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

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

[1] Division of Ecology and Evolution, Research School of Biology & Centre for Biodiversity Analysis, Australian National University, Canberra, Australian Capital Territory, Australia

[2] School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia

[3] South Australian Museum, Adelaide, South Australia, Australia

[4] School of Biological Sciences, Flinders University, Adelaide, South Australia, Australia

Corresponding Author: Paul M Oliver
Email address: paul.oliver@anu.edu.au

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 15 mm) 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 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.

1 **Mountain colonisation, miniaturisation and ecological evolution in a radiation of direct-**

2 **developing New Guinea Frogs (*Choerophryne*, Microhylidae)**

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

6 *[1] Division of Evolution and Ecology, Research School of Biology & Centre for Biodiversity*

7 *Analysis, The Australian National University, Building 116, Daley Road, Canberra, Australian*

8 *Capital Territory 2061, Australia*

9 *[2] School of Biological Sciences, University of Adelaide, Adelaide SA 5005, Australia*

10 *[3] South Australian Museum, North Terrace, Adelaide SA 5000, Australia.*

11 *[4] School of Biological Sciences, Flinders University, GPO Box 2100*

12 *Adelaide SA 5001, Australia*

14 Corresponding author: Paul Oliver. Research School of Biology, Australian National University,

15 Building 116, Canberra, 2601, Australia.

16 Email: Paul.oliver@anu.edu.au

18 **Running Header: Mountains and Miniaturised Frogs**

26 **ABSTRACT**

28 **Aims.** Mountain ranges in the tropics are characterised by high levels of localised endemism,

29 often-aberrant evolutionary trajectories, and some of the world's most diverse regional biotas.

30 Here we investigate the evolution of montane endemism, ecology and body size in a clade of

31 direct-developing frogs (*Choerophryne,* Microhylidae) from New Guinea.

32 **Methods.** Phylogenetic relationships were estimated from a mitochondrial molecular dataset

33 using Bayesian and maximum likelihood approaches. Ancestral state reconstruction was used to

34 infer the evolution of elevational distribution, ecology (indexed by male calling height), and

35 body size, and phylogenetically corrected regression was employed to examine the relationships

36 between these three traits.

37 **Results.** We obtained strong support for a monophyletic lineage comprising the majority of taxa

38 sampled. Within this clade we identified one subclade that appears to have diversified primarily

39 in montane habitats of the Central Cordillera (> 1000 m a.s.l.), with subsequent dispersal to

40 isolated North Papuan Mountains. A second subclade (characterised by moderately to very

41 elongated snouts) appears to have diversified primarily in hill forests (< 1000 m a.s.l.), with

42 inferred independent upwards colonisations of isolated montane habitats, especially in isolated

43 North Papuan Mountains. We found no clear relationship between extremely small body size

44 (adult SVL less than 15 mm) and elevation, but a stronger relationship with ecology – smaller

45 species tend to be more terrestrial.

46   **Conclusions.** Orogeny and climatic oscillations have interacted to generate high montane

47   biodiversity in New Guinea via both localised diversification within montane habitats (centric

48   endemism) and periodic dispersal across lowland regions (eccentric endemism). The correlation

49   between extreme miniaturisation and terrestrial habits reflects a general trend in frogs,

50   suggesting that ecological or physiological constraints limit niche usage by miniaturised frogs,

51   even in extremely wet environments such as tropical mountains.

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73   **INTRODUCTION**

74

75   Tropical mountains contain some of the most diverse regional biotas in the world, with high

76   levels of localised endemism and often fine elevational turnover in biodiversity (Mayr &

77   Diamond, 1976; Fjeldså et al., 2012; Merckx et al., 2015; Rosauer & Jetz, 2015). The origins of,

78   and processes shaping, this exceptional diversity are of great scientific interest, both for

79   improved understanding of the drivers of biological diversity (Janzen, 1967; Hutter et al., 2013;

80   Graham et al., 2014), and for understanding how these highly diverse biotas will be affected by

81   anthropogenic climatic change (Williams et al., 2003; La Sorte & Jetz, 2010; Freeman & Class

82   Freeman, 2014).

83   Two broad paradigms to explain high diversity in tropical mountains have been advanced

84   (Fjeldså et al., 2012), and both received support from different studies: a) mountain uplift and

85   climatic change have driven local speciation (the 'cradle' hypothesis; Weir, 2006; Price et al.,

86   2014), or b) mountains have provided refugia, often for specialised taxa that would have

87   otherwise died out due to competition or climatic change (the 'museum' hypothesis; Hutter et al.,

88   2013). In a recent study focused on understanding the biogeographic origins of montane

89   endemics Merckx et al. (2015), suggested they could also be broadly dichotomised into centric

90   endemics (derived from upslope colonisation of lowland taxa) and eccentric endemics (derived

91   via long distance colonisation of cool adapted taxa).

92   The large tropical island of New Guinea has arguably the 'most complex orogeny in the

93 world' (Baldwin et al., 2012). The collision of the leading edge of the northwards-moving

94 Australian plate with the westwards-moving southern edge of the Pacific Plate has uplifted a

95 high Central Cordillera (> 4000 m a.s.l.) extending nearly the length of the island (Baldwin et al.,

96 2012) (Fig. 1A). These ranges may date from the late Miocene, and high elevation habitats are

97 even younger (Hall, 2002; van Ufford & Cloos, 2005; Baldwin et al., 2012). Beginning in the

98 Miocene, and continuing with the ongoing rapid uplift of the Huon and Finnisterre Ranges (Fig.

99 1), additional smaller and more isolated montane regions scattered along northern New Guinea

100 are the uplifted remnants of island arcs that have accreted onto the northern edge of the

101 Australian plate (Hall, 2002; Polhemus, 2007).

102   The biota of New Guinea has been profoundly shaped by this complex orogeny. The

103 uplift of the Central Cordillera has largely isolated the biotas of lowland regions to the north and

104 south of New Guinea (Rawlings & Donnellan, 2003; Unmack et al., 2013; Georges et al., 2014).

105 It has also been suggested that emerging elevation gradients may have increased speciation rates

106 in some New Guinea radiations, inflating regional alpha diversity (Toussaint et al., 2013, 2014),

107 a species pump model similar to the uplift of the northern Andes (Weir, 2006; Santos et al.,

108 2009). In contrast the endemic montane fauna of the smaller, younger and more isolated

109 mountains of northern New Guinea is particularly poorly known, and there have been few

110 phylogenetically-informed assessments of the origins of endemic taxa in these ranges (Beehler et

111 al., 2012; Oliver et al., 2012a, 2016).

112   The New Guinea frog biota is exceptionally diverse, with > 450 recognised species, and

113 many more awaiting description (Oliver et al., 2013; Frost, 2015)—far more diverse than nearby

114 landmasses such as Borneo or Australia. Such anuran diversity is remarkable for being

115 dominated by just two major radiations, of which the most speciose and ecologically diverse is a

116 clade of nearly 250 recognised species of direct-developing microhylids, the Asterophryninae

117 Günther, 1858 (Frost et al., 2006). Their reproductive ecology, wide elevational distribution,

118 high levels of localised endemism, and overall species richness suggest that microhylid frogs

119 may provide an excellent system for understanding how the mountains may have shaped

120 diversification in New Guinea.

121   *Choerophryne* (including the previously recognised genus *Albericus*: see Peloso et al.,

122 2015) is a moderately diverse clade (31 recognised taxa) within the Asterophryninae, comprised

123 of small to miniaturised frogs endemic to New Guinea. This genus occurs from hill to upper

124 montane habitats across much of the Central Cordillera and North Papuan Mountains (although

125 they appear to be largely absent from most of the west and southern lowlands of the island)

126 (Günther, 2000; Richards et al., 2000). Broadly, taxa formerly placed in the genus *Albericus* are

127 mostly climbing frogs with well-developed finger and toe pads, while taxa formerly placed in

128 *Choerophryne* tend to be more terrestrial, although there are exceptions to this general trend

129 (Kraus & Allison, 2000; Richards et al., 2007; Günther & Richards, 2011) (Fig. 2).

130   *Choerophryne* also includes many miniaturised species, here defined as frogs less than 15

131 mm long (Yeh, 2002), some of which approach minimum size limits for tetrapods (Kraus,

132 2010a; Rittmeyer et al., 2012). The water-permeable skin of frogs plays a critical role in shaping

133 both local and regional patterns of diversity and habitat use (Scheffers et al., 2013), with smaller

134 species at greater risk of desiccation than larger species (Tracy et al., 2010). It follows therefore,

135 that smaller size in *Choerophryne* species may be correlated with occurrence in more humid

136 environments. We hypothesise that such a trend may manifest as a negative correlation between

137   body size and altitude, due to the existence of reliably moist cloud forest habitats at higher

138   elevations.

139   Here we present an analysis of the phylogenetic relationships and evolution of key traits

140   within *Choerophryne*. We initially focus on the origins of montane endemism, with a specific

141   prediction being that the older Central Cordillera will be dominated by *in situ* diversification

142   processes (centric endemism) linked to ongoing uplift, while the younger North Papuan

143   mountains may show evidence of colonisation from the older Central Cordillera (eccentric

144   endemism). We also test the prediction that ecological shifts (arboreal to terrestrial), and shifts in

145   body size (towards extreme miniaturisation) may correlate with occurrence in novel habitats and

146   climatic regimes at higher elevations.

147

148   **METHODS**

149

150   **Specimen Selection**

151

152   This study utilised whole specimens and tissue samples deposited in Museum collections (ethics

153   approval was therefore not required). Full details of samples included are given in Tables S1–2.

154   In our final analysis we included 18 of 31 recognised species, plus 11 candidate taxa. Following

155   Vieites et al. (2009) we recognised lineages as distinct OTUs (candidate species) for downstream

156   analysis if they met any two of the following three criteria: a) males with distinctive

157   advertisement calls, b) evidence of morphological differentiation or c) evidence of genetic

158   differentiation (usually greater than 3% uncorrected pairwise in the 16S rRNA gene (see Table

159   S3 for a summary). Mitochondrial DNA sequences of an additional 11 *Choerophryne* were

160   downloaded from GenBank, along with 14 outgroup sequences from 6 other New Guinean

161   microhylid genera. The taxonomic assignation of *Choerophryne* species is challenging,

162   especially in the absence of calls, so taxonomic designations used in this study should be

163   considered provisional. It is possible further work may demonstrate that some candidate species

164   are conspecific with additional recognised taxa. Full details of samples included are given in

165   Appendix S1.

166

167

168   **DNA extraction, amplification, sequencing and alignment**

169

170   Whole genome DNA was extracted from frozen or alcohol preserved liver samples using the

171   Gentra Puregene kit protocol (QIAGEN 2011). Sequence data from the 12S and 16S

172   mitochondrial genes was PCR amplified with an annealing temperature of 58°C using the

173   primers 12SAL and 12SBH (Palumbi et al., 2002) and 16SL3 and 16SAH (Vences et al., 2003),

174   then purified on MultiScreen PCR$_{384}$ Filter Plates. Sanger sequencing (forward and reverse) of

175   purified PCR product used the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied

176   Biosystems), purified using MultiScreen SEQ$_{384}$ Filter Plates and sent to the Australian Genome

177   Research Facility (AGRF) for capillary separation.

178   Geneious Pro v5.5.2 (Kearse et al., 2012) was used to align forward and reverse sequence

179   traces and reviewed by eye. The consensus sequences along with sequences from GenBank

180   (Appendix S1) were aligned with 8 iterations of the MUSCLE algorithm under default parameter

181   settings (Edgar, 2004). Hypervariable regions with poor local alignment were removed using

182 Gblocks v0.91b (Castresana, 2000); of the original 1556 aligned positions, 1347 were retained in

183 final analyses.

184

185 **Phylogenetic Analysis**

186

187 To assess congruence of topology and support values across methods, we estimated phylogenetic

188 relationships using Bayesian and maximum likelihood approaches. Based on the output of the

189 model selection program MrModeltest (Nylander, 2004) all analyses were performed using the

190 general time-reversible model, allowing for variation in the rate of evolution among sites and

191 including invariable sites (GTR+I+G). Both genes were treated as a single partition due to the

192 relatively short sequence length and similar features (i.e. mitochondrial rRNA).

193   The maximum likelihood tree with bootstrap values was produced using RAxML v

194 8.0.26 (Stamatakis, 2006) with bootstrap scores calculated using the rapid bootstrap (-f a)

195 function with 1000 replicates. The Bayesian consensus tree was generated by Mr Bayes 3.2.2

196 (Ronquist et al., 2012) using an unconstrained branch length prior, 4 chains (incrementally

197 heated at temperature 0.2), each of 5 million generations with a 1 million generation burn-in and

198 sampling every 200 generations.

199   These topology-only analyses with dense sampling across populations were compared to

200 analyses where we simultaneously estimated phylogeny, divergence dates and trait evolution on

201 species-level trees (see below).

202

203 **Trait and biogeographical scoring**

204

205   We scored each taxon for three traits of interest: i) adult male body size, ii) elevation and iii)

206   maximum calling height of males (as a proxy for arboreality vs terrestriality) (Table S4). These

207   data were scored from genotyped specimens and associated field notes, or extracted from

208   primary literature.

209   We used a typical measure of size in anurans, the distance from the tip of the snout to the

210   urostyle tip (SUL), which has been previously used in *Choerophryne* (Günther, 2008). We used

211   the maximum recorded size for males (sex determined by observations of specimens calling).

212   Although some *Choerophryne* have unusually long snouts, at most these comprised 10% of the

213   total body length.

214   The maximum elevational range (difference between lower and upper occurrences)

215   obtained for any species was just over 1000 metres, involving three taxa that occur primarily in

216   hill forests, but range into lower montane forests (*Choerophryne gracilirostris*, *C. rostellifer* and

217   *C.* sp A7). Seven taxa are also only known from single sites. To score elevation as a continuous

218   character (for use in phylogenetic regressions) we used the mid-point of records for each lineage

219   (to the nearest 100 m).

220   For discrete categorisation of elevation we used the forest classification system presented

221   by Johns (1982): hill forest and lowlands (< 1000 m a.s.l.), lower montane (1000–2000m a.s.l.),

222   mid-montane (2000–3000 m a.s.l.) and upper montane (> 3000m a.s.l.). These bands broadly

223   reflect how reducing mean temperatures with elevation shapes the transition from megathermal

224   to microthermal vegetative communities (Nix, 1982). For most taxa, the majority of records were

225   focused in just one of these bands. The small number of taxa whose distributions spanned bands

226   were placed in the band in which the majority of records were concentrated. Finally,

227   *Choerophryne laurini* is known only from typical lower montane forest on mossy ridge tops in

228   the Wondowoi mountains between 800-950 metres, although it may also occur in lower montane

229   forest in the Snow Mountains (Richards et al. 2015). This species was coded as lower montane

230   for discrete analyses.

231   To better visualise potential colonisation paths to the isolated North Papuan Mountains,

232   we also devised a further coding system of four states that combined geography and elevation:

233   southern lowland (south of Central Cordillera below 1000 m a.s.l.), central highlands (Central

234   Cordillera above 1000 m a.s.l.), northern lowland (south of Central Cordillera below 1000 m

235   a.s.l.) and northern montane (North Papuan Mountains above 1000 m a.s.l).

236   Male *Choerophryne* show extensive variation in their typical calling height, from largely

237   terrestrial (e.g. *Choerophryne alpestris*) to more than 3 metres off the ground (e.g. *Choerophryne*

238   *pandanicola*) (Günther & Richards, 2011). To score calling height as a continuous trait we used

239   the maximum recorded calling height of males, either from the literature or personal

240   observations. We also employed a second scheme for coding calling ecology, by dividing taxa

241   into two broad guilds: a) *Terrestrial* - species that called predominantly on or very close to the

242   ground on leaf litter or duff (generally less than 50 cm high); and b) *Scansorial* - species that

243   usually climb into vegetation and call from (generally more exposed) positions up to several

244   metres high. Two taxa (*C. arndtorum* and *C. microps*) for which the majority of calling records

245   are terrestrial but which have occasionally been recorded calling a metre or more above the

246   ground (Günther, 2008), were coded as terrestrial in the discrete character analyses, while the

247   maximum recorded calling height was used in continuous trait based analyses.

248

249   **Ancestral state analyses.**

250

251   We used BEAST v 1.8.2. (Drummond & Rambaut, 2007) to co-estimate trait evolution

252   (including ancestral states) with phylogeny and divergence dates. These analyses used a reduced

253   dataset comprising a single exemplar of each genetically and/or morphologically divergent

254   lineage identified in earlier phylogenetic analyses (i.e. recognised or candidate species). The

255   original molecular data for each exemplar was also included. To ensure these analyses were

256   focused on a strongly supported and well-sampled monophyletic group, in these trait analyses we

257   excluded two samples from a highly divergent clade that did not strongly associate with other

258   *Choerophryne* in estimated phylogenies (see results). Size was $\log_{10}$ transformed. The two

259   discrete variables (elevation and calling ecology) were coded using the MK + strict clock model,

260   which assumes that transformations between states are reversible and occur at the same rate

261   throughout the tree; more complex models were not feasible due to the relatively small tree and

262   number of transformations. Elevation character states were ordered–e.g. shifts from lower- to

263   upper-montane habitats were constrained to involve moving through intervening mid-montane

264   habitats. Analyses were run for 50 million generations, sampling every 50,000 generations. The

265   first 20% of trees were discarded as burnin and the remaining 800 post-burnin trees from each

266   run were combined to generate the final consensus topology. The final xml file is in Appendix

267   S2. Effective samples sizes (ESS) for all parameters (Tracer v 1.6.0; Drummond & Rambaut,

268   2007) in both individual and combined BEAST analyses were above 200.

269   BEAST automatically produces an ultrametric tree, however there are no fossil

270   calibrations within *Choerophryne,* and there has been no recent thorough assessment of rates of

271   mitochondrial DNA evolution in frogs. To provide a rough timescale for *Choerophryne,* we used

272   a broad consensus molecular evolutionary rate for mitochondrial genes of between 1–2%

273   pairwise divergence per million years, which was incorporated into the prior for the average

274   substitution (clock) rate. Rates of molecular variation vary extensively (Eo & DeWoody, 2010),

275   and thus the resultant dates from this analysis are interpreted with caution. Importantly, the

276   ancestral state analyses (above) only require relative rather than absolute branch lengths (e.g.

277   they could still be performed if root age was arbitrarily scaled to 1), so our results are robust to

278   these dating uncertainties.

279

280   **Phylogenetic Least Squares Regression**

281

282   The relationship of a) body size to calling ecology and/or elevation, and b) calling ecology to

283   elevation was analysed using BayesTraits v 2.0 (Pagel & Meade, 2013) across the concatenated

284   3200 post-burnin trees from BEAST. For these analyses all variables were included as $\log_{10}$-

285   transformed continuous states. We only included data for lineages in two well-sampled clades of

286   *Choerophryne* that were strongly supported as sister taxa (see below); other species in the trees

287   were scored as missing data. We also performed regressions on each of these two well-

288   differentiated clades. The Bayesian MCMC implementation of the continuous module was used

289   to regress a) body size against ecology and elevation; and b) ecology against elevation. Eleven

290   million steps were used with the first 1 million discarded for burnin, and 4 runs of BayesTraits

291   were performed and checked for convergence using Tracer v 1.6.0 (Drummond & Rambaut,

292   2007). Pagel & Meade (2013) state that the significance of a variable can be assessed either by

293   comparing harmonic means (for analyses with and without the variable), or observing whether

294   the estimated distribution of that variable (e.g. 95% HPD) excludes 0. Due to issues around the

295   use of harmonic means to estimate marginal likelihoods (Xie et al., 2011), we adopted the latter

296   approach.

297

298   **RESULTS**

299

300   **Phylogenetic relationships and lineage diversity.**

301

302   Bayesian and maximum likelihood analyses identified three major lineages of *Choerophryne*

303   (Fig. 3, Fig. S1). Clade A comprised the majority of sampled taxa that were formerly placed in

304   the genus *Albericus*, Clade B included all taxa with a moderate to pronounced rostral projection

305   formerly placed in *Choerophryne sensu stricto*. Clade C comprised two scansorial taxa lacking

306   distinctive rostral projections that occur south of the Central Cordillera in hill forest, and on the

307   Finnistere Ranges (north-east New Guinea) in hill to lower montane forest respectively.

308   A sister taxon relationship between Clades A and B was strongly supported in all

309   analyses (Posterior Probability 1.0, bootstrap support >90). Clade C was more divergent and

310   there was no evidence that it forms the sister group to Clade A+B (or any other microhylid

311   lineage). All basal relationships between the sampled New Guinea microhylid genera were

312   poorly supported, but these were not the focus of this study.

313   Within Clade A we identified two strongly supported primary lineages, with the major

314   split being between a clade of two lower montane and hill forest taxa from the south side of the

315   Central Cordillera, and several clusters of species from across the Central Cordillera and North

316   Papuan Mountains, including derived terrestrial taxa from mid to upper montane habitats (*C.*

317   *alpestris* and *C. brevicrus*).

318   Within Clade B there were three well supported primary lineages: one comprising three

319   deeply divergent taxa (*C. burtoni* and two unnamed taxa) from hill forest to mid-montane

320   habitats on the Central Cordillera; a further lineage of large-bodied and very long-snouted taxa

321   from hill and lower montane forest in northern New Guinea; and finally a diverse

322   conglomeration including lineages from hill and lower montane forests in northern New Guinea,

323   in addition to one taxon from south of the Central Cordillera (*C. gracilirostris*).

324   In all three major clades we identified lineages (candidate species) that were deeply

325   divergent from, and could not be confidently assigned to, recognised species. This was most

326   pronounced in Clade A, which includes a number of scansorial species that are difficult to

327   diagnose on the basis of external morphology.

328

329   **Ancestral States analyses**

330

331   The dated species tree for ancestral states analysis (Figs. 4–5) was congruent with our densely-

332   sampled, undated molecular phylogeny (Fig. 3). Character states for Clade C were not included

333   in most ancestral state analyses due to phylogenetic uncertainty and the relatively small number

334   of lineages. These analyses highlighted the contrasting evolutionary trajectories of the two 'core'

335   clades of *Choerophryne* (A & B). In all analyses including elevation, hill forest habitats (largely

336   distributed between 0–1000 m a.s.l.) were inferred as the ancestral habitat for both Clades A and

337   B. Clade A was inferred to have diversified primarily within montane habitats during the late

338   Miocene (14 out of 15 nominal taxa), including more recent upslope shifts into mid and upper-

339   montane zones (Fig. S2). Independent colonisation from eccentric origins to the North Papuan

340   Mountains is inferred when geography is included (especially in the Foja Mountains) (Fig. 4A).

341   In contrast Clade B was centred on hill forest habitats, but with 2–4 relatively recent upslope

342   (eccentric) shifts into montane habitats in mostly distantly related taxa, again mainly occurring in

343 isolated North Papuan Mountains (specifically Japen Island, and the Foja and Torricelli

344 Mountains) (Fig. 4B, Fig. S2).

345 Miniaturised species (<15mm) occurred across the phylogeny (Fig. 5), implying that

346 multiple lineages of *Choerophryne* have independently evolved very small body size. Taxa in the

347 predominantly scansorial Clade A tended to be larger than those in the more terrestrial clade B.

348 Calling ecology was relatively labile across the genus, with multiple shifts between

349 terrestrial and scansorial calling, the latter being inferred as the ancestral state for the common

350 ancestor of clades A and B (Fig. S2). However, there were contrasting patterns across the two

351 clades. Clade A was inferred as largely scansorial with a small number of shifts towards

352 terrestrial calling, Clade B included a majority of taxa (9 out of 14) that call from on, or close to,

353 the ground (this state was accordingly inferred as ancestral) with 3 transitions to scansorial

354 calling.

355

356 **Phylogenetic Regressions**

357

358 All BayesTraits runs converged well before the burnin, and the concatenated runs yielded ESS of

359 all parameters >1000. In the analysis relating body size to ecology and/or elevation, both ecology

360 and elevation (considered together following Pagel & Meade 2013) exhibited significant

361 phylogenetic structure (Lambda for all taxa was significantly positive: mean 0.55; 95% HPD =

362 0.12, 0.98). Ecology (as indexed by calling height) was positively associated with body size in

363 the all taxa analysis (mean= 0.09, 95% HPD = 0.03, 0.15). In analyses focusing on specific

364 clades this relationship was also positive, although the HPD included zero for Clade A (mean=

365 0.1; 95% HPD = -0.01, 0.19), but not Clade B (mean= 0.1; 95% HPD = 0.01, 0.20).

366   Elevation was not strongly related to body size in all relevant analyses, with a regression

367   coefficient centred almost exactly on 0 when all taxa were included (mean = 0.01, 0. 95% HPD =

368   -0.11, +0.11). Analyses of the two main clades showed positive and negative relationships,

369   although in both cases the HPD again included 0, suggesting the relationships were weak or

370   insignificant (Clade A mean= 0.24, 95% HPD = -0.06, 0.5; Clade B mean= -0.05, 95% HPD = -

371   0.20, 0.08).

372   Calling height was weakly negatively related to elevation, although in all cases the HPD

373   again spanned zero (all taxa mean= -0.65, 95% HPD = -1.27, 0.04; Clade A mean= -1.1, 95%

374   HPD = -2.49, 0.15; Clade B mean= -0.74, 95% HPD = -1.62,-0.03). Removal of three high

375   elevation (>2500 m a.s.l.) taxa in Clade A that live in mossy grasslands where there are few

376   arboreal habitats weakened this relationship further, resulting in a 95% highest probability

377   posterior distribution that more broadly included 0 (mean= -0.47, 95% HPD = -1.06, 0.16).

378

379   **DISCUSSION**

380

381   Despite the biological wealth and high endemism of the New Guinea Mountains (Tallowin et al.

382   2016) and emerging evidence for major evolutionary radiations (Toussaint et al., 2014; Givnish

383   et al., 2015), only a small number of phylogenetic studies of lineages with distributions centred

384   on the montane regions of New Guinea have been published (Meredith et al., 2010; Toussaint et

385   al., 2013; Irestedt et al., 2015). Our study complements the recent work focusing on volant or

386   large-bodied taxa, by presenting data for a lineage of small, direct-developing frogs that may be

387   presumed to have comparatively low vagility.

388

389 **Species diversity and phylogeny**

390

391 Molecular assessments of amphibian diversity on tropical islands over the last decade have

392 revealed exceptionally high levels of previously unrecognised diversity (Meegaskumbura et al.,

393 2002; Vieites et al., 2009). However, while New Guinea already has the most diverse insular

394 frog fauna in the world (over 450 recognised species; Frost, 2015), molecular assessments of

395 frog diversity in this region are scarce. While taxonomy was not the focus of this study, we

396 uncovered 12 candidate species (although it remains possible that further work will show some

397 of these to be conspecific with recognised taxa), in addition to three new taxa recently named

398 (Iannella et al. 2014; 2015). Molecular studies of other New Guinea microhylid frogs

399 (*Mantophryne*) have also revealed a diversity of deeply divergent lineages (Oliver et al., 2013),

400 and further fieldwork and integration of molecular, morphological and acoustic analyses seem

401 certain to cement New Guinea's position as a global hotspot of amphibian diversity.

402 Clades A and B together formed a strongly supported monophyletic group, but the overall

403 monophyly of all three sampled lineages of *Choerophryne* was not strongly supported (or

404 rejected). There are however morphological synapomorphies uniting all three lineages of

405 *Choerophryne* (see Burton & Zweifel, 1995), and their monophyly was also recently supported

406 based on a phylogenomic study including examplars of all three major lineages (Peloso et al.,

407 2015). The non-monophyly of *Choerophryne* in our analyses could be an artefact of rapid

408 diversification and/or the short rapidly saturating loci used in this study. Resolution and further

409 discussion of the phylogeny and generic taxonomy of *Choerophryne* will require larger nuclear

410 gene-based datasets and sampling of taxa from other regions of New Guinea. However because

411   of uncertainty in basal relationships, in this study we focused ancestral state analyses on the well-

412   sampled and supported clades A and B.

413   There were distributional gaps in our genetic sampling (Fig. 4B). However, recent

414   surveys in western New Guinea (upper Mamberamo, Fakfak mountains) have indicated that

415   *Choerophryne* (which are usually easy to locate) are absent or rare, suggesting this disjunction

416   reflects genuine absence (Günther, 2000; Richards et al., 2000). Another gap is the Papuan

417   Peninsula, where endemic *Choerophryne* are found (Fig. S3). However, none of these taxa are

418   shared with central New Guinea, suggesting that taxa in this region—which is geologically very

419   distinctive—will have their own history. Furthermore, while future addition of taxa from this

420   region into phylogenetic datasets is a research priority, we consider it unlikely to change the

421   broadly reciprocal patterns of elevational distribution and montane colonisation between clades

422   A and B in central New Guinea that we discuss below.

423

424   **Complex origins of montane endemism**

425

426   Uplifting tropical mountains have been shown to be 'cradles' of young diversity in diverse

427   regional bird communities (Weir, 2006; Price et al., 2014). Recent work on beetles, mammals

428   and birds has suggested a similar association between diversification and the recent uplift of

429   mountains in New Guinea (Meredith et al., 2010; Toussaint et al., 2014; Irestedt et al., 2015). In

430   this study we complement such work by providing the first molecular phylogeny of a vertebrate

431   clade (Clade A within *Choerophryne*) that is both moderately diverse (15 nominal taxa), and

432   almost entirely endemic to the New Guinea Highlands (>1000 m a.s.l.). Our phylogeny suggests

433   Clade A colonised lower montane habitats first, possibly by the mid-Miocene, while higher

434   altitude taxa (i.e. > 2000 m a.s.l.) in Clade A are younger (Pliocene origin). This pattern is

435   broadly consistent with progressive upslope colonisation as the Central Cordillera gained height

436   through the late Miocene, and during the Pliocene, suggesting that recent mountain uplift has

437   played a key role in the diversification of this lineage.

438   On the other hand we find weak evidence that New Guinea mountains have functioned as

439   a 'museum'. One potential example from *Choerophryne* is a clade in the Central Cordillera

440   region (*burtoni*, *sp*B2 and *sp*B3) that shows outwardly disjunct distributions and deep

441   divergences (estimated 10 mya in our analyses). However, when compared to deeply divergent

442   relict bird lineages or high phylogenetic endemism of mammals (Jønsson et al., 2010; Rosauer &

443   Jetz, 2015) in the New Guinea mountains, our data do not at this stage provide strong evidence

444   that relict taxa have inflated montane diversity in *Choerophryne*.

445   A further striking result of this study is the inference of both centric and eccentric origins

446   of montane diversity in the younger, lower elevation, more isolated and poorly known North

447   Papuan Ranges. These ranges are home to numerous endemic taxa or isolated populations

448   (Richards et al., 2009; Oliver et al., 2011, Oliver et al. 2012a,b, 2016; Beehler et al., 2012), but

449   in most cases these are clearly related to, or even conspecific with, montane taxa occurring

450   elsewhere in New Guinea (e.g. 100% of birds are allopatric isolates of lineages occurring in

451   montane habitats elsewhere; Beehler et al., 2012). In *Choerophryne* two lineages in Clade A

452   show a similar pattern; they appear to be endemic to montane habitats in the north Papuan

453   Mountains (not found below around 1000 m a.s.l.), related to taxa otherwise known only from

454   montane Central Cordillera habitats, and unknown from the intervening lowlands (Richards &

455   Suryadi, 2003). This apparent pattern of eccentric origins suggest that lower montane forests in

456   New Guinea have a dynamic climatic history, including periods of major elevational depression

457   similar to those inferred elsewhere in the tropics (Colinvaux et al., 1996; Zhuo, 1999).

458   However, ancestral state analyses of our well-sampled Clade B, also provides strong

459   evidence for at least two and potentially three independent derivations of North Papuan montane

460   endemics from surrounding lowland taxa (centric endemism) (Fig. 4A). Detailed fine scale

461   sampling is required to understand the processes that have shaped this endemism; elevational

462   segregation may be an outcome rather than a driver of speciation (Caro et al., 2013; Freeman,

463   2015). However, regardless of the exact process, this represents the first strong evidence that

464   endemic montane vertebrates have arisen *de novo* in northern New Guinea from largely lowland

465   lineages. These contrasting origins of endemism suggest that the young and isolated North

466   Papuan Mountains may provide excellent opportunities for comparative analyses of the

467   processes driving montane endemism in young tropical mountains.

468   Finally, mountain uplift may also inflate regional diversity at lower elevations by

469   isolating formerly continuous populations of lowland taxa (vicariance). In New Guinea there is

470   already compelling evidence that the uplift of the Central Cordillera has isolated northern and

471   southern vicars in lowland and aquatic taxa (Rawlings & Donnellan, 2003; Georges et al., 2014),

472   and potentially also lower montane taxa (Irestedt et al., 2015). However, our sampling of

473   *Choerophryne* did not reveal extensive north-south vicariance, although one possible exception

474   is a recently described pair of potential sister taxa in Clade B from hill and lower montane forest;

475   *C. gracilirostris* (south) and *C. grylloides* (north) that are estimated to have diverged around 10

476   mya. This general lack of signal for north-south vicariance is unsurprising given the majority of

477   species in the two clades are associated with hill and montane forest, and are less likely to be

478   isolated by mountain uplift than lowland or aquatic taxa.

479

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

481

482 A number of anuran lineages that approach the minimum size limits for vertebrates have been

483 recently described (Wollenberg et al., 2008; Kraus, 2010a; Rittmeyer et al., 2012, Lehr &

484 Coloma, 2008; Kraus, 2010, 2011; Wollenberg et al., 2011; Rittmeyer et al., 2012)**,** and it has

485 been suggested that miniaturised frogs may represent an often overlooked, but important

486 ecological guild in tropical areas (Rittmeyer et al., 2012). Broadly, three patterns are globally

487 apparent in miniaturised frogs: most lack a free-swimming tadpole stage (Estrada & Hedges,

488 1996); occur in wet tropical and usually insular regions; and are more-or-less terrestrial (Kraus,

489 2010a; Rittmeyer et al., 2012). Across the six different genera of Papuan microhylids that

490 contain miniaturised taxa (*Aphantophryne*, *Austrochaperina*, *Choerophryne*, *Cophixalus*,

491 *Oreophryne* and *Paedophryne*) all three of these correlates are evident.

492   Our analyses further indicate that within *Choerophryne* there have been at least three

493 relatively recent shifts towards extremely small body size (three lineages with SUL ~15 mm or

494 less), all of which are inferred in lineages that call on or close to the ground. This plasticity of

495 body size and ecology of *Choerophryne* contrasts with conservatism of these same features in

496 another miniaturised genus of Papuan microhylids, *Paedophryne* (Rittmeyer et al. 2012).

497 Patterns of evolution across both genera do however strongly support the hypothesis that

498 physiological or ecological constraints limit miniaturised taxa to a terrestrial lifestyle. Most

499 recognised taxa missing from our analyses are moderate sized (SUL > 15 mm) and scansorial,

500 and likely belong in Clades A and C. Their inclusion in our analyses is unlikely to change the

501 correlation between terrestriality and small size.

502   Counter to our initial prediction, we did not find a strong positive correlation between

503   elevation and either ecology (calling height) or body size, as might be expected if desiccation

504   risk is decreased at higher elevations (Scheffers et al., 2013). This lack of pattern may indicate

505   that for frogs of extremely small size, physiological or ecological pressures associated with

506   microhabitat use are a greater constraint on body sizes than elevation-related variation in

507   climates. Unlike the correlation between terrestriality and small size in which we are confident,

508   and which mirrors a broader pattern, further analysis including both *Choerophryne* taxa missing

509   from our dataset, and other microhylid genera is probably needed to refine understanding of the

510   potentially much more nuanced relationships between body size, ecology and elevation.

511   Finally, *Choerophryne* provides a striking example of an insular frog lineage that has

512   undergone ecological diversification, with repeated shifts between scansorial and relatively

513   terrestrial ecologies, reflected in significant reduction or even loss of terminal discs and

514   shortening of limbs (Günther, 2008; Kraus, 2010b; Günther & Richards, 2011). Similar

515   ecological diversity and morphological plasticity has also been observed in other microhylid

516   lineages in New Guinea, as well as in other island systems such as Madagascar and the

517   Philippines (Andreone et al., 2005; Köhler & Günther, 2008; Blackburn et al., 2013). In contrast,

518   microhylids generally seem to be peripheral (and usually terrestrial or fossorial) components of

519   frog diversity in continental regions (see Duellman, 1999).

520

521   **CONCLUSIONS**

522

523   Our new phylogeny and ecophenotypic data for the microhylid frog genus *Choerophryne*

524   indicates that montane areas have been colonised via a complex suite of biogeographic

525   processes, especially upslope colonisation and speciation in presumably novel highland habitats

526   and dispersal between montane islands, and that the relative importance of these processes has

527   differed across even closely related lineages. *Choerophryne* also shows a correlation between

528   extremely small size and utilisation of terrestrial habitats, mirroring a global pattern that suggests

529   that, in frogs, ecological or physiological constraints largely limit extremely miniaturised taxa to

530   terrestrial microhabitats in tropical areas.

531

532   **ACKNOWLEDGEMENTS**

533   We thank the numerous landholders in New Guinea for access to their land, the National

534   Research Institute and Department of Environment and Conservation (now Conservation and

535   Environment Protection Authority) in Papua New Guinea for research and export approvals, the

536   South Australian Museum for access to material in their care, and to the numerous other research

537   organisations and NGOs that facilitated the collection and examination of material used in this

538   study. We thank Mark Scherz and an anonymous reviewer for their extensive helpful comments

539   on earlier versions of this manuscript.

540

541   **REFERENCES**

542   Andreone F., Vences M., Vieites D.R., Glaw F., & Meyer A. (2005) Recurrent ecological

543   adaptations revealed through a molecular analysis of the secretive cophyline frogs of

544   Madagascar. *Molecular Phylogenetics and Evolution*, **34**, 315–322.

545   Baldwin S.L., Fitzgerald P.G., & Webb L.E. (2012) Tectonics of the New Guinea Region.

546   *Annual Review of Earth and Planetary Sciences*, **40**, 495–520.

547   Beehler B., Diamond J., Kemp N., Scholes E., Milensky C., & Laman T. (2012) Avifauna of the

548   Foja Mountains of western New Guinea. *Bulletin of the British Ornithologist's Club*, **132**,

549   1–18.

550   Blackburn D.C., Siler C.D., Diesmos A.C., Mcguire J.A., Cannatella D.C., & Brown R.M.

551   (2013) An adaptive radiation of frogs in a southeast asian island archipelago. *Evolution*, **67**,

552   2631–2646.

553   Burton T.C. & Zweifel R.G. (1995) A new genus of genyophrynine microhylid frogs from New

554   Guinea. *American Museum Novitates*, **3129.**

555   Caro L.M., Caycedo-Rosales P.C., Bowie R.C.K., Slabbekoorn H., & Cadena C.D. (2013)

556   Ecological speciation along an elevational gradient in a tropical passerine bird? *Journal of*

557   *Evolutionary Biology*, **26**, 357–374.

558   Castresana J. (2000) Selection of conserved blocks from multiple alignments for their use in

559   phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540–552.

560   Colinvaux P.A., Liu K.-B., Oliveira P., Bush M.B., Miller M.C., & Kannan M.S. (1996)

561   Temperature depression in the lowland tropics in glacial times. *Climatic Change*, **32**, 19–33.

562   Drummond A.J. & Rambaut A. (2007) BEAST: Bayesian evolutionary analysis by sampling

563   trees. *BMC evolutionary biology*, **7**, 214.

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

565   Press, Baltimore.

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

567   Eo S.H. & DeWoody J.A. (2010) Evolutionary rates of mitochondrial genomes correspond to

568   diversification rates and to contemporary species richness in birds and reptiles. *Proceedings*

569   *of the Royal Society of London: Biological sciences*, **277**, 3587–3592.

570   Estrada A.R. & Hedges S.B. (1996) At the Lower Size Limit in Tetrapods: A New Diminutive

571   Frog from Cuba (Leptodactylidae: *Eleutherodactylus*). *Copeia*, **4**, 852–859.

572   Fjeldså J., Bowie R.C.K., & Rahbek C. (2012) The Role of Mountain Ranges in the

573   Diversification of Birds. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 249–

574   265.

575   Freeman B.G. (2015) Competitive Interactions upon Secondary Contact Drive Elevational

576   Divergence in Tropical Birds. *The American Naturalist*, **186**, 470–479.

577   Freeman B.G. & Class Freeman A.M. (2014) Rapid upslope shifts in New Guinean birds

578   illustrate strong distributional responses of tropical montane species to global warming.

579   *Proceedings of the National Academy of Sciences*, **111**, 4490–4494.

580   Frost D.R. 2014. (2015) Amphibian Species of the World. Website:

581   http://research.amnh.org/vz/herpetology/amphibia/

582   Frost D.R., Grant T., Faivovich J., Bain R.H., Haas A., Haddad C.F.B., De Sá R.O., Channing

583   A., Wilkinson M., Donnellan S.C., Raxworthy C.J., Campbell J. a., Blotto B.L., Moler P.,

584   Drewes R.C., Nussbaum R. a., Lynch J.D., Green D.M., & Wheeler W.C. (2006) The

585   Amphibian Tree of Life. *Bulletin of the American Museum of Natural History*, **297**, 1–291.

586   Georges A., Zhang X., Unmack P., Reid B.N., Le M., & Mccord W.P. (2014) Contemporary

587   genetic structure of an endemic freshwater turtle reflects Miocene orogenesis of New

588   Guinea. *Biological Journal of the Linnean Society*, **111**, 192–208.

589   Givnish T.J., Spalink D., Ames M., Lyon S.P., Hunter S.J., Zuluaga A., Iles W.J.D., Clements

590   M.A., Arroyo M.T.K., Leebens-mack J., Endara L., & Kriebel R. (2015) Orchid

591   phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of*

592   *the Royal Society of London: Biological Sciences*, **282**, 1814.

593   Graham C.H., Carnaval A.C., Cadena C.D., Zamudio K.R., Roberts T.E., Parra J.L., McCain

594   C.M., Bowie R.C.K., Moritz C., Baines S.B., Schneider C.J., VanDerWal J., Rahbek C.,

595   Kozak K.H., & Sanders N.J. (2014) The origin and maintenance of montane diversity:

596   integrating evolutionary and ecological processes. *Ecography*, **37**, 711–719.

597   Günther R. (2000) *Albericus laurini* species nova, the first record of the genus *Albericus* (Anura,

598   Microhylidae) from the west of New Guinea. *Mitteilungen aus dem Museum fur*

599   *Naturkunde in Berlin Zoologische Reihe*, **76**, 167–174.

600   Günther R. (2008) Descriptions of four new species of *Choerophryne* (Anura, Microhylidae)

601   from Papua Province, Indonesian New Guinea. *Acta Zoologica Sinica*, **54**, 653–674.

602   Günther R. & Richards S.J. (2011) Five new microhylid frog species from Enga Province, Papua

603   New Guinea, and remarks on *Albericus alpestris* (Anura, Microhylidae). *Vertebrate*

604   *Zoology*, **61**, 343–372.

605   Hall R. (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific:

606   computer based reconstructions, model and animations. *Journal of Asian Earth Sciences*,

607   **20**, 353–431.

608   Hutter C.R., Guayasamin J.M., & Wiens J.J. (2013) Explaining Andean megadiversity: the

609   evolutionary and ecological causes of glassfrog elevational richness patterns. *Ecology*

610   *Letters*, **16**, 1135–1144.

611   Iannella A., Oliver P., Richards S. 2015. Two new species of *Choerophryne* (Anura,

612   Microhylidae) from the northern versant of Papua New Guinea's Central Cordillera.

613   *Zootaxa* 4058:332–340. DOI: 10.11646/zootaxa.3753.5.6.

614   Iannella A., Richards S., Oliver P. 2014. A new species of *Choerophryne* (Anura, Microhylidae)

615   from the Central Cordillera of Papua New Guinea. *Zootaxa* 3753:483–493. DOI:

616   10.11646/zootaxa.3753.5.6.

617   Irestedt M., Batalha-Filho H., Roselaar C.S., Christidis L., & Ericson P.G.P. (2015) Contrasting

618   phylogeographic signatures in two Australo-Papuan bowerbird species complexes (Aves:

619   *Ailuroedus*). *Zoologica Scripta*, **45**, 365–379.

620   Janzen D.H. (1967) Why mountain passes are higher in the tropics. *The American Naturalist*,

621   **101**, 233–249.

622   Johns R.. (1982) Plant Zonation. *Biogeography and Ecology of New Guinea* (ed. by J.. Gressitt),

623   pp. 309–330. Dr W. Junk Publishers, The Hague.

624   Jønsson K.A., Fabre P., Ricklefs R.E., & Fjeldså J. (2010) Major global radiation of corvoid

625   birds originated in the proto-Papuan archipelago. *Proceedings of the National Academy of*

626   *Sciences*, **108**, 2328–2333.

627   Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A.,

628   Markowitz S., Duran C., Thierer T., Ashton B., Mentjies P., & Drummond A. (2012)

629   Geneious Basic: an integrated and extendable desktop software platform for the

630   organization and analysis of sequence data. *Bioinformatics*, **28**, 1647–1649.

631   Köhler F. & Günther R. (2008) The radiation of microhylid frogs (Amphibia: Anura) on New

632   Guinea: A mitochondrial phylogeny reveals parallel evolution of morphological and life

633   history traits and disproves the current morphology-based classification. *Molecular*

634   *Phylogenetics and Evolution*, **47**, 353–365.

635   Kraus F. (2010a) New genus of diminutive microhylid frogs from Papua New Guinea. *ZooKeys*,

636   **48**, 39–59.

637   Kraus F. (2010b) An unusual new species of *Albericus* (Anura: Microhylidae) from Mount

638   Giluwe, Papua New Guinea. *Proceedings of the Biological Society of Washington*, **123**, 1–

639   7.

640   Kraus F. (2011) At the lower size limit for tetrapods, two new species of the miniaturized frog

641   genus *Paedophryne* (Anura, Microhylidae), **88**, 71–88.

642   Kraus F. (2013) A New Species of *Choerophryne* (Anura : Microhylidae) from Papua New

643   Guinea, with remarks on the taxonomic status of *Albericus*. *Zoosystematics and Evolution*,

644   **89**, 283–291.

645   Kraus F. & Allison A. (2000) A Review of the Endemic New Guinea Microhylid Frog Genus

646   *Choerophryne*. *Herpetologica*, **57**, 214–232.

647   La Sorte F.A. & Jetz W. (2010) Projected range contractions of montane biodiversity under

648   global warming. *Proceedings of the Royal Society of London: Biological Sciences*, **277**,

649   3401–10.

650   Lehr E. & Coloma L.A. (2008) A Minute New Ecuadorian Andean Frog (Anura:

651   Strabomantidae, *Pristimantis*). *Herpetologica*, **64**, 354–367.

652   Mayr E. & Diamond J.M. (1976) Birds on islands in the sky: Origin of the montane avifauna of

653   Northern Melanesia. *Proceedings of the National Academy of Sciences*, **73**, 1765–1769.

654   Meegaskumbura M., Bossuyt F., Pethiyagoda R., Manamendra-Arachchi K., Bahir M.,

655   Milinkovitch M.C., & Schneider C.J. (2002) Sri Lanka: an amphibian hot spot. *Science*,

656   **298**, 379.

657   Merckx V.S.F.T., Hendriks K.P., Beentjes K.K., Mennes C.B., Becking L.E., Peijnenburg

658   K.T.C.A., Afendy A., Arumugam N., de Boer H., Biun A., Buang M.M., Chen P.-P., Chung

659   A.Y.C., Dow R., Feijen F.A.A., Feijen H., Soest C.F., Geml J., Geurts R., Gravendeel B.,

660   Hovenkamp P., Imbun P., Ipor I., Janssens S.B., Jocqué M., Kappes H., Khoo E., Koomen

661   P., Lens F., Majapun R.J., Morgado L.N., Neupane S., Nieser N., Pereira J.T., Rahman H.,

662   Sabran S., Sawang A., Schwallier R.M., Shim P.-S., Smit H., Sol N., Spait M., Stech M.,

663   Stokvis F., Sugau J.B., Suleiman M., Sumail S., Thomas D.C., van Tol J., Tuh F.Y.Y.,

664   Yahya B.E., Nais J., Repin R., Lakim M., & Schilthuizen M. (2015) Evolution of endemism

665   on a young tropical mountain. *Nature*, **524**, 347–350.

666   Meredith R.W., Mendoza M.A., Roberts K.K., Westerman M., & Springer M.S. (2010) A

667   phylogeny and timescale for the evolution of Pseudocheiridae (Marsupialia: Diprotodontia)

668   in Australia and New Guinea. *Journal of Mammalian Evolution*, **17**, 75–99.

669   Nix H. (1982) Environmental determinants of biogeography and evolution in Terra Australis.

670   *Evolution in Arid Australia* (ed. by W.R. Barker and P.J.M. Greenslade), pp. 47–66.

671   Peacock Publications in association with Australian Systematic Botany Society and

672   ANZAAS, Frewville, South Australia,

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

674   Oliver L.A., Rittmeyer E.N., Kraus F., Richards S.J., & Austin C.C. (2013) Phylogeny and

675   phylogeography of *Mantophryne* (Anura: Microhylidae) reveals cryptic diversity in New

676   Guinea. *Molecular Phylogenetics and Evolution*, **67**, 600–607.

677   Oliver P.M., Krey K., Mumpuni, & Richards S. (2011) A new species of bent-toed gecko

678   (*Cyrtodactylus*, Gekkonidae) from the North Papuan Mountains. *Zootaxa*, **55**, 22–32.

679   Oliver P.M., Richards S.J., & Sistrom M. (2012a) Phylogeny and systematics of Melanesia's

680   most diverse gecko lineage (*Cyrtodactylus*, Gekkonidae, Squamata). *Zoologica Scripta*, **41**,

681   437–454.

682   Oliver P., Richards S., Mumpuni M., & Rösler H. (2016) The Knight and the King: two new

683   species of giant bent-toed gecko (*Cyrtodactylus*, Gekkonidae, Squamata) from northern

684   New Guinea, with comments on endemism in the North Papuan Mountains. *ZooKeys*, **562**,

685   105–130.

686   Oliver P., Richards S., & Tjaturadi B. (2012b) Two new species of *Callulops* (Anura:

687   Microhylidae) from montane forests in New Guinea. *Zootaxa*, **44**, 33–44.

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

689   Palumbi S.R., Martin A., Romano S., McMillan W.O., Stice L., & Grabowski G. (2002) *The*

690   *simple fool's guide to PCR version 2.* University of Hawaii, Honululu,

691   Peloso P.L. V, Frost D.R., Richards S.J., Rodrigues M.T., Donnellan S., Matsui M., Raxworthy

692   C.J., Biju S.D., Lemmon E.M., Lemmon A.R., & Wheeler W.C. (2015) The impact of

693   anchored phylogenomics and taxon sampling on phylogenetic inference in narrow-mouthed

694   frogs (Anura, Microhylidae). *Cladistics*, **32**, 113–140.

695   Polhemus D. (2007) Tectonic Geology of Papua. *The Ecology of Papua* (ed. by A. Marshall and

696   B. Beehler), pp. 137–164. EricOey, Richmond.

697   Price T.D., Hooper D.M., Buchanan C.D., Johansson U.S., Tietze D.T., Alström P., Olsson U.,

698   Ghosh-Harihar M., Ishtiaq F., Gupta S.K., Martens J., Harr B., Singh P., & Mohan D.

699   (2014) Niche filling slows the diversification of Himalayan songbirds. *Nature*, **509**, 222–5.

700   Rawlings L.H. & Donnellan S.C. (2003) Phylogeographic analysis of the green python, *Morelia*

701   *viridis*, reveals cryptic diversity. *Molecular Phylogenetics and Evolution*, **27**, 36–44.

702   Richards S.J., Dahl C.S., & Hiaso J. (2007) Another new species of *Choerophryne* (Anura:

703   Microhylidae) from Southern Highlands Province, Papua New Guinea. *Transactions of the*

704   *Royal Society of South Australia*, **131**, 135–141.

705   Richards S.J., Iskandar D.T., & Allison A. (2000) Amphibian and reptiles recorded on the RAP

706   survey in the Wapoga area, Irian Jaya, Indonesia. *A Biological Assessment of the Wapoga*

707   *River Area of Northwestern Irian Jaya, Indonesia. RAP Bulletin of Biological Assessment*

708   *14.* (ed. by A.L. Mack and L. Alonso), pp. 54–57. Conservation International, Washington,

709   DC.

710   Richards S.J., Oliver P.M., Krey K., & Tjaturadi B. (2009) A new species of *Litoria* (Amphibia:

711   Anura: Hylidae) from the foothills of the Foja Mountains, Papua Province, Indonesia.

712   *Zootaxa*, **13**, 1–13.

713   Richards S. & Suryadi S. (2003) A Biodiversity Assessment of Yongsu-Cyclops Mountains and

714   the Southern Mamberamo Basin, Papua, Indonesia. *A Biodiversity Assessment of Yongsu-*

715   *Cyclops Mountains and the Southern Mamberamo Basin, Papua, Indonesia: RAP Bulletin*

716   *of Biological Assessment 25* (ed. by S.J. Richards and S. Suryadi), pp. 76–80. Conservation

717   International, Washington, DC.

718   Richards S.J, Tjaturadi B., Mumpuni & Puradyatmika, P. (2015) *Field guide to frogs of the*

719   *Mimika region – Papua, Indonesia*. PT Freeport Indonesia, Mimika.

720   Rittmeyer E.N., Allison A., Gründler M.C., Thompson D.K., & Austin C.C. (2012) Ecological

721   guild evolution and the discovery of the world's smallest vertebrate. *PLoS ONE*, **7**, 1–11.

722   Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu

723   L., Suchard M.A., & Huelsenbeck J.P. (2012) Mrbayes 3.2: Efficient bayesian phylogenetic

724   inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

725   Rosauer D.F. & Jetz W. (2015) Phylogenetic endemism in terrestrial mammals. *Global Ecology*

726   *and Biogeography*, **24**, 168–179.

727   Santos J.C., Coloma L.A., Summers K., Caldwell J.P., Ree R., & Cannatella D.C. (2009)

728   Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages.

729   *PLoS Biology*, **7**, 0448–0461.

730   Scheffers B.R., Phillips B.L., Laurance W.F., Sodhi N.S., Diesmos A., & Williams S.E. (2013)

731   Increasing arboreality with altitude: a novel biogeographic dimension. *Proceedings of the*

732   *Royal Society: Biological Sciences*, **280**, 20131581.

733   Stamatakis A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with

734   thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

735   Toussaint E.F.A., Sagata K., Surbakti S., Hendrich L., & Balke M. (2013) Australasian sky

736   islands act as a diversity pump facilitating peripheral speciation and complex reversal from

737   narrow endemic to widespread ecological supertramp. *Ecology and Evolution*, **3**, 1031–

738   1049.

739 Toussaint E.F.A, Hall R., Monaghan M.T., Sagata K., Ibalim S., Shaverdo H. V, Vogler A.P.,

740   Pons J., & Balke M. (2014) The towering orogeny of New Guinea as a trigger for arthropod

741   megadiversity. *Nature communications*, **5**, 4001.

742 Tracy C.R., Christian K.A., & Richard Tracy C. (2010) Not just small, wet, and cold: Effects of

743   body size and skin resistance on thermoregulation and arboreality of frogs. *Ecology*, **91**,

744   1477–1484.

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

746   119–140.

747 Unmack P.J., Allen G.R., & Johnson J.B. (2013) Phylogeny and biogeography of rainbowfishes

748   (Melanotaeniidae) from Australia and New Guinea. *Molecular Phylogenetics and*

749   *Evolution*, **67**, 15–27.

750 Vences M., Kosuch J., Glaw F., Böhme W., & Veith M. (2003) Molecular phylogeny of

751   hyperoliid treefrogs: Biogeographic origin of Malagasy and Seychellean taxa and re-

752   analysis of familial paraphyly. *Journal of Zoological Systematics and Evolutionary*

753   *Research*, **41**, 205–215.

754 Vieites D.R., Wollenb, Erg K.C., Andreone F., Köhler J., Glaw F., & Vences M. (2009) Vast

755   underestimation of Madagascar's biodiversity evidenced by an integrative amphibian

756   inventory. *Proceedings of the National Academy of Sciences of the United States of*

757   *America*, **106**, 8267–8272.

758   Weir J.T. (2006) Divergent Timing and Patterns of Species Accumulation in Lowland and

759   Highland Neotropical Birds. *Evolution*, **60**, 842–855.

760   Williams S.E., Bolitho E.E., & Fox S. (2003) Climate change in Australian tropical rainforests:

761   an impending environmental catastrophe. *Proceedings of the Royal Society of London:*

762   *Biological sciences*, **27**, 1887–92.

763   Wollenberg K.C., Vieites D.R., Van Der Meijden A., Glaw F., Cannatella D.C., & Vences M.

764   (2008) Patterns of endemism and species richness in Malagasy cophyline frogs support a

765   key role of mountainous areas for speciation. *Evolution*, **62**, 1890–1907.

766   Xie W., Lewis P.O., Fan Y., Kuo L., & Chen M.-H.H. (2011) Improving Marginal Likelihood

767   Estimation for Bayesian Phylogenetic Model Selection. *Systematic Biology*, **60**, 150–160.

768   Yeh J. (2002) The effect of miniaturized body size on skeletal morphology in frogs. *Evolution;*

769   *international journal of organic evolution*, **56**, 628–41.

770   Zhuo Z. (1999) Response of altitudinal belts of vegetation to the late quaternary climatic changes

771   in tropical Asia. *Geographical Research*, **18**, 96–104.

772

773

774   **Supporting Information.**

775

776   Appendix S1. Supplementary tables and figures. Specimen numbers, locality information and

777   GenBank accession numbers for *Choerophryne* specimens included in analyses (Table S1);

778   GenBank accession details for outgroup samples (Table S2); genetic distance data for species

779   and candidate lineages (Table S3); and summary data on body, elevational distribution and

780   calling height for *Choerophryne* (Table S4). Bayesian tree for all samples (Figure S1); Trait

781   evolution in the major lineages of *Choerophryne* estimated using BEAST (Figure S2); and

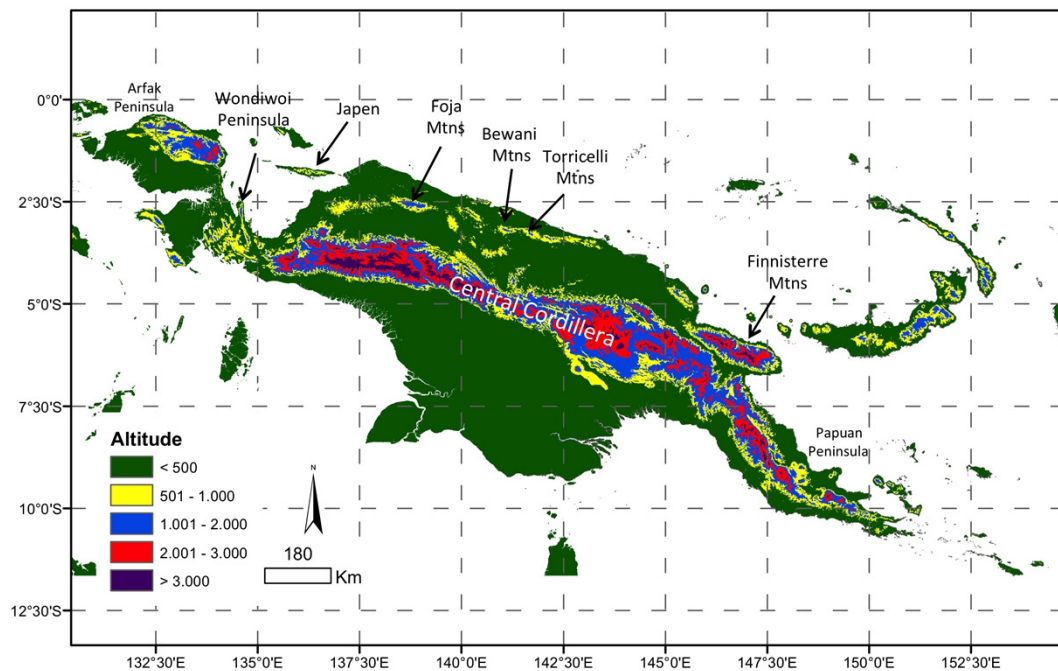
782   summary of museum records for *Choerophryne* grouped by phenotype (Figure S3).

783   Appendix S2. BEAST input file for ancestral state analyses

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

785

786   **Figure 1. Map of New Guinea.**

787   Stratified by major elevation bands and with key areas of montane forest denoted.

788

789

790

791

792

793

794

795

796

797

798

799   **Figure 2. Representative species of *Choerophryne*.**

800   A) *Choerophyrne alpestris* upper montane moss fields, Central Cordillera, terrestrial; B)

801   *Choerophyrne* spA7 hill forest, southern foothills, scansorial; C) *Choerophyrne* spB1 lower

802   montane forest, Foja Mountains, terrestrial; D) *Choerophyrne proboscidea* hill forest forest,

803   northern lowlands, scansorial. Photographs courtesy S. Richards (A,B,D) and T. Laman (C).

804

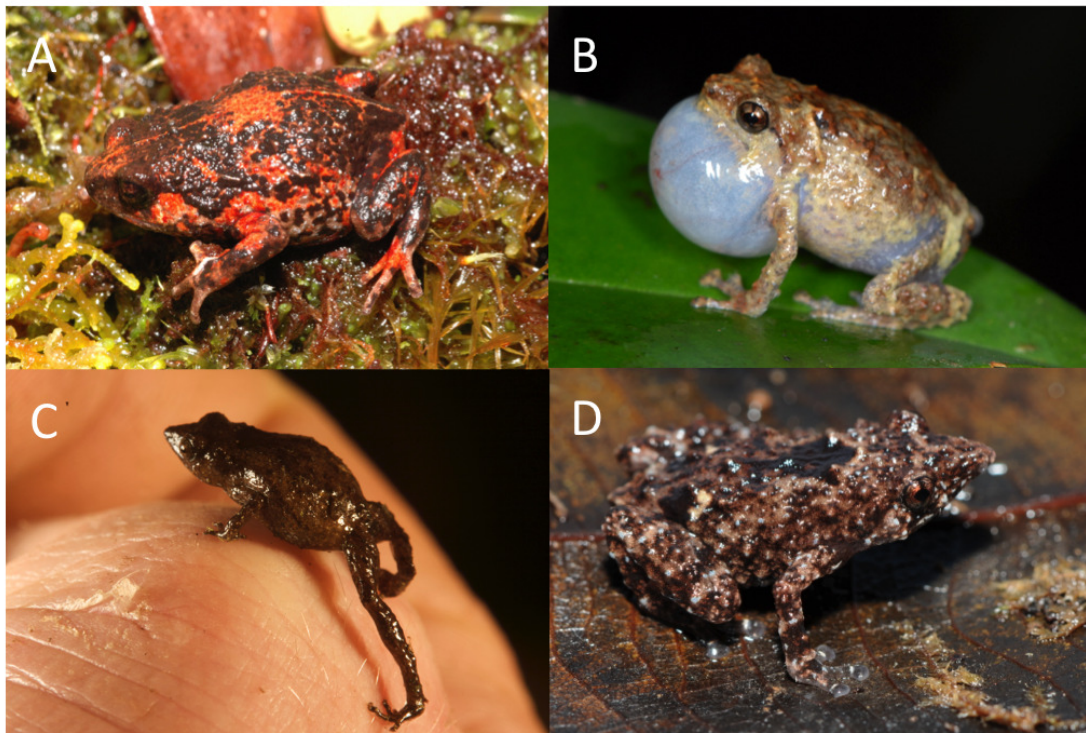
805

806

807

808

809

810

811

812 **Figure 3. Maximum Likelihood Tree of *Choerophryne*.**

813 * indicates <50% Bayesian posterior probability, interspecific nodes without support values were

814 poorly resolved in both analyses, intraspecific node supports are omitted for clarity. All

815 photographs by S. Richards.

816

817

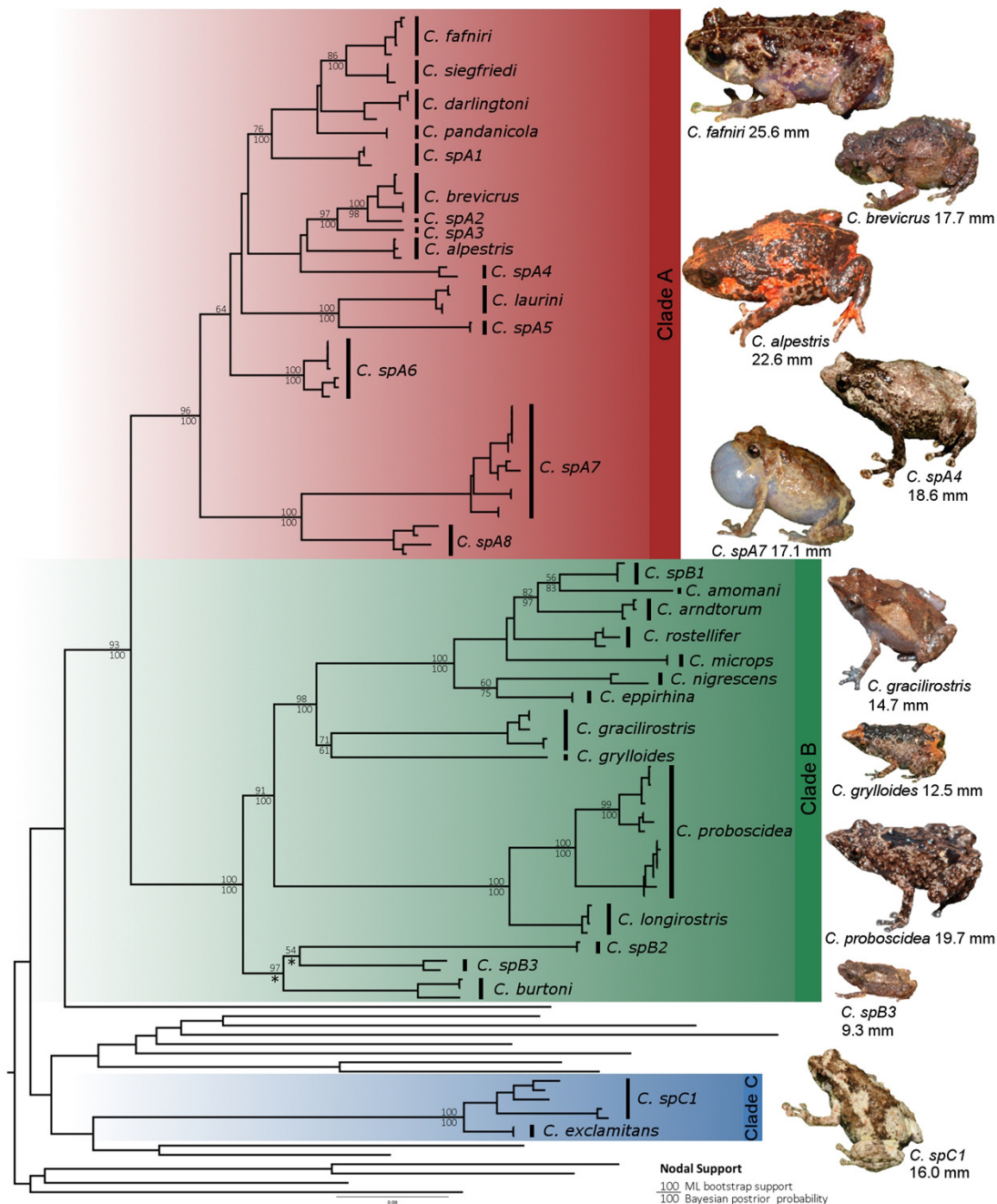



Figure 4. Chronogram and distributional summary for *Choerophryne*.

A) 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

821   years ago. Branches colour coded based on joint estimates of geographic region and elevation.

822   Four taxa under 15 mm identified by asterisk. Specific ranges in which inferred eccentric (Clade

823   A) and centric (Clade B) endemics in the North Papuan Mountain ranges are annotated. B) Map

824   of main montane areas of New Guinea, and sampling localities for the three major clades of

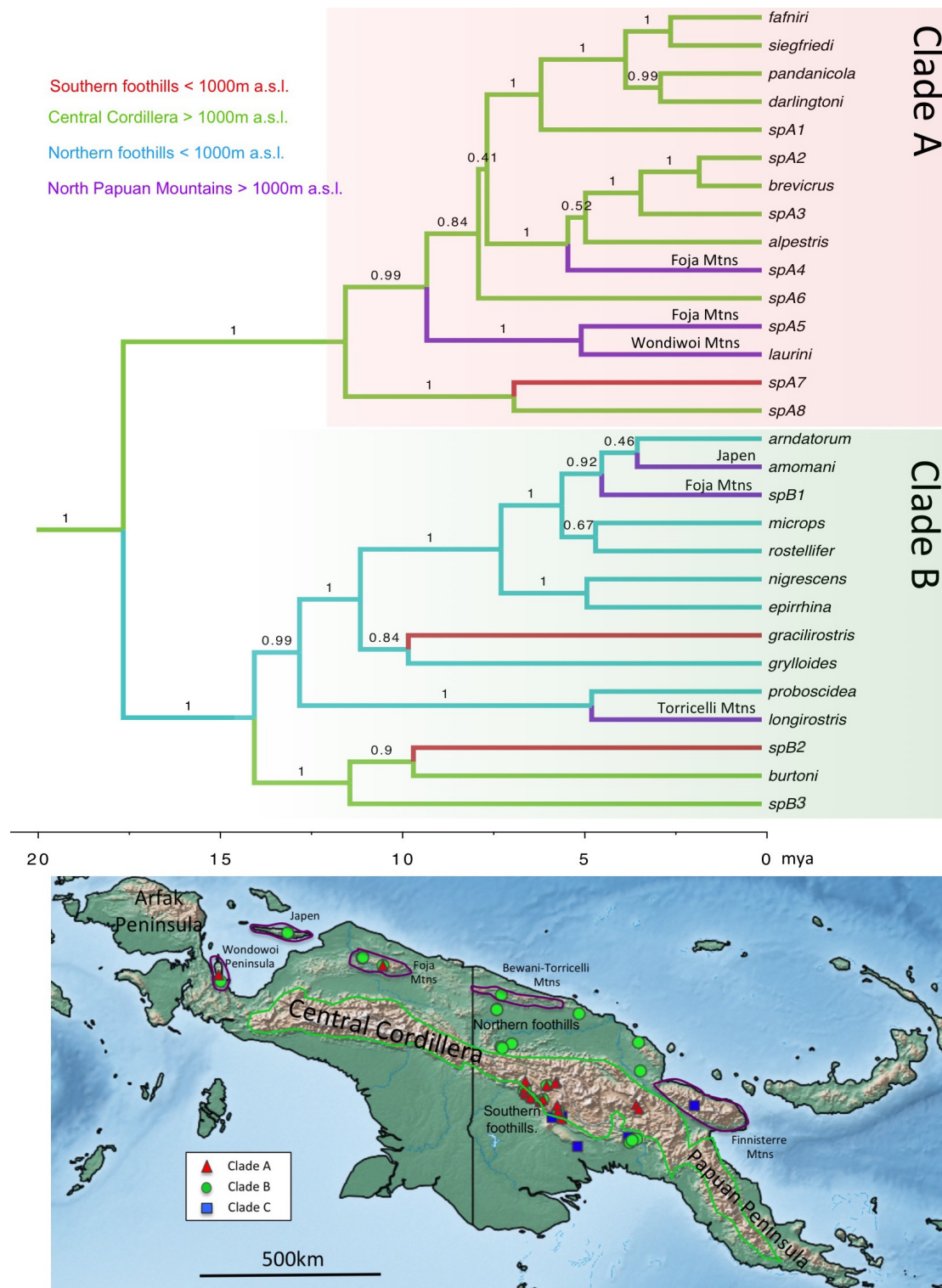
825   *Choerophryne*.

826

Southern foothills < 1000m a.s.l.

Central Cordillera > 1000m a.s.l.

Northern foothills < 1000m a.s.l.

North Papuan Mountains > 1000m a.s.l.

830

831 **Figure 5. Body size evolution in *Choerophryne*.**

832 Branch widths are proportional to maximum recorded adult male SVL. Green taxa are larger, red

833 taxa are smaller. Miniaturised taxa (<15 mm) are indicated with an asterisk. Maximum recorded

834 SUL of males in the genus *Choerophryne* ranges from 9.3 mm (spB3) up to 25.6 mm (*C. fafniri*).

835

836

837