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ORIGINAL ARTICLE

Timing and tempo of evolutionary diversification in a biodiversity hotspot: Primulaceae on Indian Ocean islands

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ABSTRACT

Aim We examined phylogenetic relationships and spatio-temporal diversification in Indian Ocean Primulaceae, assessing correlations between speciation rates, geographical expansion and ecomorphological specialization.

Location Madagascar and Indian Ocean Islands.

Methods We conducted phylogenetic analyses with plastid and nuclear DNA sequences of Primulaceae using maximum likelihood and Bayesian algorithms, and estimated divergence times using a Bayesian relaxed molecular clock. Temporal changes in diversification rate and possible correlations with the biogeographical history of the group were examined. We performed parametric ancestral area reconstruction incorporating a stratified palaeogeographical model that reflects changes in terrestrial configuration and the presence of phytogeographical connections through time in the western Indian Ocean Basin. Shifts in diversification rate were compared with ancestral area assignments and divergence age estimates.

Results Indian Ocean Primulaceae were recovered as monophyletic with a sister relationship to Asian *Ardisia*. *Oncostemum*, a genus confined to Madagascar and the Comoros, was resolved as paraphyletic by the inclusion of a monophyletic Mascarene *Badula* group consisting of single-island endemics. We found evidence for diversification bursts early in the history of Indian Ocean Primulaceae that correspond closely to the sequence of dispersal and the appearance of newly formed Mascarene Islands. Age estimates suggest a dispersal to Rodrigues that is older than the estimated geological age of the island.

Main conclusions Results suggest a Madagascan origin of Indian Ocean Primulaceae with subsequent dispersal to the Mascarenes in the middle to late Miocene, with initial establishment on either Mauritius or Rodrigues and subsequent stepping-stone dispersal to the other two Mascarene islands within the last 2 Myr. Analyses suggest that diversification has slowed over time, with significant rate changes following dispersal to new geographical areas. Onset of diversification in species-rich *Oncostemum* appears to have been recent, with major cladogenesis commencing in the early Pliocene.

Keywords

***Badula*, Bayesian relaxed clock, biodiversity hotspot, dispersal–extinction–cladogenesis, diversification rate-shifts, Indian Ocean islands, long-distance dispersal, *Oncostemum*, palaeogeographical model, Primulaceae.**

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INTRODUCTION

Madagascar and its surrounding island groups hold perhaps some of most iconic but least understood examples of evolution in isolation, for example mammals (Douady *et al.*, 2002; Yoder *et al.*, 2003; Poux *et al.*, 2005; Ali & Huber, 2010; Krause, 2010), birds (Safford & Hawkins, 2013), and vascular plants (Callmander *et al.*, 2011); for an extensive

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biogeographical account on Madagascar see Goodman & Benstead (2003), Burney *et al.* (2004) and Vences *et al.* (2009). The complex geological and spatio-temporal history of the region has facilitated the amalgamation of unique species assemblages originating through long-distance dispersal from a wide range of continental and secondary island sources (Wallace, 1876; Goodman & Benstead, 2003; Samonds *et al.*, 2012, 2013). As currently understood, the entire region, including Madagascar itself, primarily comprises species descended from recent colonizers (Yoder & Nowak, 2006; Warren *et al.*, 2010). The provenance and the patterns of diversification following initial colonization in the region, however, remain largely unknown for most taxonomic groups, especially plants (Wilmé *et al.*, 2006).

The Primulaceae, a family of *c.* 1300 species of primarily shrubs and small trees (Angiosperm Phylogeny Group, 1998, 2003; Chase *et al.*, 2009), are well represented in the Madagascar region and the more inclusive lands within and around the Indian Ocean. The study of their biogeographical history thus affords an excellent opportunity to assess the underlying history of dispersal and diversification events that have shaped plant diversity in Madagascar and neighbouring islands. Within the family, island endemism is particularly common among woody Myrsinoideae (Mez, 1902), which include the genera *Oncostemum* Juss. and *Badula* Juss., both restricted to the Madagascar region (Perrier de la Bâthie, 1953; Coode, 1981; Antoine *et al.*, 1993; Schatz, 2001). All species are regional endemics, mostly confined to single islands. The vast majority lie within *Oncostemum* (*c.* 97 species) (Perrier de la Bâthie, 1953; Schatz, 2001) confined to Madagascar and the Comoros Islands. *Badula* comprises 14 species, all endemic to the Mascarenes, an archipelago of three small islands (Coode, 1981). *Oncostemum* has been considered the most likely sister group to *Badula* on the basis of morphological characters and biogeographical distribution (Jussieu, 1789, 1830; Perrier de la Bâthie, 1952, 1953; Coode, 1978, 1979, 1981), but was resolved as paraphyletic in a recent study (Bone *et al.*, 2012).

Here we use a dataset that includes multiple molecular markers, fossil calibration points, all species of Mascarene *Badula* and a subset of Madagascan *Oncostemum* to reconstruct the historical biogeography of Indian Ocean Primulaceae. We placed particular focus on reconstructing the onset and tempo of evolution in *Badula* and assessing the effect of different scenarios of regional geographical configurations on Primulaceae diversification.

MATERIALS AND METHODS

Sampling, PCR and DNA sequencing

We included all members of Mascarene *Badula sensu* Coode (1981) and Bone *et al.* (2012) and 22 individuals of the closely related Madagascan genus *Oncostemum* (Figs 1 & 2; and see Appendix S1 in Supporting Information). Apart from the original treatment for the Flora of Madagascar (Perrier de la Bâthie, 1953), little is known about the internal relationships and evolutionary history of *Oncostemum*. In addition, new species and populations have been described, making both the treatment and descriptions outdated (Bone *et al.*, 2012). We selected a range of taxa from throughout the Primulaceae as the outgroup, with the *Androsace* + *Soldanella* + *Primula* clade employed to root the tree (Anderberg *et al.*, 1998; Bremer *et al.*, 2004; Smith & Donoghue, 2008). The nearest outgroup consists of three *Ardisia* accessions, all of which are of continental Asian origin.

We expanded a previously constructed molecular dataset (Bone *et al.*, 2012) by increasing the number of taxa and DNA regions sequenced. DNA was extracted from silica-dried material with the BioSprint DNA Plant Kit (Qiagen, Valencia, CA). Internal transcribed spacer (ITS) and *trn*SGG markers were amplified as in Bone *et al.* (2012). In addition, we sampled the plastid regions *psb*A–*trn*H, *acc*D and *rpo*B. The amplification protocol for *psb*A–*trn*H follows that of Sang (Sang *et al.*, 1997) and those for *acc*D and *rpo*B are listed on the DNA barcoding website of the Royal Botanic Gardens Kew (CBOL, 2007). The number of new sequences of each region is 63 for *acc*D, 62 for *rpo*B, 60 for *psb*A–*trn*H, 26 for ITS, and eight for *trn*SGG. Sequencing procedures follow those of Bone *et al.* (2012).

Phylogenetic reconstruction

Model selection was performed by using the Akaike information criterion (AIC) in Modeltest 3.7 (Posada & Crandall, 1998) (ITS: GTR+I+G; *trn*SGG: GTR+I; *rpo*B: HKY; *acc*D: HKY; *psb*A–*trn*H: GTR+I+G). We employed selected models in separate and combined (plastid+nuclear) maximum likelihood analyses (1000 bootstrap replicates) using RAxML (Stamatakis, 2006; Stamatakis *et al.*, 2008).

Combined Bayesian analyses were conducted by using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) with model partitioning and were run on the Vital-IT cluster of the Swiss Institute of Bioinformatics (http://www.vital-it.ch/). Indel characters, scored with Gapcoder (Young & Healy, 2003), were treated as a fifth partition with the restriction site model and 'variable' option in effect. Two separate runs were performed with four chains running for 20×10^6 generations, sampling a tree every 1000th generation and discarding the first 25% of trees as burn-in period. Convergence of the chains was examined by using the potential scale reduction factor (psrf) and assessing both the average standard deviation of split frequencies between runs (Ronquist & Huelsenbeck, 2003) and, in Tracer 1.4.1 (Rambaut & Drummond, 2007), the effective sample size (ESS). A 50% majority rule consensus tree (halfcompat) was built and converted to a chronogram in r8s (Sanderson, 1997; Sanderson & Shaffer, 2002; Sanderson & Driskell, 2003) by using the penalized likelihood (PL) approach (tree not shown).

Divergence time estimation and calibration

We used beast 1.4.8 (Drummond & Rambaut, 2007) to estimate divergence times in *Badula* and *Oncostemum* (Appendix S2).

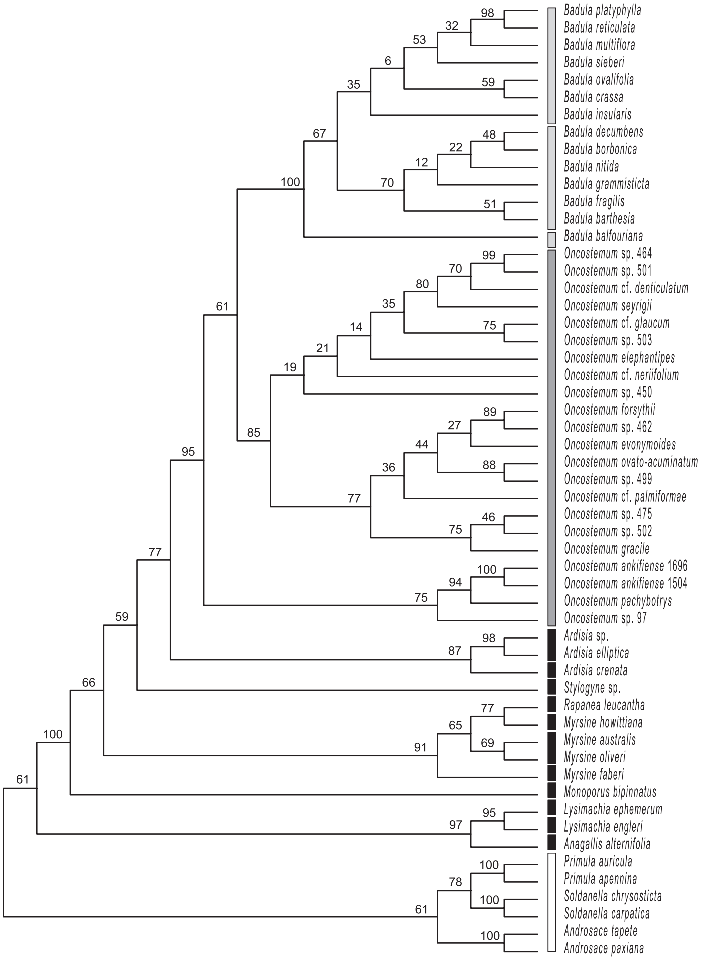

Figure 1 Maximum likelihood phylogenetic tree of Indian Ocean Primulaceae based on plastid and nuclear DNA sequence data. Numbers at nodes indicate bootstrap support values estimated with 1000 replicates. Bars indicate species groups [light grey bar, *Badula*; dark grey bar, *Oncostemum*; black bars, traditional Myrsinaceae (APGII); white bar, traditional Primulaceae (APGII) and outgroups].

Because published island age estimates for the Western Indian Ocean vary, we employed interval-based probability priors constructed with fossil records. This approach incorporates existing age uncertainty in the evaluation of colonization and diversification times. We added species from across Primulaceae by using sequences deposited in GenBank (Appendices S1 & S3) and used three calibration points to estimate absolute ages for divergence times (Appendix S2). Prior type and width were set in accordance with the source, reliability and knowledge about the evolutionary split for each target node. The geological time-scale used is that of Gradstein *et al.* (2004). The age of the split between traditionally defined Primulaceae and Myrsinaceae has been estimated as approximately 45 Ma (Bremer *et al.*, 2004). Following Smith & Donoghue (2008), and using sequences deposited in GenBank from their study, we used this split as a secondarily derived root calibration (narrow uniform prior: 30–60 Ma). We balanced the use of this secondary calibration, which can increase the amount of error introduced by the original analysis, by using other primary calibrations, a strategy justified elsewhere (Hedges & Kumar, 2004). To account for potential error in the root prior, we also tested a wider uniform prior (0–1000 Ma). With the other BEAST settings identical, we found no significant deviations in results obtained for this node, or other nodes (data not shown).

A second calibration was added at the split between *Anagallis* and *Lysimachia*. The oldest fossil seeds known for *Lysimachia* have been described from the Asian and European middle Oligocene (*c.* 28 Ma) (Chandler, 1957; Dorofeev, 1963). Additional European fossils are described from the middle Miocene (Friis, 1985; Mai, 2001) and middle Pliocene (Mai & Walther, 1988). We applied a uniform prior that covers the period from the middle Pliocene to the start of the Oligocene (3.6–33.9 Ma) to the stem node of *Lysimachia*. We included a third calibration based on *Ardisia* plant microfossils in the Miocene of New Zealand. On the basis of associated volcanic elements, these fossils are estimated to be about 20 Myr old (Pole, 1996, 2008). To take into consideration the dating uncertainty in this age estimate, we applied a wide uniform prior (5.3–23 Ma) to the stem node of *Ardisia*, incorporating the entire Miocene period. Details of all analyses and settings are given in Appendix S2.

Diversification rates and rate-shifts

We explored the temporal accumulation of lineages using lineage-through-time (LTT) curves based on the excised ingroup with GENIE 3.0 (Pybus & Rambaut, 2002). For *Oncostemum* + *Badula*, the effects of incomplete taxon sam-

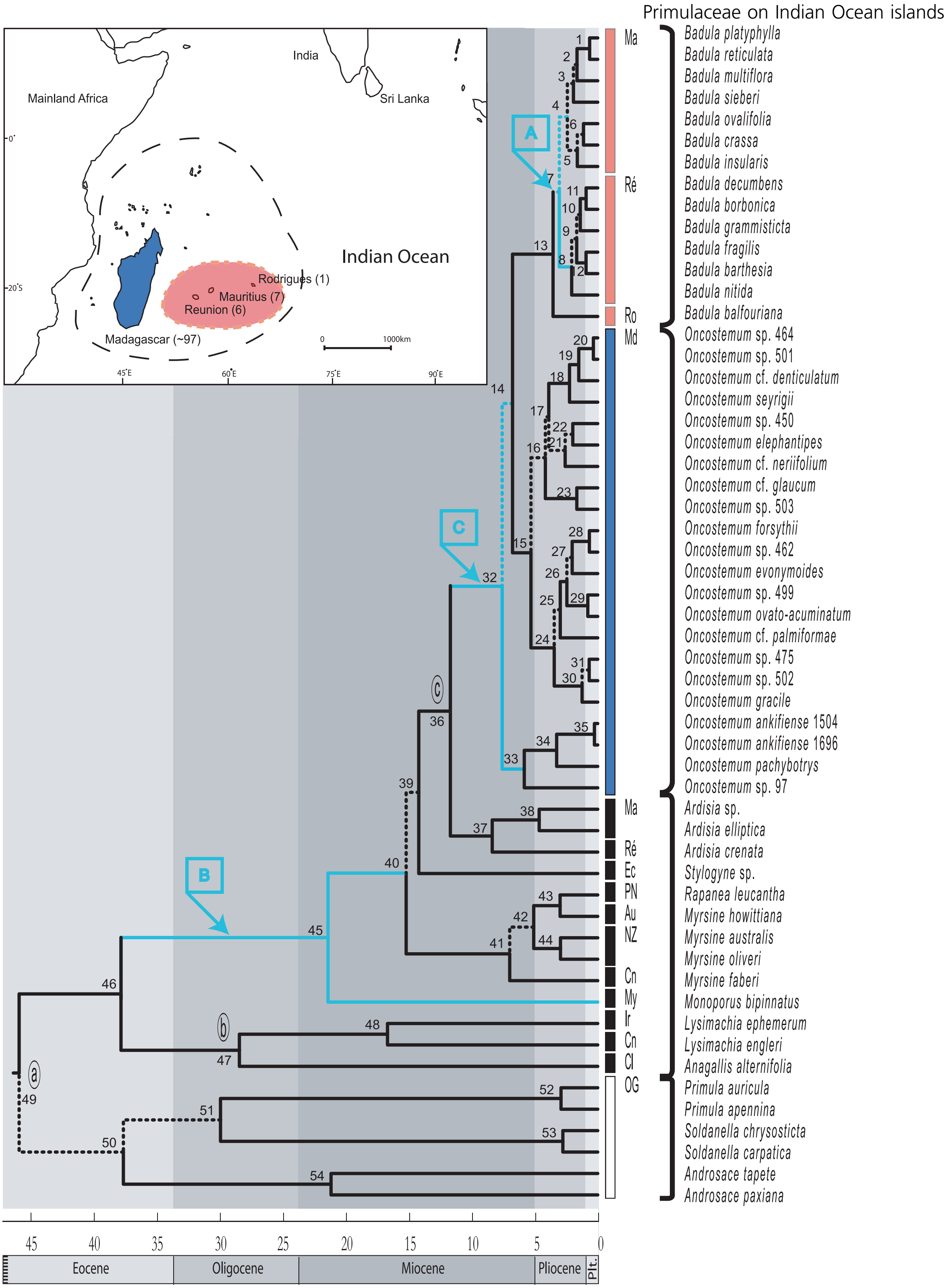

Figure 2 Maximum clade credibility chronogram obtained with BEAST estimation of divergence times of Indian Ocean Primulaceae on the combined partitioned dataset. The inset map shows biogeographical regions used in the study and species distribution in the biodiversity hotspot (blue, *Oncostemum*; red, *Badula*). Broken lines indicate branches with posterior probabilities $P < 0.90$. Blue arrows and branches indicate rate-shifts associated with results obtained with SYMMETREE (rate-shift boxes A–C). Nodes labelled a–c indicate calibrations discussed in the Materials and Methods section. Numbers at nodes refer to tabulated results in Appendix S3. Red bars, *Badula*; blue bar, *Oncostemum*; black bars, traditional Myrsinaceae (APGII); white bar, traditional Primulaceae (APGII) and outgroups. Abbreviations: Md, Madagascar; Ma, Mauritius; Ré, Réunion; Ro, Rodrigues; Ec, Ecuador; NZ, New Zealand; PN, Papua New Guinea; Au, Australia; Cn, China; My, Mayotte; Ir, Ireland; Cl, Chile; OG, outgroups.

pling on the slope of the empirical LTT curves was evaluated by generating 1000 simulated trees of the extant number of taxa (111) with a constant-rate birth–death model in Phylogen 1.1 (Rambaut, 2002). Unsampled taxa were randomly pruned, and branch lengths were rescaled to the crown age of the clade with Tree-Edit 1.0 (Rambaut & Charleston, 2002). Because monophyletic *Badula* was sampled completely, we repeated the above procedure without subsampling ($n = 14$). Simulated trees were used to construct mean LTT curves and 95% confidence intervals.

As shown here and in previous work (Bone *et al.*, 2012), *Badula* is monophyletic, but nests inside *Oncostemum*. The diversification of extant *Badula*, and therefore of the species in its sister clade, is island age-restricted as *Badula* is confined to volcanic islands for which we have an estimated maximum age limit, confining the shared node underlying these clades to un upper age limit. However, the paraphyly of *Oncostemum* complicates the comparison of shared temporal diversification in the two genera. Our limited sampling within *Oncostemum* could underestimate the root age for the entire clade (*Oncostemum* + (*Badula* + *Oncostemum*)), if significant numbers of currently unincluded species would nest in the outer *Oncostemum* clade. Hypothesizing that the crown age of the clade incorporating all taxa will not deviate significantly beyond estimates obtained in our analyses, we constructed a theoretical complete phylogeny (of *c.* 111 species) including all known *Badula* and *Oncostemum*, with which we simulated and compared three scenarios of diversification [I – rapid diversification, followed by slowdown; II – diversification closely corresponding to a constant-speciation-rate model; III – slow initial diversification, but increasing towards the present], assuming no change in root age estimate (7.59 Ma) and selecting empirical curves resembling these scenarios from the tree space of our simulation. As such, the 97 species of *Oncostemum* then diversified into a range of environments on Madagascar over at least the past 7.59 Ma (upper 95% highest posterior density, HPD: 12.38 Ma). We constructed LTT curves both for sampled *Badula* and sampled *Badula* + *Oncostemum* and constructed the simulated mean and 95% confidence interval curves for a fully simulated species set of *Badula* + *Oncostemum* (Fig. 3a–c).

To assess changes in the rate of speciation events over time, we conducted three analyses (Appendix S2). We performed the constant rate (CR) test (Harmon *et al.*, 2007) and calculated the γ-statistic in the R package LASER (Rabosky, 2006a; R Development Core Team, 2010), with additional simulations conducted to account for undersampling (mccrTest; R package LASER (Rabosky, 2006a)). We compared this analysis to the maximum likelihood method birth–death likelihood (BDL) in LASER (Rabosky, 2006a,b) and the topological method SymmeTree. The latter was also used to locate shifts in diversification rate (Chan & Moore, 2002, 2005; Moore *et al.*, 2004).

Biogeographical inference

As recommended by Buerki *et al.* (2011), geographical areas were defined with the geological criterion *sensu* Sanmartín & Ronquist (2004), together with current species ranges. Five areas were delimited: (a) Madagascar; (b) Rodrigues; (c) Mauritius; (d) Réunion; and (e) Other. The area 'Other' was designed to accommodate the distribution of the outgroup taxa that occur outside of the western Indian Ocean basin. To avoid incorrect biogeographical inference due to incomplete sampling of the more distant outgroups, we performed the analyses on a reduced dataset including only the ingroup (*Badula* and *Oncostemum*), and its closest relative (*Ardisia*).

The dispersal–extinction–cladogenesis (DEC) likelihood model implemented in Lagrange 2.0.1 (Ree *et al.*, 2005; Ree & Smith, 2008) was used to investigate biogeographical patterns (Appendix S2). This method is a parametric, extended version of dispersal–vicariance analysis (Ronquist, 1997) that

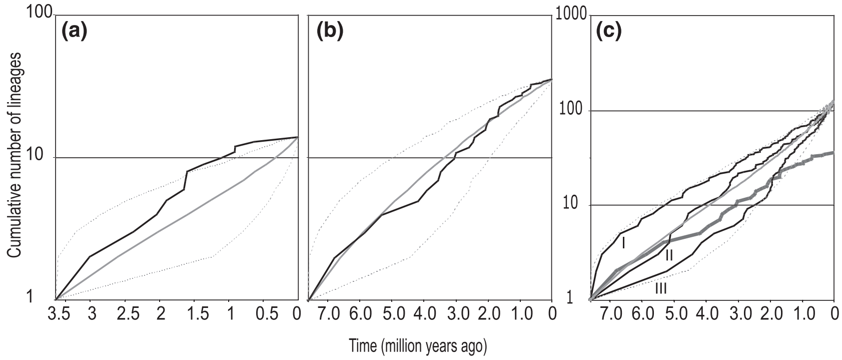

Figure 3 Lineage-through-time (LTT) curves for Indian Ocean Primulaceae: (a) *Badula* and (b) *Oncostemum* + *Badula*. Empirical curves (black) and simulated curves (grey solid lines) with 95% confidence intervals (CI, grey dashed lines) for the sampled phylogenies. (c) Simulated LTT curve (with 95% CI, grey dashed lines) constructed for a hypothesized clade size of 111 *Oncostemum* + *Badula* species (7.59 Ma) and compared to the empirical curve shown in panel b) (thick grey line). Three hypothetical curves (black lines, I–III), selected out of 1000 simulations, illustrate different evolutionary scenarios of species accumulation.

estimates ancestral ranges, transition rates between ranges, and biogeographical scenarios of range inheritance for a group of taxa in a likelihood framework (Ree & Sanmartín, 2009). With the approach described in Espindola *et al.* (2012), Lagrange analyses were performed over the reconstructed beast chronogram. All the ingroup taxa are restricted to one area (single-island endemics) and therefore Lagrange analyses were run with a maximum area set to two. Higher numbers would make increasingly less sense in an island context, as this may lead to implicitly introducing unjustifiable assumptions (improbable dispersal patterns, widespread continuous extinctions and unrealistic ancestral ranges).

An advantage of the DEC model is its ability to adapt a transition matrix (i.e. *Q*-matrix) to reflect changes in palaeogeography (e.g. land bridges) or dispersal capabilities of the group of interest through time (Buerki *et al.*, 2011). We therefore incorporated three time intervals (TI1–3) into the DEC model to cover the changes in palaeogeographical conditions in the Western Indian Ocean during the history of our study group: 11.71–8.2 Ma, 8.2–5 Ma, and 5 Ma–present. For each interval, we defined a *Q*-matrix in which transition rates accorded with the availability of source and sink areas for dispersal. Because our study deals primarily with regions that were not physically connected in the period of our group's diversification (or, in some cases, were never connected to any other land region), changing transition rates in our Q-matrices accord mostly with initial appearance of oceanic islands by volcanic activity and subsequent potential for stepping-stone dispersal (Appendix S2). Because much uncertainty exists as to the geological age of the Mascarene island of Rodrigues, which could be much older than its current estimated age (*c.* 1.5 Ma) (McDougall & Chamalaun, 1969; Saddul *et al.*, 1995) and perhaps be even older than the island of Mauritius (Cheke & Hume, 2008), we assessed the effect of the absence and presence of Rodrigues in the Indian Ocean as a potential source for arrival and departure of dispersing lineages over time by examining two separate scenarios. In scenario I ('*young Rodrigues*') we assumed a recent origin of the island by disallowing it at the root node of our phylogeny and by setting the probability of dispersal to and from the island in the matrices of the first two time intervals (TI1 and TI2) to its lowest possible setting. Only in TI3 are *Q*-matrix values for Rodrigues raised to equal that of other Mascarene Islands. In scenario II ('*old Rodrigues*'), we allowed Rodrigues to be recovered at any of the nodes, and increased the probability of dispersal to and from Rodrigues to equal that of other Mascarene Islands in all time intervals (TI1–3; details in Appendix S2).

RESULTS

Phylogenetic analyses

Individual data partitions analysed by using maximum likelihood resulted in largely congruent trees and showed no strongly supported conflicts (bootstrap support, BS $\geq$ 90). Combined analyses initially consisted of the full taxon set with multiple accessions, but a reduced set was used for results presented here (Appendix S1). Combined phylogenetic analyses resolved *Oncostemum* as paraphyletic (BS = 95), with *Badula* nested inside it as a monophyletic group (BS = 100; Fig. 1). Internal phylogenetic relationships of *Badula* break down along geographical lines, beginning with a single species on Rodrigues (*B. balfouriana*), and two distinct lineages, each restricted to a single island (Réunion, six species, and Mauritius, seven species).

Estimation of divergence times

In the maximum clade credibility tree (MCC) from beast, the Réunion (PP = 1.0) clade is sister to the Mauritian (PP = 0.62) clade, with low support (PP = 0.53). The onset of cladogenesis on Réunion (2.07 Ma; 95% HPD: 1.38–6.24 Ma) and Mauritius (2.41 Ma; 95% HPD: 0.95–4.17) is inferred as taking place nearly simultaneously (Fig. 2). The dispersal of an ancestral *Oncostemum* lineage to the Mascarene region is inferred in the late Miocene (6.77 Ma; 95% HPD: 3.14–11.05 Ma). Additional runs performed with elevated upper limits for the two uniform priors (to an arbitrary 50 Ma) did not result in any significant changes in age estimates for target nodes.

Table 1 Equal-rates Markov (ERM) nodal probabilities obtained in whole-tree tests for diversification rate-variation within Indian Ocean Primulaceae using SymmeTree. Polytomies were resolved analytically by using a taxon-size sensitive ERM random-branching model, providing 95% confidence interval limits that correspond to tail probabilities of the 0.025 and 0.975 frequentiles. Whole-tree statistics are differentially sensitive to variation in large-scale diversification rates ($B_1 < M_\Sigma < M_{\Sigma*} < M_\Pi < M_{\Pi*} < I_c$). Tests were conducted on the 50% majority rule consensus tree obtained with MrBayes and the maximum clade credibility (MCC) chronogram obtained with beast. Abbreviations: B_1, tree balance statistic; M_s, cumulative ERM probability of the sum of all individual nodal probabilities; M_{s*}, like M_s, but with differential size weighting; M_p, cumulative ERM probability of the product of all individual nodal probabilities; M_{p*}, like M_p, but with differential size weighting; I_c, Colless' index of imbalance.

Tree	No. of taxa	*Frequentiles*	B_1	M_s	M_{s*}	M_p	M_{p*}	I_c
MrBayes 50% majority rule consensus tree	57	*0.025*	0.02673	0.00137	0.00023	0.00015	0.00002	0.00002
		0.975	0.55751	0.10812	0.01154	0.00906	0.0017	0.00165
beast MCC chronogram	55	*no polytomies*	0.39608	0.19689	0.02742	0.02531	0.00302	0.00122

Table 2 Results of birth–death likelihood analysis of lineage diversification in Indian Ocean Primulaceae, for (a) *Badula* and (b) *Oncostemum* + *Badula*. Analyses were conducted with the chronogram obtained in BEAST. All non-ingroup taxa were pruned prior to analyses.

Model	LH	r1	r2	r3	*a*	*xp*	*k*	st1	st2	AIC	ΔAIC_{rc}
(a) *Badula* (*n* = 14)											
pb	1.038	0.453								−0.076	−6.350
bd	1.038	0.453			0					1.924	−8.350
DDX	2.728	2.367				0.805				−1.455	−4.971
DDL	5.213	1.739					13.872			−6.426	0
Yule2rate	3.817	1.014	0.255					1.622		−1.634	−4.792
Yule3rate	7.456	0.724	14.371	0.079				0.929	0.923	−4.912	−1.514
(b) *Oncostemum* + *Badula* (*n* = 36)											
pb	24.194	0.369								−46.389	−4.330
bd	24.194	0.369			0					−44.388	−6.330
DDX	24.814	0.710				0.235				−45.629	−5.089
DDL	26.052	0.643					50.906			−48.104	−2.614
Yule2rate	28.359	0.459	0.089					0.644		−50.718	0
Yule3rate	29.579	0.437	1.730	0.089				0.680	0.644	−49.157	−1.561

Models: pb, pure birth; bd, birth–death; DDX, exponential density dependent; DDL, logistic density dependent; Yule2rate, Yule with 2 rates; Yule3rate, Yule with 3 rates.

Parameters: LH, log-likelihood; r1/r2/r3, net diversification rates; *a*, extinction fraction; *xp*, rate change parameter DDX model; *k*, carrying capacity parameter DDL model; st1/st2, timing of rate-shift (Ma); AIC, Akaike information criterion; ΔAIC_{rc}, difference between the best rate-constant and rate-variable models.

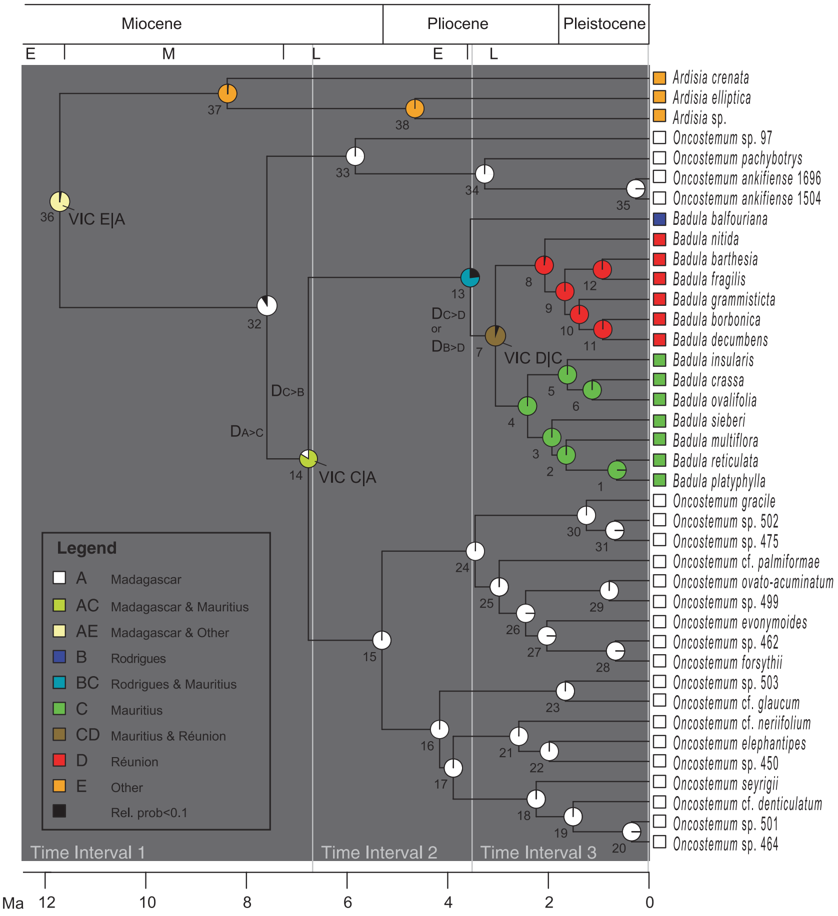

Figure 4 Dispersal–extinction–cladogenesis (DEC) scenario I (‘*young Rodrigues*’) for Indian Ocean Primulaceae, with results of the palaeogeographical model projected onto the maximum clade credibility tree created in BEAST. Pie charts represent ancestral area probabilities assigned at each node. Pie charts with a horizontal black line depict relative probabilities of $P = 1$ for the area indicated in colour; those with a vertical black line depict relative probabilities of $P < 0.1$ for the total of all areas other than that indicated in colour. Text inserts (D, dispersal; VIC, vicariance) follow most probable assignment, where applicable.

Diversification rates and rate-shifts

The LTT curve for *Badula* indicates a gradual build-up of species numbers, with a marked increase around 1.5 Ma, followed by a significant deviation from the simulated curve resembling a constant rate of diversification (Fig. 3a). In contrast, the LTT curve for *Oncostemum* + *Badula* shows a more continuously gradual curve that does not deviate significantly from the simulated constant-rate curve, with upturns around 3.9, 2.4, 2.0 and 1.7 Ma (Fig. 3b). The reconstructed curve for the sampled species mostly conforms to a steady constant-speciation-rate model (scenario II, Fig. 3c).

The CR test returned negative gamma values for both clades (*Badula*: $\gamma = -2.263$; *Badula* + *Oncostemum*: $\gamma = -1.619$), suggesting a slowdown in net diversification rates towards the present. Taking into account the effect of undersampling in *Oncostemum*, however, we failed to find significant evidence to reject the CR model ($\gamma_{\mathrm{crit}} = -2.459$, $P = 0.232$). Treating the unidentified samples more conservatively (and thus artificially increasing the undersampling fraction) did not alter this finding ($n = 26$; $\gamma = -2.230$).

Birth–death likelihood analyses of the *Badula* + *Oncostemum* phylogeny reject a constant-rate scenario ($\Delta\mathrm{AIC}_{\mathrm{rc}} = 4.330$; $P = 0.041$). For *Badula*, the best-fitting model was the density dependent logistic (DDL) model, suggesting a gradual decline of diversification rates over time. For *Oncostemum* + *Badula*, the best-fitting model was the Yule2rate model, suggesting the presence of a rate-shift very late in the clade's evolution.

Analyses involving a topological approach indicate that significant variation in diversification rates between lineages was present at intermediate levels in the phylogeny (Tables 1 & 2). Using the Δ_1 and Δ_2 likelihood rate-shift statistics, we detected significant values [$\Delta_1 = 0.0256$, $\Delta_2 = 0.0256$, Slowinski–Guyer (SG) $= 0.0256$] for an upward rate-shift along the branch leading to the split between Mauritian and Réunion *Badula* (rate-shift box A, Fig. 2). A second upward rate-shift was deeper in the tree close to the origin of the split between traditionally defined Myrsinaceae and Primulaceae ($\Delta_1 = 0.0364$, $\Delta_2 = 0.0476$, SG $= 0.3424$; rate-shift box B, Fig. 2). Statistical phylogenetic support associated with these nodes was strong (PP = 1). Rate-shift A and B were detected in both the BEAST MCC tree and the MrBayes majority rule consensus, but rate-shift C was only detected with significant support in the latter ($\Delta_1 = 0.0455$, $\Delta_2 = 0.0474$, SG $= 0.0581$; rate-shift box C, Fig. 2). We detected no significant support for a shift in diversification rate along the branch leading to *Oncostemum* ($\Delta_1 = 0.7534$, $\Delta_2 = 1.0000$, SG $= 0.9142$).

Biogeographical inference

Under scenario I (log likelihood, $-\ln L = 25.86$), the Lagrange area assignments with highest probability on the BEAST

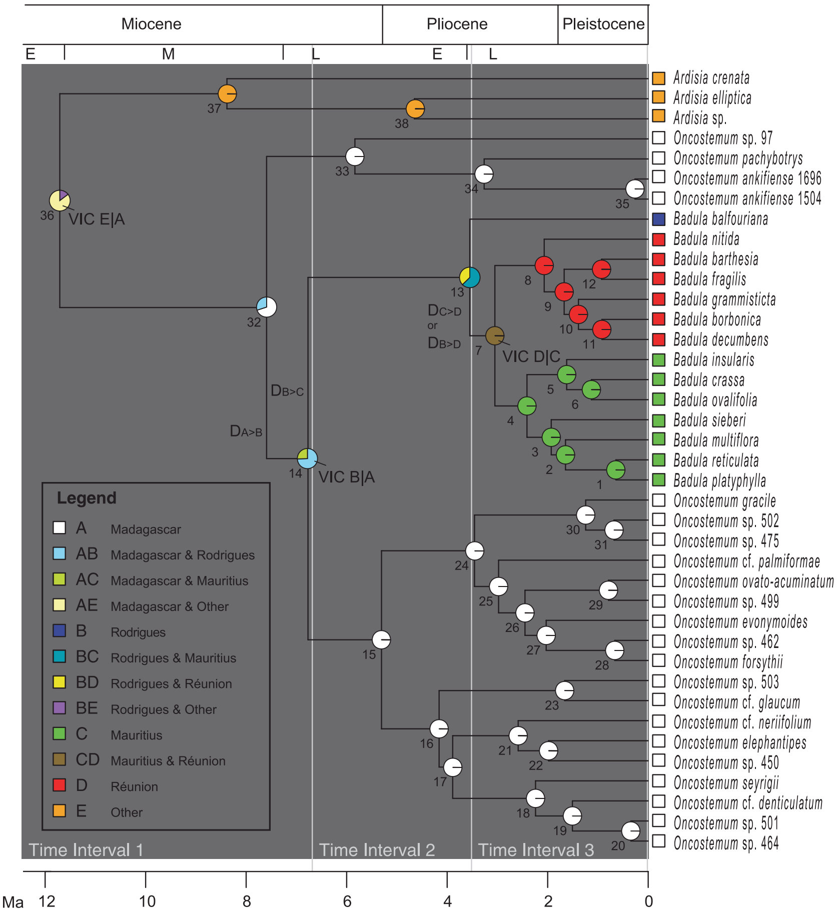

Figure 5 Dispersal–extinction–cladogenesis (DEC) scenario II ('*old Rodrigues*') for Indian Ocean Primulaceae, with results of the palaeogeographical model projected onto the maximum clade credibility tree created in BEAST. Pie charts represent ancestral area probabilities assigned at each node. Pie charts with a horizontal black line depict relative probabilities of $P = 1$ for the area indicated in colour; those with a vertical black line depict relative probabilities of $P < 0.1$ for the total of all areas other than that indicated in colour. Text inserts (D, dispersal; VIC, vicariance) follow most probable assignment, where applicable.

tree allow inference of an initial dispersal from Madagascar to Mauritius between 7.59 and 6.77 Ma (Fig. 4: node 32 to 14), followed by a dispersal from Mauritius to Rodrigues between 6.77 and 3.55 Ma (Fig. 4: node 14 to 13; late Miocene–early Pliocene) and finally a dispersal from Mauritius to Réunion between 3.55 and 3.05 Ma (Fig. 4: node 13 > 7). Under scenario II ($-\ln L = 24.4$), the LAGRANGE area assignments with highest probability allow inference of an initial dispersal from Madagascar to Rodrigues between 7.59 and 6.77 Ma, followed by a dispersal from Rodrigues to Mauritius between 6.77 and 3.55 Ma (Fig. 5: node 14 > 13) and finally a dispersal from Mauritius to Réunion between 3.55 and 3.05 Ma (Fig. 5: node 13 > 7).

DISCUSSION

Phylogenetic relationships and timing of diversification

Our analyses resolve the Indian Ocean Primulaceae as monophyletic within a broadly defined Primulaceae, and place the monophyletic Mascarene genus *Badula* as phylogenetically nested within the large Madagascan genus *Oncostemum*. The latter result corroborates that of Bone *et al.* (2012).

No matter whether we assumed a relatively old or young age for Rodrigues in our analyses, the age estimates for dispersal to Rodrigues (7.59–3.55 Ma) are substantially older than the single geological estimate available for the age of the island (*c.* 1.5 Ma) (McDougall *et al.*, 1965; Saddul *et al.*, 1995). One other recent study has also resolved Rodriguan species at basal positions (> 5–8 Ma) in Mascarene radiations (Strijk *et al.*, 2012; but see Cuenca *et al.*, 2008). When looking at the palaeographical modelling results, we find two different geographical pathways for dispersal and diversification, with very similar scores. Based on the latter, it is not possible to draw any solid conclusions on the historical range of the ancestral lineage of *Badula*, and the dispersal path used. Even so, complementary to the molecular dating results, the biological and physiogeographical features of Rodrigues are reminiscent of an island that is significantly older than 1–2 Myr, and are similar to those of volcanic islands in the later stages of their biological and geological evolution (Darwin, 1842; Whittaker & Fernández-Palacios, 2007; Whittaker *et al.*, 2007; Cheke & Hume, 2008). Regardless of the exact age of Rodrigues, and its unique features, we cannot discount the possibility that an ancestral *Badula* lineage may have once resided on the now submerged islands of the Mascarene Plateau. Such a distribution could explain older nodal estimates (see the example of Mascarene *Monimia*, diverging 32(16–48) Ma; Renner *et al.*, 2010). But because the differences in geological and molecular age estimates are relatively small in this case, testing such a hypothesis would require additional data on the extent and timing of emergence of islands on the Mascarene Plateau, as well as extensive sampling and dating of the deep basalts between Mauritius and Rodrigues, in order to better understand the origin of the latter. Only then can we hope to understand the transitory nature of the biota of such temporary islands, and the role they may have played in biotic assembly of the greater region.

Identifying underlying edaphic or climatic factors or morphological traits that might explain the geographical pattern of dispersal and diversification within islands has proven difficult in Mascarene *Badula*, partly due to the paucity of knowledge concerning pollination biology and seed dispersal. On Réunion, most species grow in cloud or submontane forest. Some have a more restricted range in the wettest part of the island (montane zone), whereas others occur more widely in mesic habitat in the western submontane zone. A correlation may exist between species distributions and the complex orographic effects found on volcanic islands such as the Mascarenes (Strasberg *et al.*, 2005; Bone *et al.*, 2012), which can be extreme. On Réunion, for example, the north-to-south orientation in the central mountain ranges (up to 3069 m a.s.l., casting a westward rain shadow) and very deep calderas have given rise to highly diverse ecological conditions over short distances and a patchwork of suitable habitats, providing ample opportunities for allopatric speciation. This appears to have affected speciation rates in ancestral *Badula* on Mauritius and Réunion shortly after dispersal to these islands (diversification rate shift A, Fig. 2). Despite their relatively recent origin, large differences in growth habit, leaf morphology and ecology occur among species of *Badula*, especially on Réunion.

Origin and dispersal of Indian Ocean Primulaceae

Phylogenetic, ancestral area and palaeogeographical reconstructions suggest the presence of a shared common ancestor of *Badula* and *Oncostemum* on Madagascar. Early dispersal into the Mascarene Basin, whether initially through Mauritius (scenario I) or Rodrigues (scenario II), precedes widespread and concurrent diversification in both *Badula* and *Oncostemum*. It appears that most ancestral lineages of Primulaceae on Madagascar did not begin to radiate until the Pliocene, which raises the question as to the causes for this apparent delayed onset of diversification. Dispersal to individual Mascarene Islands set in motion cladogenesis of unique clusters of *Badula* on each of the islands, exemplifying the speed with which speciation can occur in isolated, small areas with high habitat diversity.

The origin of the common ancestor of *Ardisia* and *Oncostemum* cannot be confidently concluded from this study because our outgroup sampling is limited and the genus *Ardisia* is species rich and widespread. There are about 200–350 recognized species of *Ardisia*, and they are spread throughout the warmer regions of Africa, Madagascar, the Americas, Asia and Australasia. Continental African *Ardisia* are restricted to the western side of the continent (Congo Basin and lower Guinea) (Cheek & Xanthos, 2012), which could make an African origin of Indian Ocean *Ardisia* seem less plausible. If the common ancestor of all Indian Ocean

Primulaceae dispersed into the Indian Ocean from the Asian mainland (at some time within the early to middle Miocene), it may have occurred through Madagascar or one of the Mascarene islands (Mauritius or Rodrigues) in either one single long-distance dispersal event or several followed by local extinction. Such a pattern would conform closely to previously reconstructed migration routes used by other taxa (Warren *et al.*, 2005; Micheneau *et al.*, 2008; Li *et al.*, 2009; Renner *et al.*, 2010). Stepping stones in the Indian Ocean (such as those in the currently submerged Mascarene Plateau) could have operated in both directions, and southern Asia (India) is likely to have played a key role in long-distance dispersal, as well as serving as a source and sink of migrating biodiversity in the basin. The total surface area of the exposed banks and the Mascarene Plateau might at times have been equivalent in size to Madagascar (Torsvik *et al.*, 2013).

Our study illustrates how successful biotic exchange throughout the Indian Ocean may seem impeded by immense distances and extreme cases of insular isolation, but could have happened repeatedly and allowed for the rapid evolution of unique lineages, on both geologically short-lived islands and ancient isolated continental fragments.

CONCLUSIONS AND PERSPECTIVES

We examined the biogeographical origins and evolutionary connections of species-rich lineages of Primulaceae in the Indian Ocean basin. The reconstructed chronogram reveals that the Indian Ocean Primulaceae are a monophyletic group, dating back to at least the early to middle Miocene, most likely stemming from long-distance dispersals into the basin through Madagascar, and from there, via a single long-distance dispersal event into the Mascarene Islands. We found that the sequence of geographical dispersal and diversification in *Badula*, a genus endemic to the Mascarene Islands, seems to correspond to the appearance of the islands forming this archipelago. Furthermore, our data indicate that dispersal to Rodrigues may have occurred at a time much older than the current geological age estimate of the island. As in many other studies on the Madagascan flora, recovered age estimates for *Oncostemum* confirm the relatively recent origin of many endemic and species-rich lineages in the region. A deeper understanding of the internal relationships within the paraphyletic *Oncostemum* may give insight into the factors that could explain the patterns of both lineage-splitting events and morphological and ecological diversification among Indian Ocean Primulaceae.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 GenBank accession numbers for taxa used in this study.

Appendix S2 Details of phylogenetic, calibration, diversification and palaeogeographical modelling analyses.

Appendix S3 Calibration scheme and results of estimation of divergence times.

BIOSKETCH

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