Mountain colonisation, miniaturisation and ecological evolution in a radiation of direct developing New Guinea Frogs (*Choerophryne*, Microhylidae)

Paul M. Oliver[1, 2], Amy Iannella[3], Stephen J. Richards[4], Michael S.Y. Lee[4,5]

[1] *Division of Evolution and Ecology, Research School of Biology & Centre for Biodiversity Analysis, The Australian National University, Building 116, Daley Road, Canberra, Australian Capital Territory 2061, Australia.*

[2] *Department of Zoology, University of Melbourne, Parkville, Vic 3052, Australia*

[3] *School of Biological Sciences, University of Adelaide, Adelaide SA 5005, Australia*

[4] *South Australian Museum, North Terrace, Adelaide SA 5000, Australia.*
[5] *School of Biological Sciences, Flinders University, GPO Box 2100*
Adelaide SA 5001, Australia.

Corresponding author: Paul Oliver. Research School of Biology, Australian National University, Building 116, Canberra, 2601, Australia.
Email: Paul.oliver@anu.edu.au

Running Header: Mountains and Miniaturised Frogs

ABSTRACT

Aims. Mountain ranges in the tropics are characterised by high levels of localised endemism, often-aberrant evolutionary trajectories, and some of the world's most diverse regional biotas. Here we investigate the evolution of montane endemism, ecology and body size in a clade of direct-developing frogs (*Choerophryne,* Microhylidae) from New Guinea.

Methods. Phylogenetic relationships were estimated from a mitochondrial molecular dataset using Bayesian and maximum likelihood approaches. Ancestral state reconstruction was used to infer the evolution of elevational distribution, ecology (indexed by male calling height), and body size, and phylogenetically corrected regression was employed to examine the relationships between these three traits.

Results. We obtained strong support for a monophyletic lineage comprising the majority of taxa sampled. Within this clade we identified one subclade that appears to have diversified primarily in montane habitats of the Central Cordillera (> 1000 m a.s.l.), with subsequent dispersal to isolated North Papuan Mountains. A second subclade (characterised by moderately to very elongated snouts) appears to have diversified primarily in hill forests (< 1000 m a.s.l.), with inferred independent upwards colonisations of isolated montane habitats, especially in isolated North Papuan Mountains. We found no clear relationship between extremely small body size (adult SVL less than 15mm) and elevation, but a stronger relationship with ecology – smaller species tend to be more terrestrial.

Conclusions. Orogeny and climatic oscillations have interacted to generate high montane biodiversity in New Guinea via both localised diversification within montane habitats (centric endemism) and periodic dispersal across lowland regions (eccentric

Mark D. Scherz 31/12/2016 12:07
Deleted: .

endemism). The correlation between extreme miniaturisation and terrestrial habits reflects a general trend in frogs, suggesting that ecological or physiological constraints limit niche usage by miniaturised frogs, even in extremely wet environments such as tropical mountains.

Keywords Central Cordillera, endemism, montane cradle, montane museum, North Papuan Mountains, terrestrial

INTRODUCTION

Tropical mountains contain some of the most diverse regional biotas in the world, with high levels of localised endemism and often fine elevational turnover in biodiversity (Mayr & Diamond, 1976; Fjeldså et al., 2012; Merckx et al., 2015; Rosauer & Jetz, 2015). The origins of, and processes shaping, this exceptional diversity are of great scientific interest, both for improved understanding of the drivers of biological diversity (Janzen, 1967; Hutter et al., 2013; Graham et al., 2014), and for understanding how these highly diverse biotas will be affected by anthropogenic climatic change (Williams et al., 2003; La Sorte & Jetz, 2010; Freeman & Class Freeman, 2014).

Two broad paradigms to explain high diversity in tropical mountains have been advanced (Fjeldså et al., 2012), and both received support from different studies: a) mountain uplift and climatic change have driven local speciation (the ‘cradle’ hypothesis) (Weir, 2006; Price et al., 2014), or b) mountains have provided refugia, often for specialised taxa that would have otherwise died out due to competition or climatic change (the ‘museum’ hypothesis) (Hutter et al., 2013). In a recent study focused on understanding the biogeographic origins of montane endemics, Merckx et al. (2015), suggested they could also be broadly dichotomised into centric endemics (derived from upslope colonisation of lowland taxa) and eccentric endemics (derived via long distance colonisation of cool adapted taxa).

The large tropical island of New Guinea has arguably the ‘most complex orogeny in the world’ (Baldwin et al., 2012). The collision of the leading edge of the northwards-moving Australian plate with the westwards-moving southern edge of the

Mark D. Scherz 31/12/2016 12:10
Deleted:

Mark D. Scherz 31/12/2016 12:10
Deleted:

Mark D. Scherz 31/12/2016 12:10
Deleted: ,

Mark D. Scherz 31/12/2016 12:10
Deleted:

Mark D. Scherz 31/12/2016 12:10
Deleted: ,

Mark D. Scherz 31/12/2016 12:11
Deleted: also

Pacific Plate has uplifted a high Central Cordillera (> 4000 m a.s.l.) extending nearly the length of the island (Baldwin et al., 2012) (Fig. 1). These ranges may date from the late Miocene, and high elevation habitats are even younger (Hall, 2002; van Ufford & Cloos, 2005; Baldwin et al., 2012). Beginning in the Miocene, and continuing with the ongoing rapid uplift of the Huon and Finnisterre Ranges (Fig. 1), additional smaller and more isolated montane regions scattered along northern New Guinea are the uplifted remnants of island arcs that have accreted onto the northern edge of the Australian plate (Hall, 2002; Polhemus, 2007).

The biota of New Guinea has been profoundly shaped by this complex orogeny. The uplift of the Central Cordillera has largely isolated the biotas of lowland regions to the north and south of New Guinea (Rawlings & Donnellan, 2003; Unmack et al., 2013; Georges et al., 2014). It has also been suggested that emerging elevation gradients may have increased speciation rates in some New Guinea radiations, inflating regional alpha diversity (Toussaint et al., 2013, 2014), a species pump model similar to the uplift of the northern Andes (Weir, 2006; Santos et al., 2009). In contrast the endemic montane fauna of the smaller, younger and more isolated mountains of northern New Guinea is particularly poorly known, and there have been few phylogenetically-informed assessments of the origins of endemic taxa in these ranges (Beehler et al., 2012; Oliver et al., 2012a, 2016).

The New Guinea frog biota is exceptionally diverse, with > 450 recognised species, and many more awaiting description (Oliver et al., 2013; Frost, 2015)—far more diverse than nearby landmasses such as Borneo or Australia. Such anuran diversity is remarkable for being dominated by just two major radiations, of which the most speciose and ecologically diverse is a clade of nearly 250 recognised species of direct developing microhylids, the Asterophryninae Günther, 1858 (Frost et al., 2006).

Mark D. Scherz 31/12/2016 12:14
Deleted:

Mark D. Scherz 31/12/2016 12:14
Deleted:

Their reproductive ecology, wide elevational distribution, high levels of localised endemism and overall species richness suggest that microhylid frogs may provide an excellent system for understanding how the mountains may have shaped diversification in New Guinea.

Choerophryne (including the previously recognised genus *Albericus*: see Peloso et al., 2015) is a moderately diverse clade (31 recognised taxa) within the Asterophryninae, comprised of small to miniaturised frogs endemic to New Guinea. This genus occurs from hill to upper montane habitats across much of Central Cordillera and North Papuan Mountains (although they appear to be absent in most of the west and southern lowlands of the island) (Günther, 2000; Richards et al., 2000). Broadly, taxa formerly placed in the genus *Albericus* are mostly climbing frogs with well-developed finger and toe pads, while taxa formerly placed in *Choerophryne* tend to be more terrestrial, although there are many exception to this general trend (Kraus & Allison, 2000; Richards et al., 2007; Günther & Richards, 2011) (Fig. 1A-D).

Mark D. Scherz 31/12/2016 12:16
Deleted: however

Choerophryne also includes many miniaturised species, here defined as frogs less than 15 mm long (Yeh, 2002), some of which approach minimum size limits for tetrapods (Kraus, 2010a; Rittmeyer et al., 2012). The water-permeable skin of frogs plays a critical role in shaping both local and regional patterns of diversity and habitat use (Scheffers et al., 2013), with smaller species at greater risk of desiccation than larger species (Tracy et al., 2010). It follows therefore, that smaller size in *Choerophryne* species may be correlated with occurrence in more humid environments. We hypothesise that such a trend may manifest as a negative correlation between body size and altitude, due to the existence of reliably moist cloud forest habitats at higher elevations that are less prone to desiccation (Nix, 1982).

Mark D. Scherz 31/12/2016 12:17
Deleted: more

Mark D. Scherz 31/12/2016 16:25
Deleted: .

Mark D. Scherz 31/12/2016 16:22
Comment [1]: I would consider adding a sentence expanding on this; if the null hypothesis that miniaturized frogs are equally distributed across altitudes cannot be rejected, this may be because of micro-habitat, with species at lower altitudes more restricted to leaf litter than higher altitude species due to the humidity constraints.

Here we present an analysis of the phylogenetic relationships and evolution of key traits within *Choerophryne*. We initially focus on the origins of montane endemism, with a specific prediction being that the older Central Cordillera will be dominated by *in situ* diversification processes (centric endemism) linked to ongoing uplift, while the younger North Papuan mountains may show evidence of colonisation from the older Central Cordillera (eccentric endemism). We also test the prediction that ecological shifts (arboreal to terrestrial), and shifts in body size (towards extreme miniaturisation) may correlate with occurrence in novel habitats and climatic regimes at higher elevations.

METHODS

Specimen Selection

This study utilised whole specimens and tissue samples deposited in Museum collections (ethics approval was therefore not required). Following Vieites et al. (2009) we recognised lineages as distinct OTUs (candidate species) for downstream analysis if they met any two of the following three criteria: a) males with distinctive advertisement calls, b) evidence of morphological differentiation or c) evidence of genetic differentiation (usually greater than 3% uncorrected pairwise in the 16S rRNA gene (see Table 6 for a summary). Mitochondrial DNA sequences of an additional 11 *Choerophryne* were downloaded from GenBank, along with 14 outgroup sequences from 6 other New Guinean microhylid genera. The taxonomic assignation of *Choerophryne* species is challenging, especially in the absence of calls, so taxonomic

Mark D. Scherz 31/12/2016 13:18
Comment [2]: There needs to be a statement of sampling completeness in the methods section. 11/13 Choerophryne s.s. are included, but only 7/18 Albericus s.s. species. This will influence interpretations. What are the chances that the several Clade A (=Albericus) 'candidates' represent already described species? How was this ruled out?

Mark D. Scherz 31/12/2016 12:28
Deleted: ;

Mark D. Scherz 31/12/2016 12:29
Deleted: SIxIX

Mark D. Scherz 31/12/2016 12:29
Deleted: –

designations used in this study should be considered provisional. Full details of all samples included are given in Appendix S1.

Mark D. Scherz 31/12/2016 12:29
Deleted:

DNA extraction, amplification, sequencing and alignment

Whole genome DNA was extracted from frozen or alcohol preserved liver samples using the Gentra Puregene kit protocol (QIAGEN 2011). Sequence data from the 12S and 16S mitochondrial genes was PCR amplified with an annealing temperature of 58°C using the primers 12SAL and 12SBH (Palumbi et al., 2002) and 16SL3 and 16SAH (Vences et al., 2003), then purified on MultiScreen PCR$_{384}$ Filter Plates. Sanger sequencing (forward and reverse) of purified PCR product used the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), purified using MultiScreen SEQ$_{384}$ Filter Plates and sent to the Australian Genome Research Facility (AGRF) for capillary separation.

Geneious Pro v5.5.2 (Kearse et al., 2012) was used to align forward and reverse sequence traces and reviewed by eye. The consensus sequences along with sequences from GenBank (Appendix S1) were aligned with 8 iterations of the MUSCLE algorithm under default parameter settings (Edgar, 2004). Hypervariable regions with poor local alignment were removed using Gblocks v0.91b (Castresana, 2000); of the original 1556 aligned positions, 1347 were retained in final analyses.

Phylogenetic Analysis

To assess congruence of topology and support values across methods, we estimated phylogenetic relationships using Bayesian and maximum likelihood approaches. Based on the output of the model selection program MrModeltest (Nylander, 2004) all analyses were performed using the general time-reversible model, allowing for variation in the rate of evolution among sites and including invariable sites (GTR+I+G). Both genes were treated as a single partition due to the relatively short sequence length and similar features (i.e. mitochondrial rRNA).

Mark D. Scherz 31/12/2016 12:31
Deleted: ig

The maximum likelihood tree with bootstrap values was produced using RAxML v 8.0.26 (Stamatakis, 2006) with bootstrap scores calculated using the rapid bootstrap (-f a) function with 1000 replicates. The Bayesian consensus tree was generated by Mr Bayes 3.2.2 (Ronquist et al., 2012) using an unconstrained branch length prior, 4 chains (incrementally heated at temperature 0.2), each of 5 million generations with a 1 million generation burn-in and sampling every 200 generations.

These topology-only analyses with dense sampling across populations were compared to analyses where we simultaneously estimated phylogeny, divergence dates and trait evolution, on species-level trees (see below).

Trait and biogeographical scoring

We scored each taxon for three traits of interest: a) adult male body size, b) elevation and c) maximum calling height of males (as a proxy for arboreality vs terrestriality) (Appendix S1). These data were scored from genotyped specimens and associated fieldnotes, or extracted from primary literature.

We used a typical measure of size in anurans, the distance from the tip of the snout to the urostyle tip (SUL), which has been previously used in *Choerophryne*

(Günther, 2008). We used the maximum recorded size for males (sex determined by observations of specimens calling). Although some *Choerophryne* have unusually long snouts, at most these comprised 10% of the total body length.

The maximum elevational range (difference between lower and upper occurrences) obtained for any species was just over 1000 metres, involving two taxa that occur primarily in hill forests, but range into lower montane forests. Seven taxa are also only known from single sites. To score elevation as a continuous character (for use in phylogenetic regressions) we used the mid-point of records for each lineage (to the nearest 100m).

Mark D. Scherz 31/12/2016 12:32
Deleted:

Mark D. Scherz 31/12/2016 12:34
Comment [4]: Please state the taxa here.

For discrete categorisation of elevation we used the forest classification system presented by Johns (1982): hill forest and lowlands (< 1000 m a.s.l.), lower montane (1000–2000 m a.s.l.), mid-montane (2000–3000 m a.s.l.) and upper montane (> 3000 m a.s.l.) (Fig. 1). These bands broadly reflect how reducing mean temperatures with elevation shapes the transition from megathermal to microthermal vegetative communities (Nix, 1982). For most taxa, the majority of records were focused in just one of these bands. The small number of taxa whose distributions spanned bands were placed in the band in which the majority of records were concentrated. Finally, *Choerophryne laurini* is known only from typical lower montane forest on mossy ridge tops in the Wondowoi mountains between 800-950 metres. This species was coded as lower montane for discrete analyses.

To better visualise potential colonisation paths to the isolated North Papuan Mountains, we also devised a further coding system of 4 states that combined geography and elevation: southern lowland (south of Central Cordillera below 1000 m. a.s.l.), central highlands (Central Cordillera above 1000 m a.s.l.), northern lowland (south of Central Cordillera below 1000 m. a.s.l.) and northern montane (North Papuan Mountains above 1000 m a.s.l).

Male *Choerophryne* show extensive variation in the typical calling height from largely terrestrial (e.g. *Choerophryne alpestris*) to more than 3 metres off the ground (e.g. *Choerophryne pandanicola*) (Günther & Richards, 2011). To score calling height as a continuous trait we used the maximum recorded calling height of males, either from the literature or personal observations. We also employed a second scheme for coding calling ecology, by dividing taxa into two broad guilds: a) *Terrestrial* - species that called predominantly on or very close to the ground on leaf litter or duff (generally less than 50 cm high), and b) *Scansorial* - species that usually climb into vegetation and call from (generally more exposed) positions up to several metres high. Two taxa (*C. arndtorum* and *C. microps*) for which the majority of calling records are terrestrial but which have occasionally been recorded calling a metre or more above the ground (Günther, 2008), were coded as terrestrial in the discrete character analyses, while the maximum recorded calling height was used in continuous trait based analyses.

Ancestral state analyses.

We used BEAST v 1.8.2. (Drummond & Rambaut, 2007) to co-estimate trait evolution (including ancestral states) with phylogeny and divergence dates. These analyses used a reduced dataset comprising a single exemplar of each genetically and/or morphologically divergent lineages identified in earlier phylogenetic analyses (i.e. recognised or candidate species). The original molecular data for each exemplar was also included. To ensure these analyses were focused on a strongly supported and well-sampled monophyletic group, in these trait analyses we excluded two samples from a highly divergent clade that did not strongly associate with other *Choerophryne* in estimated phylogenies (see results) . Size was $\log_{10}$ transformed. The two discrete variables (elevation and calling ecology) were coded using the MK + strict clock model, which assumes that transformations between states are reversible and occur at the same rate throughout the tree; more complex models were not feasible due to the relatively small tree and number of transformations. Elevation character states were ordered—e.g. shifts to from lower- to upper-montane habitats were constrained to involve moving through mid-montane habitats. Analyses were run for 50 million generations, sampling every 50,000 generations. The first 20% of trees were discarded as burn-in and the remaining 800 post-burn-in trees from each run were combined to generate the final consensus topology. The final xml file is given in Appendix S2. Effective samples sizes (ESS) for all parameters (from Tracer v 1.6.0, Drummond & Rambaut, 2007) in both individual and combined BEAST analyses were above 200.

BEAST automatically produces an ultrametric tree—however there are no fossil calibrations within *Choerophryne*, and there has been no recent thorough assessment of rates of mitochondrial DNA evolution in frogs. However, to provide a rough timescale for *Choerophryne,* we used a broad consensus molecular evolutionary rate for mitochondrial genes of between 1–2% pairwise divergence per million years, which was incorporated into the prior for average substitution (clock) rate. Rates of molecular variation vary extensively (Eo & DeWoody, 2010), and thus the resultant dates from this are interpreted with caution. Importantly, the ancestral state analyses (above) only require relative rather than absolute branch lengths (e.g. they could still be performed if root age was arbitrarily scaled to 1), so our results are robust to these dating uncertainties.

Mark D. Scherz 31/12/2016 12:39
Deleted: (see results)

Mark D. Scherz 31/12/2016 12:40
Deleted: –

Mark D. Scherz 31/12/2016 12:42
Deleted:

Mark D. Scherz 31/12/2016 12:42
Deleted: –

Mark D. Scherz 31/12/2016 12:42
Deleted: -

Mark D. Scherz 31/12/2016 12:47
Deleted: this

Phylogenetic Least Squares Regression

The relationship of a) body size to calling ecology and/or elevation and b) calling ecology to elevation was analysed using BayesTraits v 2.0 (Pagel & Meade, 2013), across the concatenated 3200 post-burnin trees from BEAST. For these analyses all variables were included as $\log_{10}$-transformed continuous states. We only included data for lineages in two well-sampled clades of *Choerophryne* that were strongly supported as sister taxa (see below), other species in the trees were scored as missing data. We also performed regressions on each these two well-differentiated clades. The Bayesian MCMC implementation of the continuous module was used to regress a) body size against ecology and elevation, and b) ecology against elevation. Eleven million steps were used with the first 1 million discarded for burn-in, and 4 runs of BayesTraits were performed and checked for convergence using Tracer v 1.6.0 (Drummond & Rambaut, 2007). Pagel & Meade (2013) state that the significance of a variable can be assessed either by comparing harmonic means (for analyses with and without the variable), or observing whether the estimated distribution of that variable (e.g. 95% HPD) excludes 0. Due to issues around the use of harmonic means to estimate marginal likelihoods (Xie et al., 2011), we adopted the latter approach.

RESULTS

Phylogenetic relationships and lineage diversity.

Mark D. Scherz 31/12/2016 12:49
Formatted: Font:Not Bold, Not Highlight

Mark D. Scherz 31/12/2016 12:49
Formatted: Not Highlight

Mark D. Scherz 31/12/2016 12:49
Formatted: Font:Not Bold, Not Highlight

Mark D. Scherz 31/12/2016 12:49
Formatted: Not Highlight

Bayesian and maximum likelihood analyses identified three major lineages of *Choerophryne* (Fig. 2, Fig. SI1). Clade A comprised the majority of sampled taxa that were formerly placed in the genus *Albericus*, Clade B included all taxa with a moderate to pronounced rostral projection formerly placed in *Choerophryne sensu stricto*. Clade C comprised two scansorial taxa lacking distinctive rostral projections and occurring to south of the Central Cordillera in hill forest, and on the Finnistere Ranges (north-east New Guinea) in hill to lower montane forest respectively.

A sister taxon relationship between Clades A and B was strongly supported in all analyses (Posterior Probability 1.0, bootstrap support >90). Clade C was more divergent and there was no evidence that it forms the sister group to Clade A+B (or any other microhylid lineage). All basal relationships between the sampled New Guinea microhylid genera were poorly supported, but these were not the focus of this study.

Within Clade A we identified two strongly supported primary lineages, with the major split being between a clade of two lower montane and hill forest taxa from the south side of Central Cordillera, and several clusters of species from across the Central Cordillera and North Papuan Mountains, including derived terrestrial taxa from mid to upper montane habitats (*C. alpestris* and *C. brevicrus*).

Within Clade B there were three well supported primary lineages: one comprising three deeply divergent taxa (*C. burtoni* and two unnamed taxa) from hill forest to mid-montane habitats on the Central Cordillera; a further lineage of large-bodied and very long-snouted taxa from hill and lower montane forest in northern New Guinea; and finally a diverse conglomeration including lineages from hill and lower montane forests in northern New Guinea, in addition to one taxon from south of the Central Cordillera (*C. gracilirostris*).

Mark D. Scherz 31/12/2016 12:50
Formatted: Indent: First line: 0 cm

Mark D. Scherz 31/12/2016 13:14
Comment [6]: Epirrhina is misspelt

Mark D. Scherz 31/12/2016 13:01
Comment [7]: Currently this is the wrong name.

In all three major clades we identified lineages (candidate species) that were deeply divergent from, and could not be confidently assigned to, recognised species. This was most pronounced in Clade A, which includes a number of scansorial species that are difficult to diagnose on the basis of external morphology.

Ancestral States analyses

The dated species tree for ancestral states analysis (Figs. 3–4) was congruent with our densely-sampled, undated molecular phylogeny (Fig. 2). Character states for Clade C were not included in most ancestral state analyses due to phylogenetic uncertainty and the relatively small number of lineages. These analyses highlighted the contrasting evolutionary trajectories of the two 'core' clades of *Choerophryne* (A & B). In all analyses including elevation, hill forest habitats (largely distributed between 0–1000 m a.s.l.) were inferred as the ancestral habitat for both Clades A and B. Clade A was inferred to have diversified primarily within montane habitats during the late Miocene (14 out of 15 nominal taxa), including more recent upslope shifts into mid and upper-montane zones (Fig. SI2). Independent colonisation from eccentric origins to the North Papuan Mountains is inferred when geography is included (especially in the Foja Mountains) (Fig. 3). In contrast Clade B was centred on hill forest habitats, but with 2–4 relatively recent upslope (eccentric) shifts into montane habitats in mostly distantly related taxa, again mainly occurring in isolated North Papuan Mountains (specifically Japen Island and the Foja and Torricelli Mountains) (Fig. 3, Fig. SI2).

Miniaturised species (< 15 mm) occurred across the phylogeny (Fig. 4), implying that multiple lineages of *Choerophryne* have independently evolved very

Mark D. Scherz 31/12/2016 13:08
Deleted:

Mark D. Scherz 31/12/2016 13:12
Deleted: -

Mark D. Scherz 31/12/2016 13:26
Deleted:

Mark D. Scherz 31/12/2016 13:29
Deleted: .

Mark D. Scherz 31/12/2016 13:32
Comment [8]: This sentence was missing some prepositions. Does it make sense now?

Mark D. Scherz 31/12/2016 13:34
Formatted: Font:Not Bold

small body size. Taxa in the predominantly scansorial Clade A tended to be larger than those in the more terrestrial clade B.

Calling ecology was relatively labile across the genus, with multiple shifts between terrestrial and scansorial calling, the latter being inferred as the ancestral state for the common ancestor of clades A and B (Fig. SI2). However there were again somewhat contrasting patterns across the two clades. Clade A was inferred as largely scansorial with a small number of shifts towards terrestrial calling, Clade B included a majority of taxa (9 out of 14) that call from on or close to the ground; this state was accordingly inferred as ancestral, with 3 transitions to scansorial calling.

Phylogenetic Regressions

All BayesTraits runs converged well before the burn-in, and the concatenated runs yielded ESS of all parameters >1000. In the analysis relating body size to ecology and/or elevation, both ecology and elevation (considered together: Pagel & Meade 2013) exhibited significant phylogenetic structure, as expected (Lambda for all taxa was significantly positive: mean 0.55; 95% HPD = 0.12, 0.98). Ecology (as indexed by calling height) was positively associated with body size in the all taxa analysis, with a regression coefficient that was always estimated as positive (mean= 0.09, 95% HPD = 0.03, 0.15). In analyses focusing on specific clades this relationship was also positive, although the HPD included zero for Clade A (mean= 0.1, 95% HPD = -0.01, 0.19), but not Clade B (mean = 0.1, 95% HPD = 0.01, 0.20).

Elevation was not strongly related to body size in all relevant analyses, with a regression coefficient centred almost exactly on 0 when all taxa were included (mean = 0.01, 0. 95% HPD = -0.11, +0.11). Analyses of the two main clades showed

Mark D. Scherz 31/12/2016 13:35
Deleted:

Mark D. Scherz 31/12/2016 13:35
Formatted: Font:Not Bold

Mark D. Scherz 31/12/2016 13:36
Deleted:

Mark D. Scherz 31/12/2016 13:36
Deleted:

Mark D. Scherz 31/12/2016 13:39
Deleted: d

Mark D. Scherz 31/12/2016 13:39
Deleted: ifferent

positive and negative relationships, although in both cases the HPD again included 0, suggesting the relationships were weak or insignificant: Clade A (mean = 0.24, 95% HPD = -0.06, 0.5) and Clade B (mean = -0.05, 95% HPD = -0.20, 0.08).

Calling height was weakly negatively related to elevation, although in all cases the HPD again spanned zero; all taxa (mean = -0.65, 95% HPD = -1.27, 0.04); Clade A (mean = -1.1, 95% HPD = -2.49, 0.15) and Clade B (mean = -0.74, 95% HPD = -1.62, -0.03). Removal of three high elevation taxa (> 2500 m a.s.l) in Clade A that live in mossy grasslands where there are few arboreal habitats weakened this relationship further, resulting in a 95% highest probability posterior distribution that more broadly included 0 (mean = -0.47, 95% HPD = -1.06, 0.16).

DISCUSSION

Despite the biological wealth and high endemism of the New Guinea Mountains (Tallowin et al. 2016) and emerging evidence for major evolutionary radiations (Toussaint et al., 2014; Givnish et al., 2015), only a small number of phylogenetic studies of lineages with distributions centred on the montane regions of New Guinea have been published (Meredith et al., 2010; Toussaint et al., 2013; Irestedt et al., 2015). Our study complements the recent work focusing on volant or large-bodied taxa, by presenting data for a lineage of small, direct-developing frogs that may be presumed to have comparatively low vagility.

Species diversity and phylogeny

Mark D. Scherz 31/12/2016 13:40
Deleted: however

Mark D. Scherz 31/12/2016 13:40
Deleted: not

Mark D. Scherz 31/12/2016 13:40
Deleted:

Mark D. Scherz 31/12/2016 14:01
Comment [9]: It is very interesting that elevation is positively correlated with size in one lineage and negatively in the other. This is worthy of discussion, even if the results are insignificant.

Mark D. Scherz 31/12/2016 13:59
Deleted:

Mark D. Scherz 31/12/2016 14:02
Deleted: 34

Mark D. Scherz 31/12/2016 14:03
Deleted: 585

Mark D. Scherz 31/12/2016 14:03
Deleted: 589

Mark D. Scherz 31/12/2016 14:07
Deleted: this

Molecular assessments of amphibian diversity on tropical islands over the last decade have revealed exceptionally high levels of previously unrecognised diversity (Meegaskumbura et al., 2002; Vieites et al., 2009). However, while New Guinea already has the most diverse insular frog fauna in the world (over 450 recognised species [Frost, 2015]), molecular assessments of frog diversity in this region are scarce. While taxonomy was not the focus of this study, we uncovered 12 candidate species, in addition to three new taxa recently named (Iannella et al. 2014, 2015). Molecular studies of other New Guinea microhylid frogs (*Mantophryne*) have also revealed a diversity of deeply divergent lineages (Oliver et al., 2013) and further fieldwork and integration of molecular, morphological and acoustic analyses seem certain to cement New Guinea's position as a global hotspot of amphibian diversity.

Mark D. Scherz 31/12/2016 14:10
Comment [10]: Again, how certain are we that these are not just the Albericus s.s. and Choerophryne s.s. species that were not included in the study?

Clades A and B together formed a strongly supported monophyletic group, but the overall monophyly of all three sampled lineages of *Choerophryne* was not strongly supported (or rejected). There are however morphological synapomorphies uniting all three lineages of *Choerophryne* (see Burton & Zweifel, 1995), and their monophyly was also recently supported based on a phylogenomic study including examplars of all three major lineages (Peloso et al., 2015). The non-monophyly of *Choerophryne* in our analyses could be an artefact of rapid diversification and/or the short rapidly saturating loci used in this study. Resolution and further discussion of the phylogeny and generic taxonomy of *Choerophryne* will require larger nuclear-gene-based datasets and sampling of taxa from other regions of New Guinea. However because of uncertainty in basal relationships, in this study we focused ancestral state analyses on the well-sampled and supported clades A and B.

Mark D. Scherz 31/12/2016 13:19
Deleted:

Mark D. Scherz 31/12/2016 14:11
Deleted: -

Mark D. Scherz 31/12/2016 14:11
Deleted:

Mark D. Scherz 31/12/2016 14:12
Deleted:

Mark D. Scherz 31/12/2016 14:12
Deleted:

There were also distributional gaps in our genetic sampling (Fig. 3). Recent surveys in western New Guinea (upper Mamberamo, Fak Fak mountains) have indicated that *Choerophryne* (which are usually easy to locate) are absent or rare, suggesting this disjunction reflects genuine absence (Günther, 2000; Richards et al., 2000). Another gap is the Papuan Peninsula, where endemic *Choerophryne* are found (Fig. SI3). However, none of these taxa are shared with Central New Guinea, suggesting that taxa in this region—which is geologically very distinctive—will have their own history. Furthermore, while future addition of taxa from this region into phylogenetic datasets is a research priority, we consider it unlikely to change the broadly reciprocal patterns of elevational distribution and montane colonisation between clades A and B in Central New Guinea that we discuss below .

Complex origins of montane endemism

Uplifting tropical mountains have been shown to be ‘cradles’ of young diversity in diverse regional bird communities (Weir, 2006; Price et al., 2014). Recent work on beetles, mammals and birds has suggested a similar association between the recent uplift of mountains in New Guinea and diversification (Meredith et al., 2010; Toussaint et al., 2014; Irestedt et al., 2015). In this study we complement this work by providing the first molecular phylogeny of a vertebrate clade that is both moderately diverse (15 nominal taxa), and almost entirely endemic to the New Guinea Highlands (> 1000 m). Our phylogeny suggests Clade A colonised lower montane habitats first (possibly by the mid-Miocene), while higher altitude taxa (i.e., > 2000 m a.s.l.) in Clade A are relatively young (Pliocene). This pattern is broadly consistent with progressive upslope colonisation as the Central Cordillera gained height through the late Miocene and Pliocene, and suggests that recent mountain uplift has played a key role in the diversification of this lineage.

Mark D. Scherz 31/12/2016 14:13
Deleted: -

Mark D. Scherz 31/12/2016 14:13
Deleted:

Mark D. Scherz 31/12/2016 14:13
Deleted: -

Mark D. Scherz 31/12/2016 14:15
Deleted: (Clade A)

Mark D. Scherz 31/12/2016 14:16
Comment [11]: There are 31 nominal taxa in Choerophryne

Mark D. Scherz 31/12/2016 14:17
Deleted: Furthermore,

Mark D. Scherz 31/12/2016 14:17
Deleted: o

Mark D. Scherz 31/12/2016 14:18
Deleted: perhaps

Mark D. Scherz 31/12/2016 14:16
Deleted:

Mark D. Scherz 31/12/2016 14:17
Deleted: ,

On the other hand we find weak evidence that New Guinea mountains have functioned as a ‘museum’. One potential example from *Choerophryne* is a clade in the Central Cordillera region (*C. burtoni*, *sp*B2 and *sp*B3) that shows outwardly disjunct distributions and deep divergences (estimated 10 mya in our analysis). However overall, when compared to deeply divergent relict bird lineages or high phylogenetic endemism of mammals (Jønsson et al., 2010; Rosauer & Jetz, 2015) in the New Guinea mountains, our data do not at this stage provide strong evidence that relict taxa have inflated montane diversity in *Choerophryne*.

A further striking result of this study is the inference of both centric and eccentric origins of montane diversity in the younger, lower elevation, more isolated and poorly known North Papuan Ranges. These ranges are home to numerous endemic taxa or isolated populations (Richards et al., 2009; Oliver et al., 2011, 2012a,b, 2016; Beehler et al., 2012), but in most cases these are clearly related to, or even conspecific with, montane taxa occurring elsewhere in New Guinea (e.g. 100% of birds are allopatric isolates of lineages occurring in montane habitats elsewhere; Beehler et al., 2012). In *Choerophryne* two lineages in Clade A show a similar pattern; they appear to be endemic to montane habitats in the north Papuan Mountains (not found below around 1000 m a.s.l.), related to taxa otherwise known only from montane Central Cordillera habitats, and unknown from the intervening lowlands (Richards & Suryadi, 2003). This apparent pattern of eccentric origins suggest that lower montane forests in New Guinea have a dynamic climatic history, possibly including periods of major elevational depression similar to those inferred elsewhere in the tropics (Colinvaux et al., 1996; Zhuo, 1999).

However, ancestral state analyses of well-sampled Clade B also provide strong evidence for at least two and potentially three independent derivations of North

Mark D. Scherz 31/12/2016 14:19
Formatted: Font:Italic

Mark D. Scherz 31/12/2016 14:19
Deleted: the tree

Mark D. Scherz 31/12/2016 14:20
Deleted: Oliver et al.

Mark D. Scherz 31/12/2016 14:20
Deleted: ;

Mark D. Scherz 31/12/2016 14:20
Deleted: Oliver et al.

Mark D. Scherz 31/12/2016 14:20
Deleted:

Mark D. Scherz 31/12/2016 14:21
Deleted: ,

Papuan montane endemics from surrounding lowland taxa (centric endemism) (Fig. 3). Detailed fine scale sampling is required to understand the processes that have shaped this endemism; elevational segregation may be an outcome rather than a driver of speciation (Caro et al., 2013; Freeman, 2015). However, regardless of the exact process, this represents the first strong evidence that endemic montane vertebrates have arisen *de novo* in northern New Guinea from largely lowland lineages. These contrasting origins of endemism suggest that the young and isolated North Papuan Mountains may provide excellent opportunities for comparative analyses of the processes driving montane endemism in young tropical mountains.

Finally, mountain uplift may also inflate regional diversity at lower elevations by isolating formerly continuous populations of lowland taxa (vicariance). In New Guinea there is already compelling evidence that the uplift of the Central Cordillera has isolated northern and southern vicars in lowland and aquatic taxa (Rawlings & Donnellan, 2003; Georges et al., 2014), and potentially also lower montane taxa (Irestedt et al., 2015). However, our sampling of *Choerophryne* did not reveal extensive north-south vicariance, although one possible exception is a recently described pair of potential sister taxa in Clade B from hill and lower montane forest (*C. gracilirostris* [south] and *C. grylloides* [north]) that are estimated to have diverged around 10 mya. This general lack of signal for north-south vicariance is not surprising given the majority of species in the two clades are associated with hill and montane forest and are less likely to be isolated by mountain uplift than lowland or aquatic taxa.

At the lower size limits of vertebrates; correlates of repeated miniaturisation

A number of new lineages of tiny frogs that approach minimum size limits for vertebrates have discovered recently (Wollenberg et al., 2008; Kraus, 2010a; Rittmeyer et al., 2012, Lehr & Coloma, 2008; Kraus, 2010, 2011; Wollenberg et al., 2011; Rittmeyer et al., 2012)**,** and it has been suggested that miniaturised frogs may represent an often overlooked, but important ecological guild in tropical areas (Rittmeyer et al., 2012). Broadly, three patterns are globally apparent in miniaturised frogs: most lack a free-swimming tadpole stage (Estrada & Hedges, 1996), most occur in wet tropical and usually insular regions, and most are more-or-less terrestrial (Kraus, 2010a; Rittmeyer et al., 2012). Across the six different genera of Papuan microhylids that contain miniaturised taxa (*Aphantophryne*, *Austrochaperina*, *Choerophryne*, *Cophixalus*, *Oreophryne* and *Paedophryne*) all three of these correlates are evident.

Our analyses further indicate that within *Choerophryne* there have been at least three relatively recent shifts towards extremely small body size (three lineages ~15 mm or less), all of which are inferred in lineages that call on or close to the ground. This plasticity of body size and ecology of *Choerophryne* contrasts with conservatism of these same features in another miniaturised genus of Papuan microhylids, *Paedophryne* (Rittmeyer et al. 2012). Patterns of evolution across both genera do however strongly support the hypothesis that physiological or ecological constraints limit miniaturised taxa to a terrestrial lifestyle. Most recognised taxa missing from our analyses are moderate sized and scansorial, and likely belong in Clades A and C. Their inclusion would also be unlikely to change the correlation between terrestriality and small size.

Mark D. Scherz 31/12/2016 14:26
Deleted:

Mark D. Scherz 31/12/2016 14:27
Deleted:

Counter to our initial prediction, we did not find a strong positive correlation between elevation and either ecology (calling height) or body size, as might be

Mark D. Scherz 31/12/2016 14:27
Deleted: Contra

Mark D. Scherz 31/12/2016 14:28
Formatted: Font:Not Bold

Mark D. Scherz 31/12/2016 14:28
Formatted: Font:Not Bold

expected if desiccation risk is decreased at higher elevations (Scheffers et al., 2013). This lack of pattern may indicate that for frogs of extremely small size, physiological or ecological pressures associated with microhabitat use are a bigger constraint on body sizes than elevation-related variation in climates. Unlike the correlation between terrestriality and small size in which we are confident and which mirrors a broader pattern, further analysis including both *Choerophryne* taxa missing from our dataset, and other genera of microhylid is probably needed to refine understanding of the potentially much more nuanced three-way relationships between body size, ecology and elevation.

Finally, *Choerophryne* provides a striking example of an insular frog lineage that has undergone ecological diversification, with repeated shifts between scansorial and relatively terrestrial ecologies, reflected in significant reduction or even loss of terminal discs and shortening of limbs (Günther, 2008; Kraus, 2010b; Günther & Richards, 2011). Similar ecological diversity and morphological plasticity has also observed in other microhylid lineages in New Guinea, as well as in other island systems such as Madagascar and Philippines (Andreone et al., 2005; Köhler & Günther, 2008; Blackburn et al., 2013). In contrast, microhylids generally seem to be peripheral (and usually terrestrial or fossorial) components of frog diversity in continental regions (see Duellman, 1999). This suggests that microhylids might be comparatively good colonists of islands (in some cases perhaps associated with direct development) and have great adaptive potential in these regions, but may be poorer competitors in diverse continental frog communities (perhaps due to their unique feeding apparatus: Meyers et al., 2004).

CONCLUSIONS

Our new phylogeny and ecophenotypic data for the microhylid frog genus *Choerophryne* indicates that montane areas have been colonised via a complex suite of biogeographic processes, especially upslope colonisation and speciation in presumably novel highland habitats and dispersal between montane islands, and that the relative importance of these processes has differed across even closely related lineages. *Choerophryne* also shows a correlation between extremely small size and utilisation of terrestrial habitats, mirroring a global pattern that suggests that, in frogs, ecological or physiological constraints largely limit extremely miniaturised taxa to terrestrial microhabitats in tropical areas.

ACKNOWLEDGEMENTS

We thank numerous landholders in New Guinea and Indonesia, permitting agencies, research organisations and NGO’s (especially Conservation International) for the help and assistance in facilitating the collection material used in this study. This work was supported by grants from the Australian Research Council to Paul Oliver, a McKenzie Postdoctoral fellowship to Paul Oliver from Melbourne University, and grant from the Australia Pacific Science Foundation to Paul Oliver, Mike Lee and Steve Richards.

REFERENCES

Andreone F., Vences M., Vieites D.R., Glaw F., & Meyer A. (2005) Recurrent ecological adaptations revealed through a molecular analysis of the secretive cophyline frogs of Madagascar. *Molecular Phylogenetics and Evolution*, **34**, 315–322.

Baldwin S.L., Fitzgerald P.G., & Webb L.E. (2012) Tectonics of the New Guinea Region. *Annual Review of Earth and Planetary Sciences*, **40**, 495–520.

Beehler B., Diamond J., Kemp N., Scholes E., Milensky C., & Laman T. (2012) Avifauna of the Foja Mountains of western New Guinea. *Bulletin of the British Ornithologist's Club*, **132**, 1–18.

Blackburn D.C., Siler C.D., Diesmos A.C., Mcguire J. a., Cannatella D.C., & Brown R.M. (2013) An adaptive radiation of frogs in a southeast asian island archipelago. *Evolution*, **67**, 2631–2646.

Burton T.C. & Zweifel R.G. (1995) A new genus of genyophrynine microhylid frogs from New Guinea. *American Museum novitates*, **3129**, 1.

Caro L.M., Caycedo-Rosales P.C., Bowie R.C.K., Slabbekoorn H., & Cadena C.D. (2013) Ecological speciation along an elevational gradient in a tropical passerine bird? *Journal of Evolutionary Biology*, **26**, 357–374.

Castresana J. (2000) Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. *Molecular Biology and Evolution*, **17**, 540–552.

Colinvaux P. a., Liu K.-B., Oliveira P., Bush M.B., Miller M.C., & Kannan M.S. (1996) Temperature depression in the lowland tropics in glacial times. *Climatic Change*, **32**, 19–33.

Drummond A.J. & Rambaut A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology*, **7**, 214.

Duellman W.E. (1999) *Patterns of Distribution of Amphibians.* The Johns Hopkins University Press, Baltimore.

Edgar R.C. (2004) MUSCLE User Guide. *Nucleic Acids Research*, **32**, 1–15.

Eo S.H. & DeWoody J.A. (2010) Evolutionary rates of mitochondrial genomes correspond to diversification rates and to contemporary species richness in birds and reptiles. *Proceedings of the Royal Society of London: Biological sciences*, **277**, 3587–3592.

Estrada A.R. & Hedges S.B. (1996) At the Lower Size Limit in Tetrapods: A New Diminutive Frog from Cuba (Leptodactylidae: *Eleutherodactylus*). *Copeia*, **4**, 852–859.

Fjeldså J., Bowie R.C.K., & Rahbek C. (2012) The Role of Mountain Ranges in the Diversification of Birds. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 249–265.

Freeman B.G. (2015) Competitive Interactions upon Secondary Contact Drive Elevational Divergence in Tropical Birds. *The American Naturalist*, **186**, 470–479.

Freeman B.G. & Class Freeman A.M. (2014) Rapid upslope shifts in New Guinean birds illustrate strong distributional responses of tropical montane species to global warming. *Proceedings of the National Academy of Sciences*, **111**, 4490–4494.

Frost D.R. 2014. (2015) Amphibian Species of the World. Website: http://research.amnh.org/vz/herpetology/amphibia/

Frost D.R., Grant T., Faivovich J., Bain R.H., Haas A., Haddad C.F.B., De Sá R.O., Channing A., Wilkinson M., Donnellan S.C., Raxworthy C.J., Campbell J. a., Blotto B.L., Moler P., Drewes R.C., Nussbaum R. a., Lynch J.D., Green D.M., & Wheeler W.C. (2006) The Amphibian Tree of Life. *Bulletin of the American Museum of Natural History*, **297**, 1–291.

Georges A., Zhang X., Unmack P., Reid B.N., Le M., & Mccord W.P. (2014) Contemporary genetic structure of an endemic freshwater turtle reflects Miocene orogenesis of New Guinea. *Biological Journal of the Linnean Society*, **111**, 192–208.

Givnish T.J., Spalink D., Ames M., Lyon S.P., Hunter S.J., Zuluaga A., Iles W.J.D., Clements M.A., Arroyo M.T.K., Leebens-mack J., Endara L., & Kriebel R. (2015) Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society of London: Biological Sciences*, **282**, .

Graham C.H., Carnaval A.C., Cadena C.D., Zamudio K.R., Roberts T.E., Parra J.L., McCain C.M., Bowie R.C.K., Moritz C., Baines S.B., Schneider C.J., VanDerWal J., Rahbek C., Kozak K.H., & Sanders N.J. (2014) The origin and maintenance of montane diversity: integrating evolutionary and ecological processes. *Ecography*, **37**, 711–719.

Günther R. (2000) *Albericus laurini* species nova, the first record of the genus *Albericus* (Anura, Microhylidae) from the west of New Guinea. *Mitteilungen aus dem Museum fur Naturkunde in Berlin Zoologische Reihe*, **76**, 167–174.

Günther R. (2008) Descriptions of four new species of *Choerophryne* (Anura, Microhylidae) from Papua Province, Indonesian New Guinea. *Acta Zoologica Sinica*, **54**, 653–674.

Günther R. & Richards S.J. (2011) Five new microhylid frog species from Enga Province, Papua New Guinea, and remarks on *Albericus alpestris* (Anura, Microhylidae). *Vertebrate Zoology*, **61**, 343–372.

Hall R. (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer based recinstructions, model and animations. *Journal of Asian Earth Sciences*, **20**, 353–431.

Hutter C.R., Guayasamin J.M., & Wiens J.J. (2013) Explaining Andean megadiversity: the evolutionary and ecological causes of glassfrog elevational richness patterns. *Ecology Letters*, **16**, 1135–1144.

Irestedt M., Batalha-Filho H., Roselaar C.S., Christidis L., & Ericson P.G.P. (2015) Contrasting phylogeographic signatures in two Australo-Papuan bowerbird species complexes (Aves: *Ailuroedus*). *Zoologica Scripta*, **45**, 365–379.

Janzen D.H. (1967) Why mountain passes are higher in the tropic. *The American Naturalist*, **101**, 233–249.

Johns R.. (1982) Plant Zonation. *Biogeography and Ecology of New Guinea* (ed. by J.. Gressitt), pp. 309–330. Dr W. Junk Publishers, The Hague.

Jønsson K.A., Fabre P., Ricklefs R.E., & Fjeldså J. (2010) Major global radiation of corvoid birds originated in the proto-Papuan archipelago. *Proceedings of the National Academy of Sciences*, **108**, 2328–2333.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Mentjies P., & Drummond A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647–1649.

Köhler F. & Günther R. (2008) The radiation of microhylid frogs (Amphibia: Anura) on New Guinea: A mitochondrial phylogeny reveals parallel evolution of morphological and life history traits and disproves the current morphology-based classification. *Molecular Phylogenetics and Evolution*, **47**, 353–365.

Kraus F. (2010a) New genus of diminutive microhylid frogs from Papua New Guinea. *ZooKeys*, **48**, 39–59.

Kraus F. (2010b) An unusual new species of *Albericus* (Anura: Microhylidae) from Mount Giluwe, Papua New Guinea. *Proceedings of the Biological Society of Washington*, **123**, 1–7.

Kraus F. (2011) At the lower size limit for tetrapods, two new species of the miniaturized frog genus *Paedophryne* (Anura, Microhylidae), **88**, 71–88.

Kraus F. (2013) A New Species of *Choerophryne* (Anura : Microhylidae) from Papua New Guinea, with remarks on the taxonomic status of Albericus. *Zoosystematics and Evolution*, **89**, 283–291.

Kraus F. & Allison A. (2000) A Review of the Endemic New Guinea Microhylid Frog Genus *Choerophryne*. *Herpetologica*, **57**, 214–232.

La Sorte F.A. & Jetz W. (2010) Projected range contractions of montane biodiversity under global warming. *Proceedings of the Royal Society of London: Biological Sciences*, **277**, 3401–10.

Lehr E. & Coloma L.A. (2008) A Minute New Ecuadorian Andean Frog (Anura: Strabomantidae, *Pristimantis*). *Herpetologica*, **64**, 354–367.

Mayr E. & Diamond J.M. (1976) Birds on islands in the sky: Origin of the montane avifauna of Northern Melanesia. *Proceedings of the National Academy of Sciences*, **73**, 1765–1769.

Meegaskumbura M., Bossuyt F., Pethiyagoda R., Manamendra-Arachchi K., Bahir M., Milinkovitch M.C., & Schneider C.J. (2002) Sri Lanka: an amphibian hot spot. *Science*, **298**, 379.

Merckx V.S.F.T., Hendriks K.P., Beentjes K.K., Mennes C.B., Becking L.E., Peijnenburg K.T.C. a., Afendy A., Arumugam N., de Boer H., Biun A., Buang M.M., Chen P.-P., Chung A.Y.C., Dow R., Feijen F. a. a., Feijen H., Soest C.F., Geml J., Geurts R., Gravendeel B., Hovenkamp P., Imbun P., Ipor I., Janssens S.B., Jocqué M., Kappes H., Khoo E., Koomen P., Lens F., Majapun R.J., Morgado L.N., Neupane S., Nieser N., Pereira J.T., Rahman H., Sabran S., Sawang A., Schwallier R.M., Shim P.-S., Smit H., Sol N., Spait M., Stech M., Stokvis F., Sugau J.B., Suleiman M., Sumail S., Thomas D.C., van Tol J., Tuh F.Y.Y., Yahya B.E., Nais J., Repin R., Lakim M., & Schilthuizen M. (2015) Evolution of endemism on a young tropical mountain. *Nature*, **524**, 347–350.

Meredith R.W., Mendoza M.A., Roberts K.K., Westerman M., & Springer M.S. (2010) A phylogeny and timescale for the evolution of Pseudocheiridae (Marsupialia: Diprotodontia) in Australia and New Guinea. *Journal of Mammalian Evolution*, **17**, 75–99.

Meyers J.J., O'Reilly J.C., Monroy J. a, & Nishikawa K.C. (2004) Mechanism of tongue protraction in microhylid frogs. *The Journal of experimental biology*, **207**, 21–31.

Nix H. (1982) Environmental determinants of biogeography and evolution in Terra Australis. *Evolution in Arid Australia* (ed. by W.R. Barker and P.J.M. Greenslade), pp. 47–66. Peacock Publications in association with Australian Systematic Botany Society and ANZAAS, Frewville, South Australia,

Nylander J.A.A. (2004) MrModeltest v2. *Evolutionary Biology Centre, Uppsala University*, .

Oliver L.A., Rittmeyer E.N., Kraus F., Richards S.J., & Austin C.C. (2013) Phylogeny and phylogeography of *Mantophryne* (Anura: Microhylidae) reveals cryptic diversity in New Guinea. *Molecular Phylogenetics and Evolution*, **67**, 600–607.

Oliver P., Krey K., Mumpuni, & Richards S. (2011) A new species of bent-toed gecko (*Cyrtodactylus*, Gekkonidae) from the North Papuan Mountains. *Zootaxa*, **55**, 22–32.

Oliver P.M., Richards S.J., & Sistrom M. (2012a) Phylogeny and systematics of Melanesia's most diverse gecko lineage (*Cyrtodactylus*, Gekkonidae, Squamata). *Zoologica Scripta*, **41**, 437–454.

Oliver P., Richards S., & Tjaturadi B. (2012b) Two new species of *Callulops* (Anura: Microhylidae) from montane forests in New Guinea. *Zootaxa*, **44**, 33–44.

Oliver P., Richards S., Mumpuni M., & Rösler H. (2016) The Knight and the King: two new species of giant bent-toed gecko (*Cyrtodactylus*, Gekkonidae, Squamata) from northern New Guinea, with comments on endemism in the

Mark D. Scherz 31/12/2016 14:31
Formatted: Font:Italic

Mark D. Scherz 31/12/2016 14:31
Formatted: Not Highlight

Mark D. Scherz 31/12/2016 14:31
Formatted: Font:Italic

North Papuan Mountains. *ZooKeys*, **562**, 105–130.

Pagel M. & Meade A. (2013) *BayesTraits: Manual.*

Palumbi S.R., Martin A., Romano S., McMillan W.O., Stice L., & Grabowski G. (2002) *The simple fool's guide to PCR version 2.* University of Hawaii, Honululu,

Peloso P.L. V, Frost D.R., Richards S.J., Rodrigues M.T., Donnellan S., Matsui M., Raxworthy C.J., Biju S.D., Lemmon E.M., Lemmon A.R., & Wheeler W.C. (2015) The impact of anchored phylogenomics and taxon sampling on phylogenetic inference in narrow-mouthed frogs (Anura, Microhylidae). *Cladistics*, **32**, 113–140.

Polhemus D. (2007) Tectonic Geology of Papua. *The Ecology of Papua* (ed. by A. Marshall and B. Beehler), pp. 137–164. EricOey, Richmond.

Price T.D., Hooper D.M., Buchanan C.D., Johansson U.S., Tietze D.T., Alström P., Olsson U., Ghosh-Harihar M., Ishtiaq F., Gupta S.K., Martens J., Harr B., Singh P., & Mohan D. (2014) Niche filling slows the diversification of Himalayan songbirds. *Nature*, **509**, 222–5.

Rawlings L.H. & Donnellan S.C. (2003) Phylogeographic analysis of the green python, *Morelia viridis*, reveals cryptic diversity. *Molecular Phylogenetics and Evolution*, **27**, 36–44.

Richards S. & Suryadi S. (2003) A Biodiversity Assessment of Yongsu-Cyclops Mountains and the Southern Mamberamo Basin, Papua, Indonesia. *A Biodiversity Assessment of Yongsu-Cyclops Mountains and the Southern Mamberamo Basin, Papua, Indonesia: RAP Bulletin of Biological Assessment 25* (ed. by S.J. Richards and S. Suryadi), pp. 76–80. Conservation International, Washington, DC.

Richards S.J., Dahl C.S., & Hiaso J. (2007) Another new species of *Choerophryne* (Anura: Microhylidae) from Southern Highlands Province, Papua New Guinea. *Transactions of the Royal Society of South Australia*, **131**, 135–141.

Richards S.J., Oliver P.M., Krey K., & Tjaturadi B. (2009) A new species of *Litoria* (Amphibia: Anura: Hylidae) from the foothills of the Foja Mountains, Papua Province, Indonesia. *Zootaxa*, **13**, 1–13.

Richards S.J., Iskandar D.T., & Allison A. (2000) Amphibian and reptiles recorded on the RAP survey in the Wapoga area, Irian Jaya, Indonesia. *A Biological Assessment of the Wapoga River Area of Northwestern Irian Jaya, Indonesia. RAP Bulletin of Biological Assessment 14.* (ed. by A.L. Mack and L. Alonso), pp. 54–57. Conservation International, Washington, DC.

Rittmeyer E.N., Allison A., Gründler M.C., Thompson D.K., & Austin C.C. (2012) Ecological guild evolution and the discovery of the world’s smallest vertebrate. *PLoS ONE*, **7**, 1–11.

Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M. a., & Huelsenbeck J.P. (2012) Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

Rosauer D.F. & Jetz W. (2015) Phylogenetic endemism in terrestrial mammals. *Global Ecology and Biogeography*, **24**, 168–179.

Santos J.C., Coloma L.A., Summers K., Caldwell J.P., Ree R., & Cannatella D.C.

(2009) Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. *PLoS Biology*, **7**, 0448–0461.

Scheffers B.R., Phillips B.L., Laurance W.F., Sodhi N.S., Diesmos A., & Williams S.E. (2013) Increasing arboreality with altitude: a novel biogeographic dimension. *Proceedings of the Royal Society: Biological Sciences*, **280**, 20131581.

Stamatakis A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Toussaint E.F.A., Sagata K., Surbakti S., Hendrich L., & Balke M. (2013) Australasian sky islands act as a diversity pump facilitating peripheral speciation and complex reversal from narrow endemic to widespread ecological supertramp. *Ecology and Evolution*, **3**, 1031–1049.

Toussaint E.F. a, Hall R., Monaghan M.T., Sagata K., Ibalim S., Shaverdo H. V, Vogler A.P., Pons J., & Balke M. (2014) The towering orogeny of New Guinea as a trigger for arthropod megadiversity. *Nature communications*, **5**, 4001.

Tracy C.R., Christian K.A., & Richard Tracy C. (2010) Not just small, wet, and cold: Effects of body size and skin resistance on thermoregulation and arboreality of frogs. *Ecology*, **91**, 1477–1484.

van Ufford A.Q. & Cloos M. (2005) Cenozoic tectonics of New Guinea. *AAPG Bulletin*, **89**, 119–140.

Unmack P.J., Allen G.R., & Johnson J.B. (2013) Phylogeny and biogeography of rainbowfishes (Melanotaeniidae) from Australia and New Guinea. *Molecular*

Mark D. Scherz 31/12/2016 14:31
Deleted:

Phylogenetics and Evolution, **67**, 15–27.

Vences M., Kosuch J., Glaw F., Böhme W., & Veith M. (2003) Molecular phylogeny of hyperoliid treefrogs: Biogeographic origin of Malagasy and Seychellean taxa and re-analysis of familial paraphyly. *Journal of Zoological Systematics and Evolutionary Research*, **41**, 205–215.

Vieites D.R., Wollenb, Erg K.C., Andreone F., Köhler J., Glaw F., & Vences M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 8267–8272.

Weir J.T. (2006) Divergent Timing and Patterns of Species Accumulation in Lowland and Highland Neotropical Birds. *Evolution*, **60**, 842–855.

Williams S.E., Bolitho E.E., & Fox S. (2003) Climate change in Australian tropical rainforests: an impending environmental catastrophe. *Proceedings of the Royal Society of London: Biological sciences*, **27**, 1887–92.

Wollenberg K.C., Vieites D.R., Van Der Meijden A., Glaw F., Cannatella D.C., & Vences M. (2008) Patterns of endemism and species richness in Malagasy cophyline frogs support a key role of mountainous areas for speciation. *Evolution*, **62**, 1890–1907.

Xie W., Lewis P.O., Fan Y., Kuo L., & Chen M.-H.H. (2011) Improving Marginal Likelihood Estimation for Bayesian Phylogenetic Model Selection. *Systematic Biology*, **60**, 150–160.

Yeh J. (2002) The effect of miniaturized body size on skeletal morphology in frogs. *Evolution; international journal of organic evolution*, **56**, 628–41.

Zhuo Z. (1999) Response of altitudinal belts of vegetation to the late quaternary climatic changes in tropical Asia. *Geographical Research*, **18**, 96–104.

Supporting Information.

Appendix S1. Supplementary tables and figures. Specimen numbers, locality information and GenBank accession numbers for *Choerophryne* specimens included in analyses (Table S1); GenBank accession details for outgroup samples (Table S2); and summary data on body, elevational distribution and calling height for *Choerophryne* (Table S3).

Genetic distances data

Supp Figures – 1. Bayestrees

Appendix S2. BEAST input file for ancestral state analyses

Appendix S3. Treefile for chronogram estimated in BEAST with ancestral states.

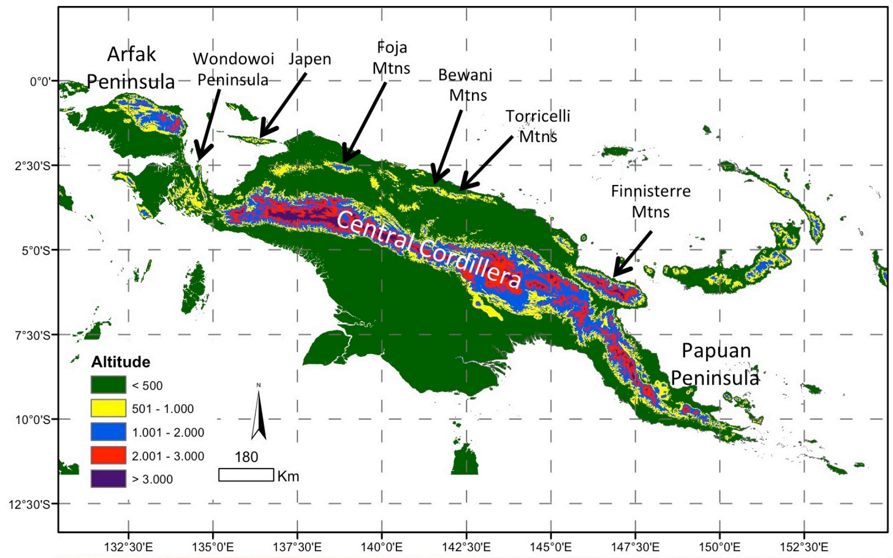
Mark D. Scherz 31/12/2016 14:31
Deleted: s

Mark D. Scherz 31/12/2016 14:32
Comment [15]: These supplemental files need to be submitted as individual tables and figures to comply with PeerJ requirements.

Figure 1. Top. Map of New Guinea stratified by major elevation bands and with key areas of montane forest denoted. Bottom. Images of selected *Choerophryne* species: A) *Choerophyrne alpestris* upper montane moss fields, Central Cordillera, terrestrial; B) *Choerophyrne* spA7 hill forest, southern foothills, scansorial; C) *Choerophyrne* spB1 lower montane forest, Foja Mountains, terrestrial; D) *Choerophyrne proboscidea* hill forest forest, northern lowlands, scansorial. Photographs courtesy S. Richards and T. Laman.

Mark D. Scherz 31/12/2016 14:33
Deleted: .

Mark D. Scherz 31/12/2016 14:33
Deleted: .



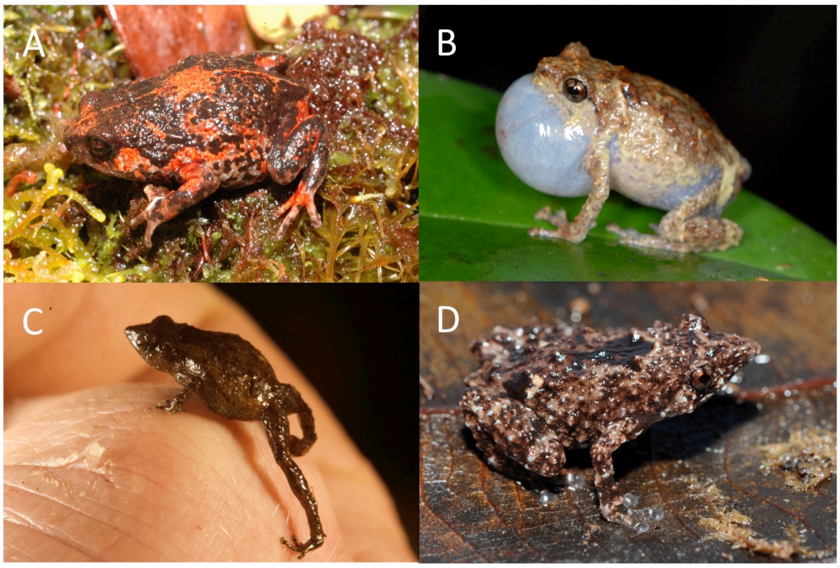


Figure 2. Maximum likelihood tree of *Choerophryne*. * indicates <50% Bayesian posterior probability, interspecific nodes without support values were poorly resolved in both analyses, intraspecific node supports are omitted for clarity. Pictures are scaled to actual size. All pictures taken by S. Richards.

Mark D. Scherz 31/12/2016 14:35
Comment [16]: Nodal support is given in the figure, and PeerJ requires figures have proper titles.

Mark D. Scherz 31/12/2016 14:34
Deleted: L

Mark D. Scherz 31/12/2016 14:34
Deleted: T

Mark D. Scherz 31/12/2016 14:34
Deleted: with bootstrap nodal support above the line and Bayesian posterior probabilities below the line;

Mark D. Scherz 31/12/2016 14:35
Comment [17]: You must give a scalebar if photos are to scale. You don't know what size the figure will be printed at.

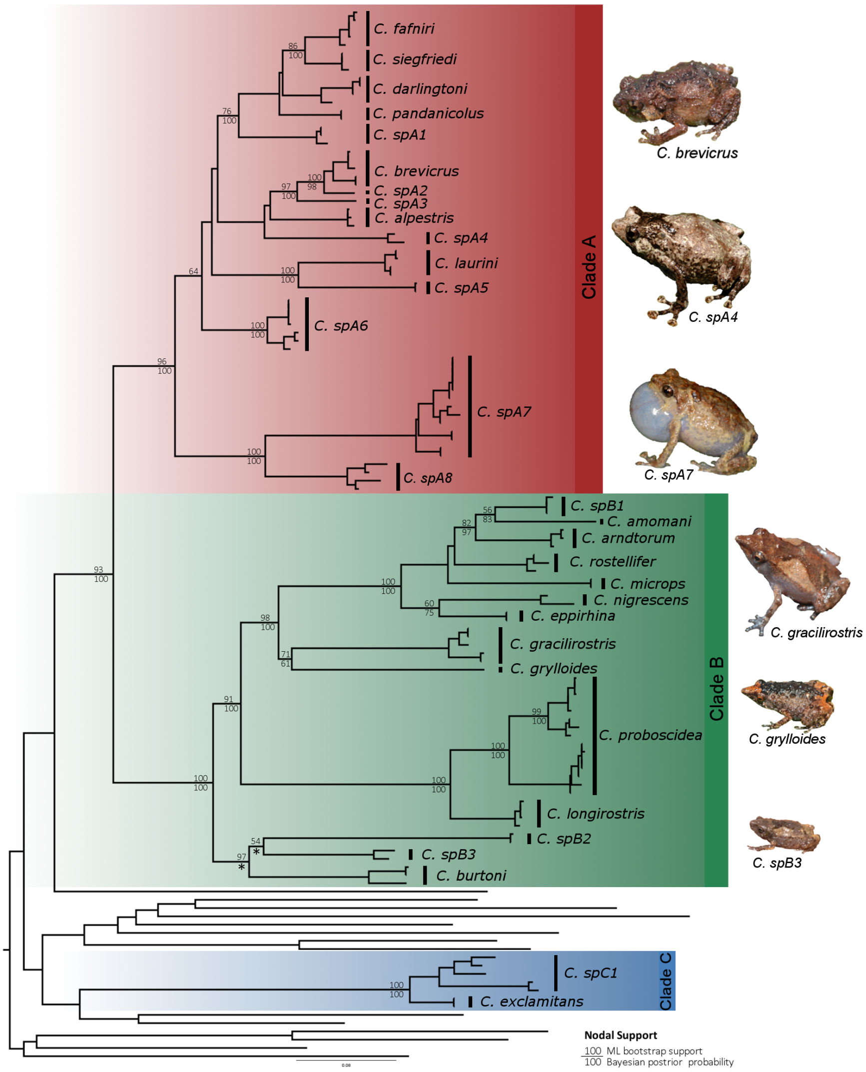


Figure 3. Top. Chronogram for *Choerophryne* and outgroups estimated using 12S and 16S data and rate-based calibration. Node values are Bayesian Posterior Support values from BEAST analysis. Axes along bottom indicate time in millions of years ago. Branches colour coded based on joint estimates of geographic region and elevation. Four taxa under 15mm further identified by an asterisk. Specific ranges in which inferred eccentric (Clade A) and centric (Clade B) endemics in the North

Mark D. Scherz 31/12/2016 14:36
Deleted: ,

Papuan Mountain ranges are annotated. Bottom. Map summarising the main montane areas of New Guinea, and sampling localities for the three major clades of *Choerophyrne*.

Mark D. Scherz 31/12/2016 14:36
Deleted:

Mark D. Scherz 31/12/2016 14:36
Deleted:

Mark D. Scherz 31/12/2016 14:38
Deleted: S

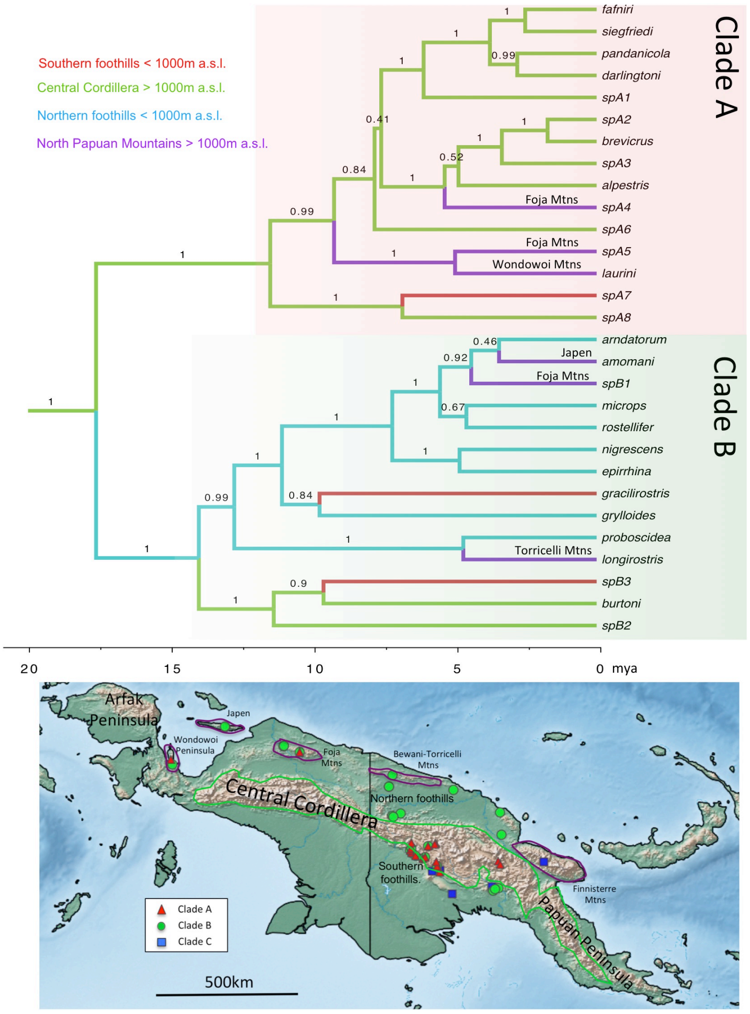


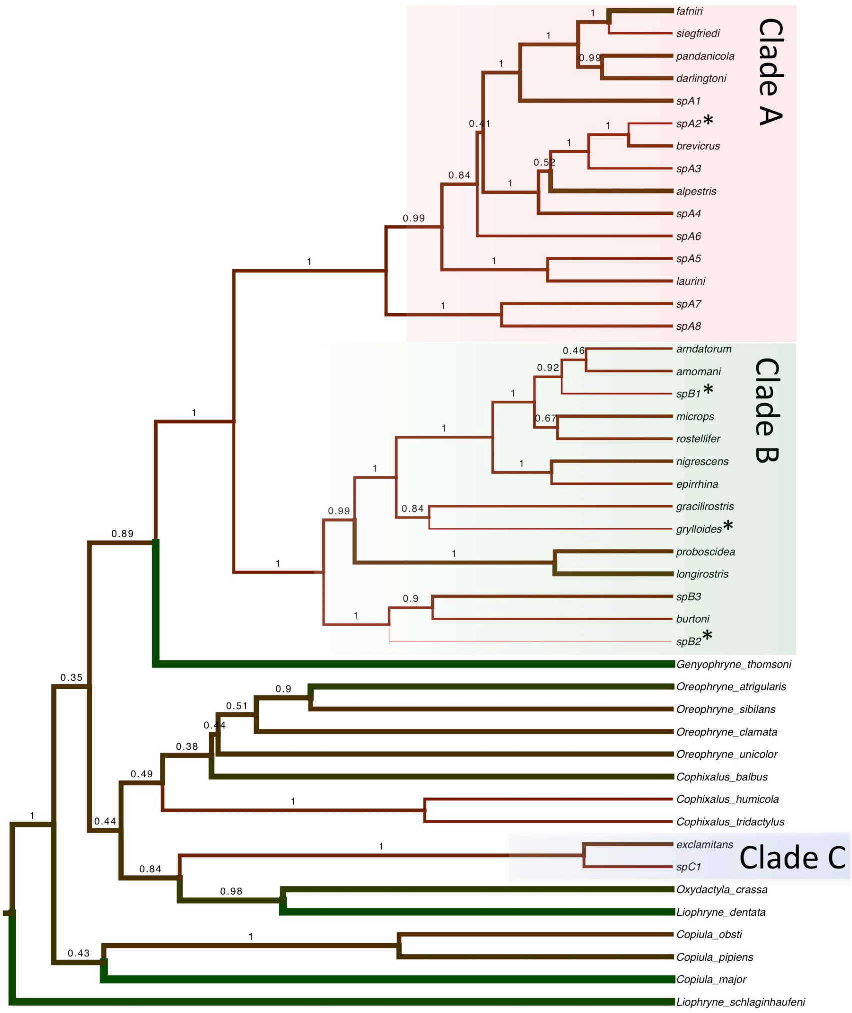
Figure 4. Body size evolution estimated using BEAST. Branch widths are proportional to maximum recorded adult male SVL. Green taxa are larger, red taxa are smaller. Miniaturised taxa (< 15 mm) are indicated with an asterisk.

Mark D. Scherz 31/12/2016 14:40

Comment [18]: Adding the sizes as branch labels would be a nice touch.

Mark D. Scherz 31/12/2016 14:39

Deleted: Optional: There is no scale here - you could pull out the actual size (log size) in figtree and put that against each branch, instead of posterior - maybe as an additional SI figure.


Clade A
Clade B
Clade C
fafniri
siegfriedi
pandanicola
darlingtoni
spA1
spA2*
brevicrus
spA3
alpestris
spA4
spA6
spA5
laurini
spA7
spA8
arndatorum
amomani
spB1*
microps
rostellifer
nigrescens
epirrhina
gracilirostris
grylloides*
proboscidea
longirostris
spB3
burtoni
spB2*
Genyophryne_thomsoni
Oreophryne_atrigularis
Oreophryne_sibilans
Oreophryne_clamata
Oreophryne_unicolor
Cophixalus_balbus
Cophixalus_humicola
Cophixalus_tridactylus
exclamitans
spC1
Oxydactyla_crassa
Liophryne_dentata
Copiula_obsti
Copiula_pipiens
Copiula_major
Liophryne_schlaginhaufeni
1
1
1
0.99
0.41
1
0.52
1
0.84
1
0.99
1
1
1
0.46
0.92
1
0.67
1
1
1
0.84
0.99
1
1
0.9
1
0.89
0.35
0.9
0.51
0.44
0.38
0.49
1
1
0.44
1
0.84
0.98
1
0.43