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ORIGINAL ARTICLE

Persistence and stability of Eastern Afromontane forests: evidence from brevicipitid frogs

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ABSTRACT

Aim The persistence and stability of habitats through time are considered predictors of high levels of biodiversity in some environments. Long-term habitat persistence and stability may explain the species-rich, endemic forest fauna and flora of the Eastern Afromontane Biodiversity Region (EABR). Using complementary phylogenetic and biogeographical approaches, we examine evolutionary patterns in EABR brevicipitid frogs. Using these data, we test whether brevicipitid history reflects patterns of long-term forest persistence and/or stability across the EABR.

Location East Africa.

Methods A dated phylogeny for brevicipitids was constructed using two nuclear and three mitochondrial markers. Alternative diversification models were used to determine signal for constant or varying net diversification rates. Using our dated tree, we identified areas of high phylogenetic diversity (PD), and inferred ancestral areas using likelihood and Bayesian approaches.

Results Brevicipitids have a long history, with generic diversification among extant lineages pre-dating the Oligocene (> 33 Ma). Ancestral-area reconstructions indicate the presence of brevicipitids in the EABR since the Oligocene, and support a scenario of palaeoendemics surviving in EABR refugia. Ancestral-area reconstructions indicate that the central Eastern Arc Mountains (EAM) formed the initial centre of diversification of forest brevicipitids. Measures of PD show that diversity varies across the EABR but is highest in the EAM. Constant net diversification rate in brevicipitids is a significantly better fit than alternative, rate-variable models.

Main conclusions The degree of persistence of forest habitats appears to be a contributing factor to the varying levels of diversity across the EABR in brevicipitids (and other organisms). In contrast to the Southern Highlands and Ethiopian Bale Mountains, the EAM stands out as an area that enabled the constant accumulation of brevicipitid species over a long period of time.

Keywords
Africa, ancestral area reconstruction, biogeography, Brevicipitidae, diversification models, diversification rates, Eastern Afromontane, forest persistence, phylogenetic diversity, radiation.

INTRODUCTION

The exceptionally biodiverse Eastern Afromontane Biodiversity Region (EABR) is highly fragmented (Fig. 1) and is considered to be among the most important areas for conservation globally (e.g. Mittermeier *et al.*, 2004). Patterns of biodiversity within the EABR are generally poorly understood, but local species richness varies substantially across

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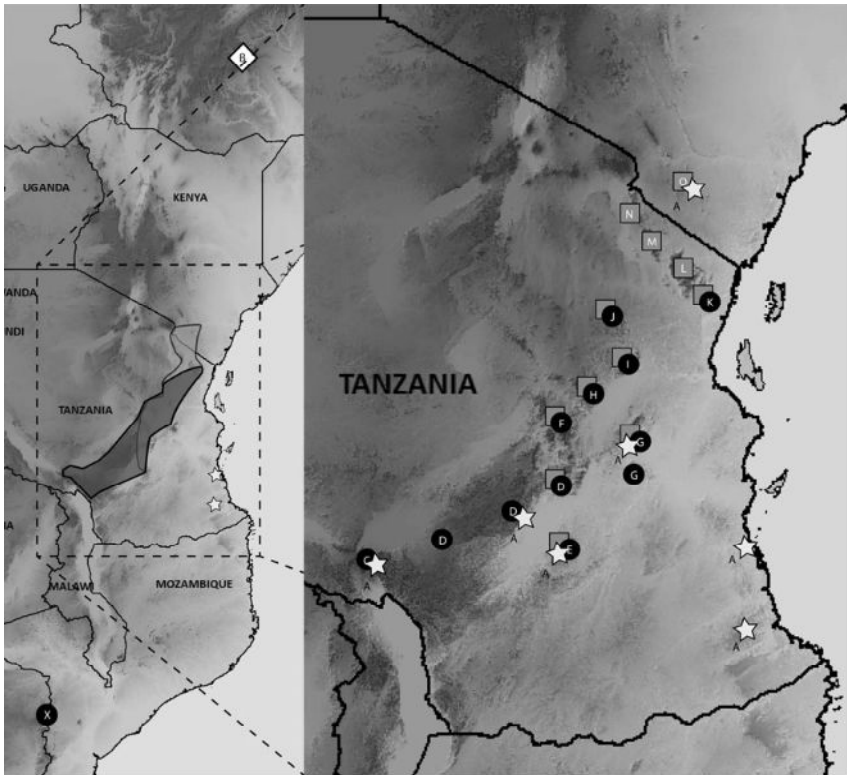


Figure 1 Topographical map of the Eastern Afromontane Biodiversity Region (EABR) (left) and with expanded area of Eastern Arc Mountains (right) showing the distribution of brevicipitids. Light grey squares, range of *Callulina*; dark grey circles, range of *Probreviceps*; grey, overlapping ranges of *Probreviceps* and *Callulina*; white diamond, *Balebreviceps* (Bale Mountains, Ethiopia); white star, *Spelaeophryne*. Species found in particular EABR fragments are as follows. A, Lowlands: *S. methneri*; B, Ethiopia: *Balebreviceps hillmani*; C, Southern Highlands: *P. rungwensis*, *S. methneri*; D, Udzungwa: *C.* 'lowland', *P. loveridgei*, *P. rungwensis*, *P.* 'kigogo', *S. methneri*; E, Mahenge: *C.* 'lowland', *P. rungwensis*; F, Rubeho: *C.* 'rubeho', *P.* 'rubeho'; G, Uluguru/Malundwe: *C.* 'lowland', *P. loveridgei*, *P. uluguruensis*, *S. methneri*; H, Ukaguru: *C.* 'lowland', *P. durirostris*, *P.* 'rubeho'; I, Nguru: *C. meteora*, *C. hanseni*, *C. kanga*, *C.* 'lowland', *P. macrodactylus*, *P.* cf. *durirostris* 'nguru'; J, Nguu: *C.* 'lowland', *P.* cf. *durirostris* 'nguu'; K, East Usambara: *C. krefftii*, *P. macrodactylus*; L, West Usambara: *C. kisiwamsitu*; M, South Pare: *C. stanleyi*, *C. shengena*; N, North Pare: *C. laphami*; O, Taita Hills: *C. dawida*, *S. methneri*; X, Zimbabwe: *P. rhodesianus*.

the region (Mittermeier *et al.*, 2004), with some areas holding disproportionate amounts of diversity (e.g. Eastern Arc Mountains; Lovett & Wasser, 1993; Burgess *et al.*, 2007). Various historical factors have been suggested as causes of local differences in diversity across the EABR (e.g. Lovett & Wasser, 1993; Burgess *et al.*, 2007). Generally, however, the most prevalent hypothesis is that higher diversity is explained by long-term persistence of habitat, as indicated by the presence of multiple 'old' lineages (Fjeldså *et al.*, 1999; Lovett *et al.*, 2005). This has, however, rarely been examined in detail across the geographically complex and fragmented EABR.

Palaeoenvironmental data have provided important insights into the persistence and stability of forest habitats in East Africa, including the EABR, during the last 150,000 years. Dramatic shifts between lowland and highland forest and savanna habitats have been documented across numerous sites in Malawi, western Tanzania, southern and western Uganda, Kenya and Ethiopia (e.g. Hamilton, 1982; DeBusk, 1998; Lamb *et al.*, 2007; Scholz *et al.*, 2007; Umer *et al.*, 2007; Tiercelin *et al.*, 2008). Interestingly, in contrast to most East African sites, rain forest habitats persisted in the Eastern Arc Mountains (EAM; at the heart of the EABR) even during the Last Glacial Maximum, although it should be noted that marked compositional assemblage changes have been detected in montane forest habitats (Mumbi *et al.*, 2008; Finch *et al.*, 2009; Finch & Marchant, 2010). Although only a few EAM areas have been sampled, such insights provide evidence to support the longer-term persistence of rain forest in at least some parts of the EABR.

Phylogenetic data have provided complementary information to the palaeoenvironmental records in East Africa (e.g. Janssens *et al.*, 2009; Ruiz Guajardo *et al.*, 2010). Such studies have confirmed the presence of palaeoendemics, but have also revealed species-rich radiations in forests (e.g. Lovett *et al.*, 2005; Dimitrov *et al.*, 2012). Because most palaeoenvironmental and/or phylogenetic studies thus far have been localized and focused mainly on the EAM (e.g. Dimitrov *et al.*, 2012), few patterns can be compared across areas within the EABR (Kebede *et al.*, 2007; Blackburn & Measey,

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2009; Ruiz Guajardo *et al.*, 2010; Tolley *et al.*, 2011). Further data on species radiations across the region will be important in gaining additional phylogenetic insights into the persistence and stability of habitats. Of particular significance are areas for which palaeoenvironmental data are absent or relatively incomplete, which is the case for large parts of the EABR.

In this paper, we study the frog family Brevicipitidae, in particular a clade comprising the genera *Balebreviceps*, *Callulina*, *Probreviceps* and *Spelaeophryne*, which are distributed across the EABR. Species of these brevicipitid genera are generally 'cool-preferring' (Poynton, 2000) and are therefore expected to show evolutionary patterns that reflect changes in the relative permanence of Afrotemperate forest habitats (Poynton, 2013). We test the hypothesis that habitat persistence and stability are major determinants of present-day forest species richness by investigating the historical biogeography of brevicipitids. In support of this hypothesis, we predict a combination of characteristics that might indicate long-term persistence and stability, including: old diversification dates; high phylogenetic diversity; constant rates of diversification; and the presence of EABR localities as areas of origin in ancestral-area reconstructions.

MATERIALS AND METHODS

Taxon sampling

The family Brevicipitidae contains five genera – *Balebreviceps*, *Breviceps*, *Callulina*, *Probreviceps* and *Spelaeophryne* – and is concentrated in the Eastern Afromontane region and South Africa. Specimens of East African brevicipitids were obtained by fieldwork in Tanzania and Ethiopia between 2000 and 2012 (see Appendix S1 in Supporting Information). For EABR brevicipitids, we sampled multiple populations of all known species of the family Brevicipitidae except for *Probreviceps rhodesianus*, and a *Callulina* population from the Shimba Hills that might be a new species (Loader *et al.*, 2010a). We surveyed all EAM for brevicipitids and included samples of all known populations (Fig. 1). *Balebreviceps hillmani* was collected from the Bale Mountains, Ethiopia, the only known locality (Gower *et al.*, 2013). Two species in the genus *Breviceps* occur in the EABR and these were also included. The monophyly of *Breviceps* is strongly supported by molecular (e.g. Loader *et al.*, 2004, 2009; Frost *et al.*, 2006; Roelants *et al.*, 2007) and morphological (Parker, 1934) evidence, and we assume that the incomplete sampling of *Breviceps* is unlikely to unduly influence the results of our study (Appendix S1).

Phylogenetics

Alignments of partial 12S, 16S, cytochrome *b* (*cytb*), recombination-activating gene 1 (*rag1*) and chemokine (CXC motif) receptor 4 (*cxcr4*) sequences were assembled based on previously published (Loader *et al.*, 2006, 2010a,b; Menegon *et al.*, 2011) and newly generated data. We sampled all specimens for three mitochondrial gene sequences and sampled seven representatives of the main EABR lineages for *rag1* and *cxcr4* (Appendix S1). Extraction, amplification and sequencing followed protocols reported elsewhere (Loader *et al.*, 2004; Roelants *et al.*, 2007). We produced two alignments: one including all data, and a second, reduced-taxon dataset comprising all samples with complete nuclear and mitochondrial data partitions (i.e. representatives of the main lineages). The methods for alignment construction are given in Appendix S1 together with voucher and GenBank accession numbers.

The datasets were analysed with maximum likelihood (ML) and Bayesian inference (BI) using the Bioportal at the University of Oslo (http://www.bioportal.uio.no/). ML analyses were conducted with RAxML 7.0.4 (Stamatakis, 2006) using the rapid hill-climbing algorithm and the GTRGAMMA substitution model (Stamatakis *et al.*, 2007). For BI we used MrBayes 3.2.1 (Ronquist *et al.*, 2012) with parallel runs of four simultaneous Markov chains for 10 million generations, sampling every 1000 generations, and discarding the first one million generations as burn-in to prevent sampling before reaching stationarity, determined using Tracer 1.5 (Rambaut & Drummond, 2007). Two independent parallel BI runs were performed to check for convergence, using the default settings. For both ML and BI analyses, model parameters were independently optimized for each partition ('un-link' option in effect). Support for groupings was evaluated by nonparametric bootstrapping (Felsenstein, 1985) with 1000 replicates, performed with RAxML (ML) (Stamatakis *et al.*, 2008), and with posterior probabilities (BI) (Ronquist *et al.*, 2012).

Biogeography

Ancestral areas and events were reconstructed using Markov chain Monte Carlo (MCMC), statistical dispersal–vicariance analysis (S-DIVA) and dispersal–extinction–cladogenesis (DEC) algorithms (see below). The first two approaches can use either multiple phylograms (i.e. account for topological uncertainties, as described above) or an ultrametric tree, whereas the DEC algorithm optimally requires trees with node ages. A time-calibrated tree was reconstructed by BI using the program beast 1.7.4 (Drummond *et al.*, 2012) and running the MCMC simulation for 10 million generations, sampling trees every 1000 generations (see Appendix S1 for further details of the analyses and calibration points).

Ancestral areas were reconstructed using a Bayesian method implemented in the program rasp 2.1 (Nylander *et al.*, 2008; Yu *et al.*, 2013). Bayesian binary MCMC analyses were set to 100,000 cycles, 10 chains, estimated state frequencies and gamma among-site rate variation, implementing codes for either 15 different areas or seven different areas (see Appendix S1 for further details). The seven-area coding was applied so that we could directly compare reconstructions from rasp and Lagrange (see below). These areas

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were defined on the basis of 12 EAM fragments (currently discontinuous rain forests), combining North + South Pare, and Malundwe + Uluguru (on the basis of close geographical proximity). In addition, some large areas were collectively grouped into single, composite areas: the Southern Highlands (Madehani and Rungwe), Ethiopian Highlands (Bale Mountains) and East African lowlands (Rondo plateau, Kilombero Valley, and other sites adjacent to EAM forest localities). The East African lowlands are environmentally heterogeneous, including forest and non-forest habitats, but contain a distinctive amphibian community (Poynton *et al.*, 2006; Müller *et al.*, 2013). Because of the extensive lowland area across East Africa, and its relatively poor geographical sampling in this study, a direct comparison with EABR areas was not undertaken or relevant to the focus of the paper. We also performed a statistical dispersal–vicariance analysis (S-DIVA), using the default settings, by setting the maximum number of ancestral areas to four. Both MCMC and S-DIVA analyses were carried out with the chronogram obtained from BEAST, as well as the last 3000 trees obtained from the MRBAYES analysis.

We carried out DEC analyses on the time-calibrated tree obtained from BEAST using LAGRANGE 20120508 (Ree & Smith, 2008). The input files for LAGRANGE were assembled using the web-based configurator (http://www.reelab.net/lagrange/configurator/index) and the area codes used for the DEC analyses are given in Appendix S1. Software constraints limit the maximum number of areas that can be defined to seven (Appendix S1). Four scenarios with different dispersal probabilities between areas at various time intervals and different numbers of maximum ancestral areas were reconstructed and their likelihoods compared. The probability of dispersal between areas was lowered with increasing geographical distance, independently of the time. Additionally, time-dependent dispersal constraints were enforced, based on periods of aridification and geological activity (Appendix S1) (Couvreur *et al.*, 2008).

Diversification rates

To investigate diversification rates in brevicipitids, we used the complete time-calibrated ultrametric tree obtained from BEAST reduced to one representative per species and removing the outgroup taxa and *Breviceps* individuals, leaving a total of 27 terminal taxa. Diversification-rate analyses were carried out with the LASER package (Rabosky, 2006) in R 2.10.1 (R Development Core Team, 2009), first determining the overall diversification rates under no ($\varepsilon = 0$) and high ($\varepsilon = 0.9$) extinction rates. Next, ΔAIC (difference in values of the Akaike information criterion of two models) values were calculated for our phylogeny compared to five different models of diversification (two constant-rate models, 'pure birth' and 'birth–death', as well as three rate-variable models: exponential and linear density dependent models 'DDX' and 'DDL' and two-rate Yule model). Ten thousand trees were simulated under a Yule speciation model, to obtain a *P*-value for the diversification rates of our phylogeny. We also fitted the 'SPVAR', 'EXVAR' and 'BOTHVAR' models, alternatively employing time-varying speciation and constant extinction, time-varying extinction and constant speciation, and time-varying speciation and extinction, to investigate which model best explains our data (Rabosky & Lovette, 2008). The gamma statistic (Pybus & Harvey, 2000) was also applied, to check whether diversification rates have changed over time. Our data include almost all species for the genera in question, and it was therefore not necessary to investigate the effect of missing species on diversification rates. To visualize the accumulation of lineages over time in the brevicipitid phylogeny, we compared 10,000 simulated trees (generated under a pure-birth model), and lineages-through-time (LTT) plots were drawn in the R package APE (Paradis *et al.*, 2004).

Phylogenetic diversity

We estimated Faith's phylogenetic diversity (PD; Faith, 1992) for the same areas that were assigned for the RASP and DEC analyses, using the time-calibrated tree obtained from BEAST. PD was estimated using a script written by R. Greyner (University of Oxford) in APE. To obtain a null model for testing whether the PD of any given area was different from that expected by chance, 10,000 randomizations of subsets of phylogenetic trees were carried out, obtaining quantiles (q) of the randomized PD distribution for significance testing (Forest *et al.*, 2007; Kembel *et al.*, 2010). To visualize PD across EABR, we used the software BIODIVERSE (Laffan *et al.*, 2010).

RESULTS

Phylogenetics

The Bayesian and ML trees reconstructed for all 83 ingroup brevicipitids display five major lineages, corresponding to the genera *Balebreviceps*, *Breviceps*, *Callulina*, *Probreviceps* and *Spelaeophryne* (Fig. 2). The analyses provide generally strong support for the relationships (*Breviceps*, (*Balebreviceps*, (*Spelaeophryne*, (*Callulina*, *Probreviceps*)))). The position of *Spelaeophryne* is, however, not well supported, with weak bootstrap (< 50%) and posterior-probability (0.71) support for the clade comprising it, *Callulina* and *Probreviceps*. Analyses of the reduced dataset with representative samples of each major lineage including all mitochondrial and nuclear data (Appendix S1) provide stronger support for the same set of relationships, including high posterior probability (1.0) and moderate ML bootstrap support (87%) for the *Spelaeophryne* + *Callulina* + *Probreviceps* clade.

The genera *Spelaeophryne* and *Balebreviceps* are currently monotypic. Sampling across a large geographical range for *S. methneri* (Uluguru, Mahenge, Rondo Plateau and Kilombero Valley) did not reveal genetic variation comparable to that within the genera *Callulina* and *Probreviceps*. *Balebreviceps* is known from only four sites in Harenna forest (Gower *et al.*, 2013)

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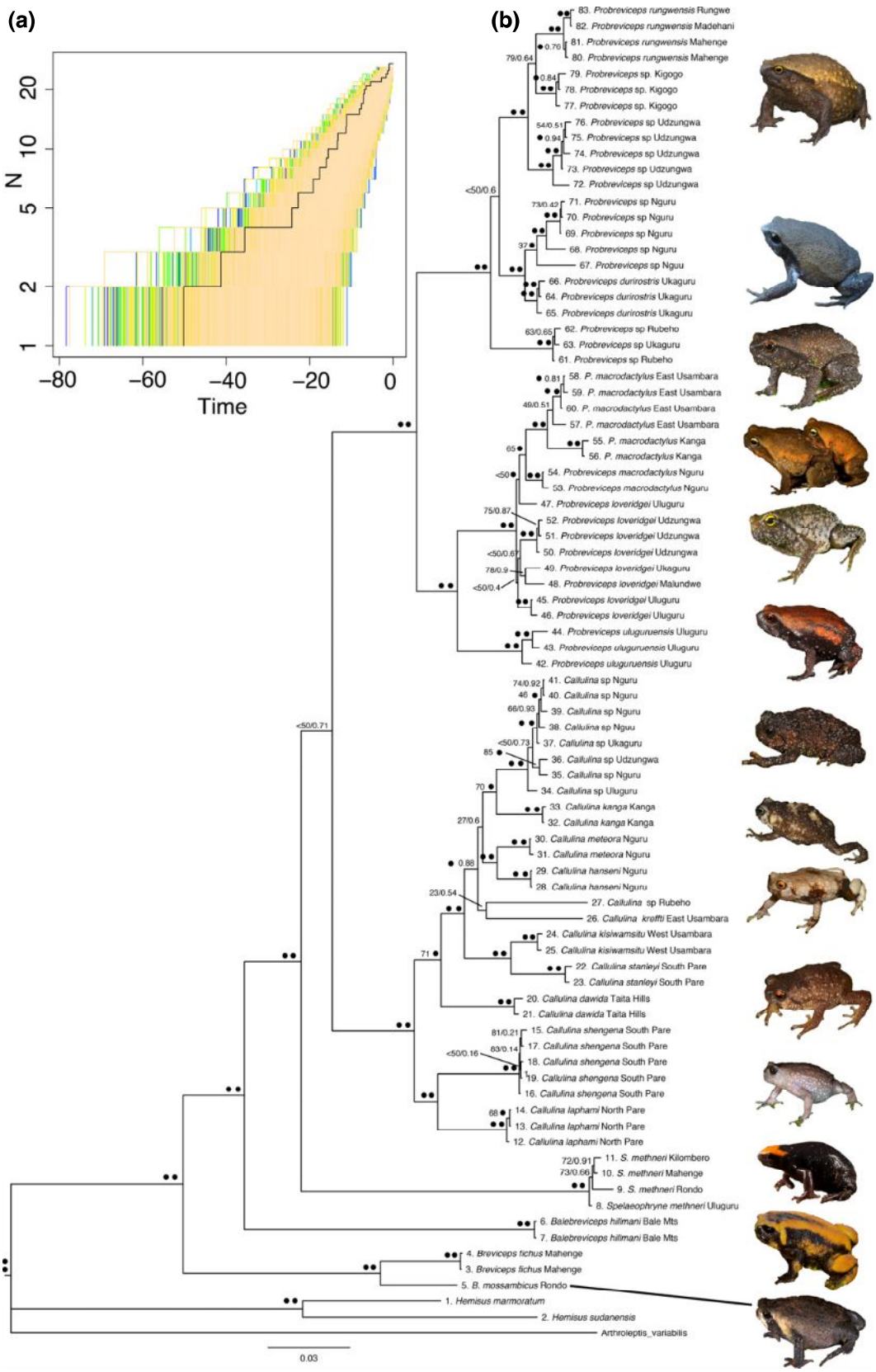


Figure 2 (a) Lineages-through-time plot of 10,000 simulated Yule-process trees (coloured lines) of brevicipitid species used in the diversification rate analyses; (b) Bayesian consensus phylogram for brevicipitids. Node support is shown by maximum-likelihood (ML) bootstrap values and posterior probabilities above each branch. Solid black circles indicate branch support > 90% for RAxML, and > 0.95 for Bayesian posterior probabilities.

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Table 1 Output from LASER diversification analyses for maximum-likelihood ΔAIC test statistic for brevicipitid data from the Eastern Afromontane Biodiversity Region.

Model	*r*1	*r*2	Model parameters	−LH	AIC	ΔAIC
Rate-constant (rc) and variable-rate models						
Pure birth	0.06124014			33.56211	69.12422	0
Birth–death	0.05445070		0.1864549 (*a*)	33.52542	71.05085	−1.93
Yule 2-rate	0.02521620	0.0699269	24.44377 (*st*)	32.28585	70.57170	−1.45
DDX	0.05481209		−0.04549467 (*x*)	33.54734	71.09469	−1.97
DDL	0.06554311		217.1553 (*k*)	33.54633	71.09265	−1.97
$\Delta AIC_{rc} = -1.447475$; $n = 27$; $P = 0.6583342$						
Variable speciation/extinction models						
SPVAR				33.55332	73.10664	−3.98
EXVAR				33.53023	73.06046	−3.94
BOTHVAR				33.55332	75.10664	−3.98

*r*1, rates (in lineages per Myr) of the first model; *r*2, rates (in lineages per Myr) of the second model (for Yule 2-rate only); LH, log-likelihood; AIC, Akaike information criterion.

and we recovered no genetic variation in our limited geographical sampling at one site. In contrast to the very limited extant intrageneric genetic diversity exhibited in *Spelaeophryne* and *Balebreviceps*, both *Callulina* and *Probreviceps* are substantially more diverse (Appendices S1 & S2).

Analysis of the full dataset yielded a tree with divergence estimates similar to those produced from a reduced dataset (Appendix S1). The larger dataset was used to estimate PD (see below). The main patterns of diversification among the major lineages are not altered substantially by the data or the calibrations used. The dating estimates using the complete dataset (mitochondrial and nuclear datasets) are slightly older than those based on a nuclear-only dataset, but the differences are not substantial (Appendix S1). Importantly, the chronogram supports the existence of all the major lineages (genera) of East African brevicipitids across a long timescale, at least over the past 30 Myr (Appendix S1).

The net accumulation of lineages over time is shown in the LTT plots (Fig. 2a). The net diversification rate for brevicipitids (excluding *Breviceps*) was found to be 0.053 Myr^{-1} assuming no extinction, and 0.026 Myr^{-1} assuming high rates of extinction ($\varepsilon = 0.9$). Based on the likelihood-ratio tests, our data fit a pure-birth model better than a birth–death model, and the data are better explained by models with variable extinction (EXVAR) than with variable speciation (SPVAR) rates (Table 1). The negative ΔAIC_{rc} (rate-constant ΔAIC) value obtained indicates that our data are best explained by a constant (rather than variable) rate model. When our data were compared to 10,000 Yule-process-simulated trees, the difference was not significant ($P = 0.66$). Based on the gamma statistic (calculated gamma = 0.169; $P = 0.57$) diversification rates in brevicipitids have not decreased over time.

Biogeography

The DEC run with the highest log-likelihood score ($-\ln L = 66.33$) in LAGRANGE analyses had the maximum number of areas set to three (allowing for the ancestral area to comprise up to three of our coded areas), and only distance-based dispersal constraints (as opposed to distance- and time-based constraints) (Appendix S1). With areas coded into seven regions, the overall LAGRANGE analysis detected 23 instances of vicariant speciation, five dispersal events and no extinctions. The MCMC analysis carried out in RASP using the same number of regions identified 12 vicariant speciation events, 29 dispersal events, and one extinction event (for further details, see Appendices S1 & S3). In summary, the area reconstructions using seven regions in RASP and LAGRANGE indicate a lowland ancestral area for brevicipitids with diversification events of the most recent common ancestor (MRCA) of *Callulina* and *Probreviceps* focused in the central EAM region and subsequent dispersal into northern areas of the EAM (parts of the *Callulina* radiation) (Fig. 3a). The main source of conflict between the results from the two methods is the split between lowland *Breviceps* and Ethiopian *Balebreviceps* – reconstructed as being either lowland or as a vicariance event between Ethiopia and highland Tanzania. This conflict occurs because LAGRANGE identifies highland Tanzania as part of the ancestral area at an earlier time than RASP does (where the lowlands are recovered as the main ancestral area of the most recent common ancestor of *Balebreviceps* and the remaining brevicipitids, albeit with a low probability).

The MCMC analysis carried out in RASP using 15 areas identified 31 events of vicariance, 63 of dispersal, and a single extinction (Appendix S1). The ancestral areas reconstructed using RASP mostly lie within the more general areas identified using larger block coding in the LAGRANGE and RASP analyses (Appendix S1). Overall, the RASP reconstruction using 15 areas reconstructs similar patterns and ancestral areas to those using seven areas, but provides more precision for the geographical location within the central EAM (e.g. Nguru and Uluguru) area where the initial diversification of the extant forest lineages occurred.

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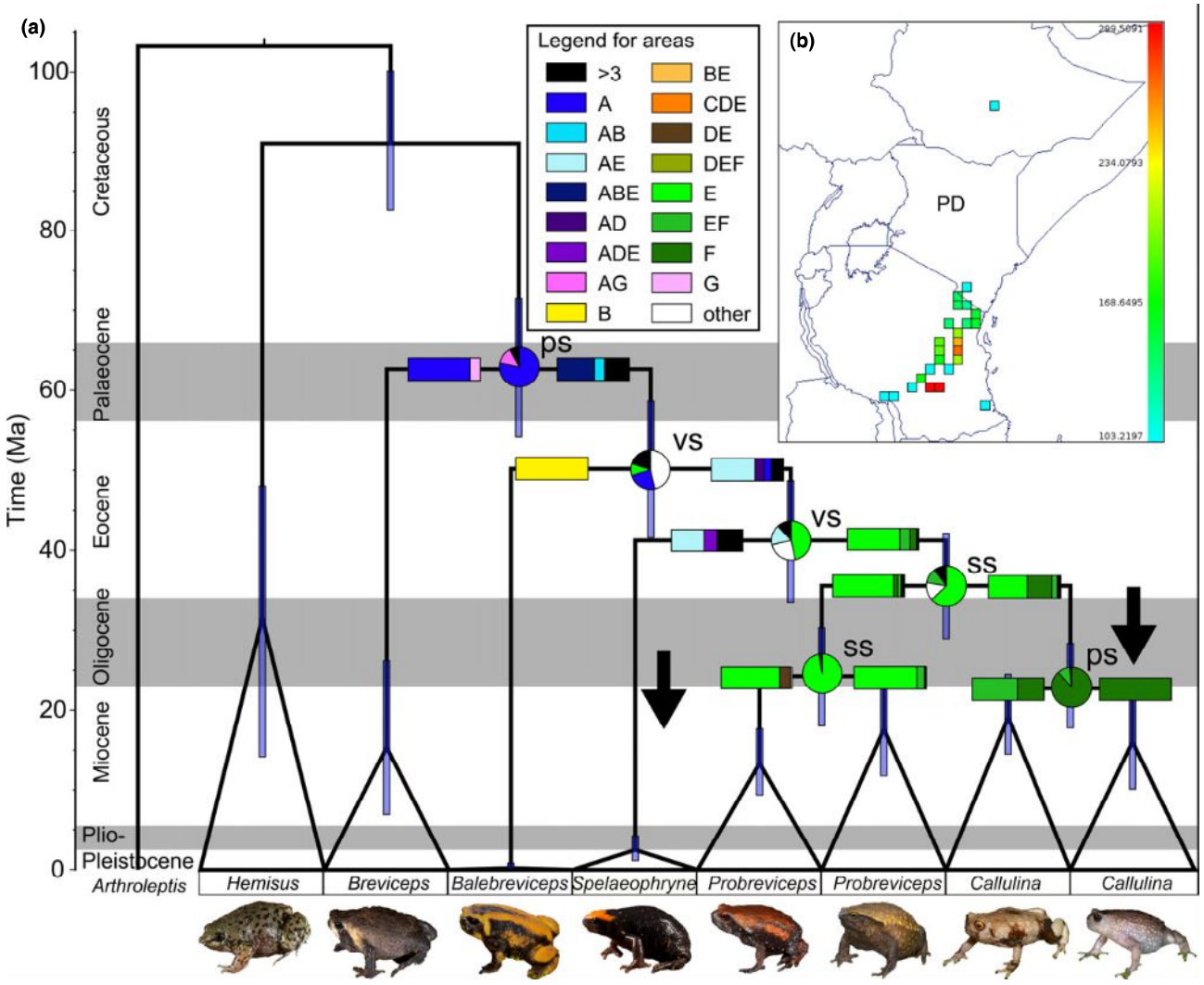


Figure 3 (a) Time-calibrated ultrametric tree of major brevicipitid lineages and their ancestral-area reconstructions, with 95% confidence intervals at nodes (blue bars). Pie charts at nodes represent the ancestral areas determined by the RASP MCMC analysis on multiple Bayesian trees. Coloured rectangles on the branches represent the descendant areas for each node as relative probabilities obtained from LAGRANGE. Areas are colour-coded as in the legend (A, Lowlands; B, Ethiopia; C, Southern Highlands; D, Udzungwa/Mahenge; E, Rubeho/Uluguru/Malundwe/Ukaguru/Nguru/Nguu; F, East Usambara/West Usambara/South Pare/North Pare/Taita Hills; G, Southern Africa). Two-letter codes at nodes represent the cladogenetic events (vs, speciation through vicariance; ss, sympatric speciation; ps, peripatric speciation) and black arrows represent major dispersal events. (b) Distribution of brevicipitid phylogenetic diversity across the eastern Afromontane biodiversity region with colour codes corresponding to the scale to the right of the map (see Appendix S1 for further details).

Phylogenetic diversity

Across East Africa, brevicipitid PD is highest in the lowlands, North and South Pare, West Usambara, Nguu, Nguru and Ukaguru Mountains (Table 2, Fig. 3b). Lower brevicipitid PD occurs in Ethiopia, the Taita Hills, Rubeho, Mahenge and the Southern Highlands. Two-tailed comparison of observed PD and a randomized distribution indicates that most areas have lower PD than expected by chance ($P < 0.01$) (Table 2). Exceptions to this are West Usambara, which has an expectedly low PD, and the lowlands, which has higher PD than expected.

DISCUSSION

Biological exploration of the EABR, which began over a century ago, has revealed a remarkable array of species, with some highly distinctive and divergent taxa (Gregory, 1896; Barbour & Loveridge, 1928; Loveridge, 1933, 1937; Moreau, 1935, 1963; Basilewsky, 1976; partly summarized by Lovett & Wasser, 1993). An ancient ancestry was suggested for some taxa because their closest living relatives are from distant areas and/or they are morphologically divergent (Loveridge, 1933; Moreau, 1963; Lovett & Wasser, 1993; Dinesen *et al.*, 1994; Lovett *et al.*, 2005). Such observations were of biogeo-

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Table 2 Faith's phylogenetic diversity (PD; from highest to lowest) for brevicipitids in the Eastern Afromontane Biodiversity Region (also see Fig. 3b). *q*, significance value obtained from the quantile of randomized PD distributions.

Area	PD	*q*
Lowlands	409.74	0.8
Nguu	315.15	0.025
Ukaguru	305.47	0.005
West Usambara	283.48	0.2
Pares	282.14	0.005
Nguru	279.01	0.01
Malundwe	244.85	0.005
East Usambara	242.91	0.025
Uluguru	242.49	0.025
Udzungwa	231.41	0.005
Southern Highlands	207.72	0.005
Rubeho	207.31	0.05
Mahenge	206.82	0.005
Taita Hills	206.72	0.005
Ethiopia (Bale Mountains)	206.63	0.005

graphical significance, because they prompted the idea that the EABR is old, and that this might explain the high biological diversity of the region. Our phylogenetic study provides a quantitative assessment of these earlier speculations for a particular lineage.

All extant non-*Breviceps* brevicipitids (a monophyletic group) are taxa of moist forests. This, combined with the phylogenetic results (which show little evidence within lineages for locality-switching or widespread distributions), dating estimates and LTT plots, suggests that (1) brevicipitid lineages have been steadily accumulating in the EABR over the last 30 Myr, and (2) that this implies that moist forest habitats have persisted in the EABR for at least this long. Of course, habitat tolerances and preferences can change substantially during evolution (see also Tolley *et al.*, 2011), but there is no evidence for such changes in this case, and palaeoenvironmental data also provide support for the persistence of some moist forest in the EABR over this period (e.g. Mumbi *et al.*, 2008). Overall, this evidence supports the hypothesis that forest habitats in the EABR, and EAM in particular, have persisted for a long time (Lovett *et al.*, 2005). The long-term, constant accumulation of diversity also supports the view that this environmental history might explain the current high species diversity across other taxonomic groups.

The three main EABR regions from which brevicipitids were sampled in this study (EAM, Southern Highlands and Bale Mountains) have uneven levels of brevicipitid diversity (Appendix S1). PD values for brevicipitids in Ethiopia (Bale) and the Southern Highlands highlight lower net diversification rates (Table 2, Fig. 3b) in these regions than in most areas in the EAM. Beyond brevicipitids, there are numerous other EABR taxa that are more species-rich in the EAM than in Ethiopia (e.g. for amphibians, compare Howell, 1993; Largen, 2001). In contrast to the EAM, palaeoenvironmental data (Lamb *et al.*, 2007; Tiercelin *et al.*, 2008) and tectonic and volcanic activity (summarized by Corti, 2009) indicate that Ethiopian forest habitats have not remained stable (and might not have persisted) throughout the last 30 Myr. Overall, the available evidence points to variation in the persistence and stability of forest habitats across the EABR, with a positive correlation between environmental persistence and extant species richness, supportive of a causal link.

The Southern Highlands have a different history to that of the nearby EAM – being affected in parts by recent periods of volcanism (Harkin, 1960) – and thus potentially provides an interesting test of the biotic impact of habitat persistence and stability. Our phylogenetic results indicate that the single extant brevicipitid in the Southern Highlands region (*Probreviceps rungwensis*) is the product of a recent divergence and dispersal from an EAM ancestor. Thus, there is no evidence for brevicipitids having been in the Southern Highlands for more than 1.22 Myr, though it is not immediately clear whether this can be attributed to a lack of habitat persistence/stability or to restricted opportunities for dispersal from the more central parts of the EAM. Although the Southern Highlands are generally species-rich (Bjørndalen, 1992; Lovett & Wasser, 1993), suggesting some degree of habitat persistence/stability, recent dispersals from adjoining areas, for example the EAM (Gravlund, 2002; Davenport *et al.*, 2006; Fjeldså *et al.*, 2006; Roberts *et al.*, 2010), Livingstone Mountains (Lawson, 2013) and Malawi Highlands (Fjeldså *et al.*, 2006; Lawson, 2010), might explain at least some of this diversity. Further understanding of the biodiversity and biogeography of the Southern Highlands will require additional palaeoenvironmental data and phylogenetic studies of more taxa.

Within the EAM, brevicipitid species richness and phylogenetic diversity are uneven, with PD estimated to be significantly less than expected by chance in all regions except West Usambara. This is interpreted as lineages surviving for a long time without accumulating as much extant diversity as expected. Similar patterns have also been recovered for EAM chameleons, and this has been interpreted to be indicative of palaeoendemic lineages (Tolley *et al.*, 2011). In contrast, Tolley *et al.* (2011) found particularly high values of PD in chameleons across locations closer to the coastal margin (e.g. East Usambara, Nguru and Uluguru) and speculated that this was caused by more climatically stable habitats there. Our study of brevicipitids shows that PD in this group is higher in the North and South Pare, West Usambara, Nguu, Nguru and Ukaguru. The limited degree of congruence in PD between our study and that of Tolley *et al.* (2011) is difficult to interpret, given that the two studies are of relatively small, single radiations, each with their idiosyncratic histories. Larger-scale multitaxon sampling is required to test the broader patterns of phylogenetic diversity across this region, but our results indicate potential differences that might be of relevance to phylogenetic and palaeoenvironmental studies.

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Despite differences between precise area relationships among various EAM taxa, reconstructions of ancestral areas show congruence among various studies (e.g. Lindqvist & Albert, 2001). Relatively higher PDs and the predominance of central EAM fragments in ancestral-area reconstructions for brevicipitids might both reflect the long-term persistence of lineages in the central EAM (as also indicated by palaeoenvironmental data – Finch *et al.*, 2009). Other factors that might be important when comparing diversity and diversification in central and marginal EAM areas include the availability of nearby refugia, the size of habitat fragments (Burgess *et al.*, 2007), climatic instability (Burgess *et al.*, 2007), volcanic effects at the EAM margins (Loader *et al.*, 2011) and potential mid-domain effects (Colwell & Lees, 2000).

CONCLUSIONS

Our analysis has identified wide variation in the phylogenetic diversity of forest brevicipitids across the EABR. The areas with the highest diversity and longest evolutionary history correspond to locations previously identified as areas where forest habitats are likely to have persisted over a long period of time. The EAM has famously been dubbed the 'oldest rainforest in Africa' (Lovett *et al.*, 2005) and our data, along with other lines of evidence, are consistent with this and its implications for diversification. In contrast, Ethiopia (Bale Mountains) is an area where environmental instability might have limited the net constant diversification of lineages, such that its amphibian fauna is characterized by several distinct, but not diverse palaeoendemics. Within the EABR, persistent forests have accumulated more diversity than regions with intermittent or less stable forests.

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SUPPORTING INFORMATION

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

Appendix S1 Taxon sampling, species delimitation and phylogenetic and biogeographical analyses.
Appendix S2 Genetic pairwise differences in brevicipitids.
Appendix S3 LAGRANGE reconstruction of nodes on brevicipitids tree.

BIOSKETCH

Simon Loader is head of the Historical Biogeography and Systematics research group at the University of Basel, Switzerland. His interests include historical biogeography and systematics, particularly of African amphibians.

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