
Cyanella cygnea G. Scott	*Goldblatt & Manning 9940* (NBG)	KC161447	KC161468	KC161487	KC161507	KC161525	KC161543
Cyanella hyacinthoides Royen ex L.	*Goldblatt & Manning 8583* (NBG)	KC161448	KC161469	KC161488	KC161508	KC161526	KC161544
Cyanella lutea L.f. subsp. *lutea*	*Manning 2195* (NBG)	KC161449	KC161470	KC161489	–	KC161527	KC161545
Cyanella orchidiformis Jacq.	*Goldblatt & Manning 9121* (NBG)	KC161450	KC161471	KC161490	KC161509	KC161528	KC161546
Eremiolirion amboense (Schinz) J.C. Manning & C.A. Mannheimer	*Mannheimer 2510* (NBG)	KC161451	–	KC161491	KC161510	KC161529	KC161547
Kabuyea hostifolia (Engl.) R.K. Brummitt	*Chase 1378* (K)	KC161452	KC161472	KC161492	KC161511	AJ290278	AJ290312
Odontostomum hartwegii Torr.	*Chase 491* (K)	KC161453	KC161473	KC161493	KC161512	KC161530	KC161548
Walleria gracilis (Salisb.) S. Carter	*Manning 2180* (NBG)	KC161454	KC161474	KC161494	KC161513	KC161531	KC161549
Walleria mackenzii J. Kirk	No voucher	KC161455	KC161475	KC161495	KC161514	AJ290279	AJ290313
Walleria nutans J. Kirk	*Goldblatt & Manning 8802* (NBG)	KC161456	KC161476	KC161496	–	KC161532	KC161550
Zephyra cyanocrocus (Leyb.) Ravenna	*Chase 447* (K)	KC161457	KC161477	KC161497	–	AJ290276	AJ290310
Zephyra elegans D. Don	*Chase 1575* (K)	KC161458	AJ579994	KC161498	–	AJ290277	AJ290311
Zephyra violiflora (Bertero ex Colla) Ravenna	*Chase 1498* (K)	KC161459	KC161478	KC161499	KC161515	KC161533	KC161551
Doryanthaceae – *Doryanthes excelsea* Correa	–	–	AJ580616	Z73697	–	AJ290281	AJ290315
Iridaceae – *Isophysis tasmanica* (Hook.) T. Moore	–	–	AJ579963	Z77287	–	AJ290283	AJ290317
Ixioliriaceae – *Ixiolirion tataricum* (Pall.) Herb.	–	–	AJ579965	Z73704	–	AJ290280	AJ290314

APPENDIX 2

List of species of Tecophilaeaceae, including information on the scoring for the biogeographical (LAGRANGE) and diversification (GeoSSE) inferences. Conanthera sabulosa *and* C. variegata *(indicated with *) are considered as synonyms of* C. campanulata *by some authors; our analyses showed that some further investigations are needed.*

		Biogeographical areas (LAGRANGE)				Ecology (GeoSSE)		
Taxon	Sampled?	(A) Chile	(B) California	(C) Tropical Africa	(D) Greater Cape region	Arid	Mediterranean	Sub-tropical
Conanthera bifolia Ruiz. & Pav.	Yes	1	0	0	0	0	1	0
Conanthera campanulata Lindl.	Yes	1	0	0	0	1	1	0
Conanthera sabulosa Ravenna*	Yes	1	0	0	0	1	1	0
Conanthera parvula (Phil.) Muñoz-Schick	No	1	0	0	0	0	1	0
Conanthera trimaculata (D.Don) F. Meigen	Yes	1	0	0	0	0	1	0
Conanthera urceolata Ravenna	No	1	0	0	0	1	0	0
Conanthera variegata Fenzl. ex Reichardt*	Yes	1	0	0	0	1	1	0
Cyanastrum cordifolium Oliv.	Yes	0	0	1	0	0	0	1
Cyanastrum goetzeanum Engl.	No	0	0	1	0	0	0	1
Cyanastrum johnstonii Baker	No	0	0	1	0	0	0	1
Cyanella alba L.f. subsp. *alba*	Yes	0	0	0	1	0	1	0
Cyanella alba L.f. subsp. *flavescens* J.C. Manning	Yes	0	0	0	1	0	1	0
Cyanella alba L.f. subsp. *minor* J.C. Manning	No	0	0	0	1	0	1	0
Cyanella aquatica Oberm ex G. Scott	Yes	0	0	0	1	0	1	0
Cyanella cygnea G. Scott	Yes	0	0	0	1	0	1	0
Cyanella hyacinthoides Royen ex L.	Yes	0	0	0	1	1	1	0
Cyanella lutea L.f.	Yes	0	0	0	1	1	1	0
Cyanella marlothii J.C.Manning & Goldblatt	No	0	0	0	1	0	1	0
Cyanella orchidiformis Jacq.	Yes	0	0	0	1	0	1	0
Cyanella pentheri Zahlbr	No	0	0	0	1	0	1	0
Cyanella ramosissima (Engl. & Krause) Engl. & K. Krause	No	0	0	0	1	0	1	0
Eremiolirion amboense (Schinz) J.C.Manning & C.A. Mannheimer	Yes	0	0	0	1	1	0	0
Kabuyea hostifolia (Engl.) R.K.Brummitt	Yes	0	0	1	0	0	0	1
Odontostomum hartwegii Torr.	Yes	0	1	0	0	0	1	0
Walleria gracilis (Salisb.) S. Carter	Yes	0	0	0	1	1	1	0
Walleria mackenzii J. Kirk	Yes	0	0	1	0	0	0	1
Walleria nutans J. Kirk	Yes	0	0	1	1	1	0	1
Zephyra compacta C. Ehrh.	No	1	0	0	0	1	1	0
Zephyra cyanocrocus (Leyb.) Ravenna	Yes	1	0	0	0	0	1	0
Zephyra elegans D. Don	Yes	1	0	0	0	1	1	0
Zephyra violiflora (Bertero ex Colla) Ravenna	Yes	1	0	0	0	0	1	0