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Evolutionary biology

A single origin of extreme matrotrophy in African mabuyine skinks

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Most mammals and approximately 20% of squamates (lizards and snakes) are viviparous, whereas all crocodylians, birds and turtles are oviparous. Viviparity evolved greater than 100 times in squamates, including multiple times in Mabuyinae (Reptilia: Scincidae), making this group ideal for studying the evolution of nutritional patterns associated with viviparity. Previous studies suggest that extreme matrotrophy, the support of virtually all of embryonic development by maternal nutrients, evolved as many as three times in Mabuyinae: in Neotropical Mabuyinae (63 species), *Eumecia* (2 species; Africa) and *Trachylepis ivensii* (Africa). However, no explicit phylogenetic hypotheses exist for understanding the evolution of extreme matrotrophy. Using multilocus DNA data, we inferred a species tree for Mabuyinae that implies that *T. ivensii* (here assigned to the resurrected genus *Lubuuya*) is sister to *Eumecia*, suggesting that extreme matrotrophy evolved only once in African mabuyine skinks.

1. Background

The amniotic egg is an evolutionary novelty that likely allowed vertebrates to diversify across terrestrial landscapes. Remarkably, live birth (viviparity) has evolved multiple times in amniotes, such that most mammals and approximately 20% of squamates (lizards and snakes) are viviparous [1,2]. Viviparity likely evolved only once or twice in mammals [2], but greater than 100 times in squamates [3], including multiple times in Mabuyinae (Scincidae) (see Pyron & Burbrink [4] for an alternative hypothesis). Therefore, mabuyine skinks are an ideal system for understanding the evolution of nutritional patterns associated with viviparity.

Viviparity differs among species, especially with respect to the sources of nutrients used for embryonic development. Embryos of many viviparous species use maternal nutrients (matrotrophy), which supplement or replace the use of yolk nutrients (lecithotrophy) to fuel development [5–7]. Additionally, all viviparous amniotes have a placenta, a specialized organ that transfers maternal nutrients to the embryo [8]. Eutherian mammals have complex placentae that bring maternal and embryonic circulatory systems into close contact, in some cases facilitating haemotrophy (matrotrophy through blood–blood transfer) [9]. Haemotrophy has been suggested for some mabuyine species, but most matrotrophic reptiles are likely histotrophic, with maternal nutrients secreted from uterine epithelial cells prior to uptake by placental cells, rather than being transferred from the maternal circulatory system [8,10].

Extreme matrotrophy, in which maternally supplied nutrients account for virtually all nutrients used for embryonic development, is found in at least

three different mabuyine lineages: Neotropical Mabuyinae (63 species), *Eumecia* (2 species; Africa) and *Trachylepis ivensii* (Africa) [8]. Nutrient transfer is best understood for several species of Neotropical Mabuyinae that transport large amounts of water, ions, lipids and proteins to the embryo via the placenta and have tiny ovulated eggs indicative of little or no lecithotrophy [7,11,12]. *Eumecia* and *T. ivensii* also ovulate tiny eggs with little or no yolk mass, strongly suggesting extreme matrotrophy in each of these lineages [5,6,10]. Recent studies have investigated whether extreme matrotrophy in Mabuyinae evolved independently, or if two, or all three of these lineages shared a common ancestor with extreme matrotrophy [8,10]. However, *T. ivensii* has not previously been included in any phylogenetic study [13–15]. Therefore, the evolution of extreme matrotrophy has been unclear.

Fresh tissues of *T. ivensii* were collected during expeditions to Angola and Zambia [16], allowing us to construct a new higher level mabuyine phylogeny and test hypotheses regarding the evolution of extreme matrotrophy. We inferred a species tree from 11 loci (2 mitochondrial and 9 nuclear) sampled from 16 species in 9 genera to determine the phylogenetic placement of Neotropical Mabuyinae, *T. ivensii* and *Eumecia* within Mabuyinae and to test whether extreme matrotrophy arose fewer than three times in Mabuyinae.

2. Material and methods

We extracted DNA using a previously described ethanol precipitation protocol [17]. We used polymerase chain reaction (PCR) and Sanger sequencing to amplify and sequence 2 mitochondrial and 9 nuclear genes for 14 mabuyine species plus 2 outgroup species (electronic supplementary material, table S1) and aligned sequences in Geneious v. 6.1 [18]. We supplemented new sequence data with sequences from GenBank for three species. To minimize missing data, we included chimeric sequences for *Caledoniscincus austrocaledonicus*, *Cryptoblepharus novocaedonicus* and *Trachylepis depressa*, using individuals confidently identified at the species level.

We inferred the species tree using *BEAST v. 1.8.2 implemented through CIPRES Science Gateway [19–21]. Pre-Pleistocene fossils are not known for Mabuyinae, and therefore to infer a time tree, we used uncorrelated relaxed molecular clocks [22] applying previously used parameter settings to generate substitution rate prior distributions (electronic supplementary material) [23]. We used GTR + Π substitution models and birth–death gene tree models for each locus, ran *BEAST for two billion generations (first 200 million generations omitted as burn-in), checked for posterior convergence in Tracer v1.6 [24], and summarized the posterior with TreeAnnotator v1.8.2 (maximum clade credibility and median node height settings) [25]. We used Mesquite v3.04 [26] to infer the presence or absence of extreme matrotrophy in ancestral Mabuyinae (electronic supplementary material).

3. Results

DNA sequence alignments included portions of 16S (545 bp), *ND2* (1029 bp), *BDNF* (692 bp), *BRCA1* (697 bp), *BRCA2* (1191 bp), *CMOS* (374 bp), *EXPH5* (877 bp), *KIF24* (565 bp), *MC1R* (663 bp), *MXRA5* (627 bp) and *RAG1* (1152 bp) loci (total missing data = 28.5%; electronic supplementary material).

*BEAST analysis reached posterior convergence after 200 million generations, and all parameters had high estimated

sample sizes (greater than 200). We recovered a phylogeny with high posterior probabilities (greater than or equal to 0.95) at most nodes (figure 1) in which *Trachylepis* is the only genus recovered as paraphyletic. *Trachylepis ivensii* is strongly supported as sister to *Eumecia*, and remaining *Trachylepis* are strongly supported as sister to *Chioninia*. The *Trachylepis* + *Chioninia* clade is sister to a Neotropical Mabuyinae clade. The *T. ivensii* + *Eumecia* clade is sister to the *Chioninia* + *Trachylepis* + Neotropical Mabuyinae clade, together forming an Afro-Neotropical clade. *Dasia* is weakly supported as sister to *Eutropis*, together forming an Asian Mabuyinae clade recovered as sister to the Afro-Neotropical clade.

Ancestral state reconstruction suggests that a single origin of extreme matrotrophy, followed by a loss in the *Trachylepis* + *Chioninia* clade, is equally parsimonious to having separate origins in the *T. ivensii* + *Eumecia* clade and in Neotropical Mabuyinae. A likelihood method favours the latter scenario (electronic supplementary material, figures S1 and S2).

4. Discussion

Skeletal data have long been recognized to support close affinities between *Eumecia* and the *Mabuya* group [27] and more recently have been interpreted to support a close relationship between *Eumecia* and *T. ivensii* [8,28]. Our results corroborate this and explicitly support their sister relationship. The two forms share a semiaquatic existence, an elongate body, and highly matrotrophic placentation [6,10,16,29]. However, they are deeply divergent genetically (table S2) and differ substantially in pholidosis [30]. To reflect generic monophyly, we resurrect the genus *Lubuya* Horton, 1972 [30] to accommodate *T. ivensii* and modify its generic diagnosis to accommodate comparison with *Eumecia* and fully limbed *Mabuya* group taxa (see appendix A).

Skinks with extreme matrotrophy ovulate tiny eggs with yolk sacs that lack yolk mass and have fetal and maternal tissues in close contact which function in nutrient transfer [5,7,10,11,12,31]. These characteristics (i) may be shared, derived characters reflecting a single origin of extreme matrotrophy, (ii) may have evolved once in Neotropical Mabuyinae and separately in the ancestor of *Lubuya* and *Eumecia* or (iii) may have evolved independently in all three lineages. Flemming & Blackburn [8] favoured a single origin of extreme matrotrophy, but also noted that Neotropical Mabuyinae differ from *Eumecia* and *Lubuya* in the morphology of the placenta and that shared reproductive features might be functionally linked, and could have arisen convergently during the evolution of extreme matrotrophy. Additionally, histological data suggest that placentae of *Eumecia* and *Lubuya* differ in their modes of formation, cellular composition and functional attributes [5,6,10]. However, *Eumecia* and *Lubuya* embryos examined by Blackburn & Flemming [5,10] and Flemming & Branch [6] mostly cover different developmental stages and therefore an examination of broader developmental series of *Eumecia* and *Lubuya* placentae would be useful to support or reject a common origin of extreme matrotrophy in African Mabuyinae. Our results suggest a single origin for extreme matrotrophy in African Mabuyinae, but are ambiguous with respect to Neotropical Mabuyinae, which may have evolved this condition independently, or may share ancestral extreme matrotrophy, with a subsequent loss in the *Trachylepis*

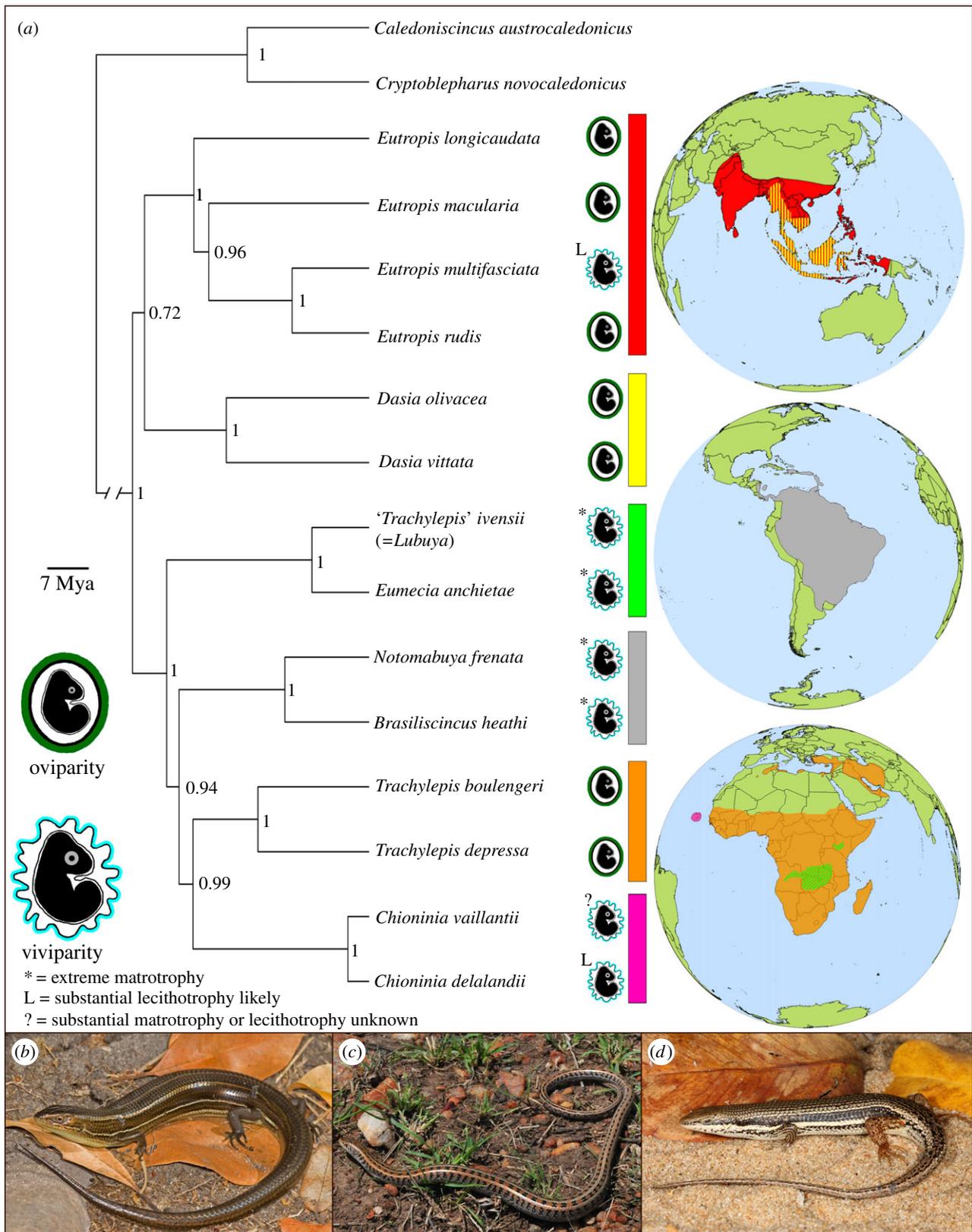


Figure 1. (a) Species tree of major mabuyine lineages (Scincidae). Node values posterior probabilities. Cartoon embryos depict parity mode; nutritional pattern is indicated for viviparous species; colours of vertical bars at tips correspond to range map colours: grey, Neotropical Mabuyinae; orange, *Trachylepis*; red, *Eutropis*; yellow, *Dasia*; pink, *Chioninia*; green, *Eumecia* and *Lubuya*; * = extreme matrotrophy; L = substantial lecithotrophy likely; ? = substantial matrotrophy or lecithotrophy unknown. (b) *Lubuya ivensii* (formerly *Trachylepis*), Zambia; (c) *Eumecia anchietae*, Kenya; (d) *Trachylepis depressa*, Mozambique. See electronic supplementary material, table S3, for parity and nutritional pattern sources.

(viviparous and oviparous forms exist) + *Chioninia* (viviparous) clade. However, the latter interpretation requires a switch from viviparity (and extreme matrotrophy) to oviparity one or more times in *Trachylepis*—a transition for which there are no convincing cases among squamates [3,32]. We,

therefore, favour the hypothesis that extreme matrotrophy in Mabuyinae evolved once in Africa and once in the Neotropics.

Ethics. All procedures involving animals and sample collection were performed in accordance with the appropriate regulations and carried out under the project 'Linking climate forcing,

plant physiology, and lizard extinctions on a global scale' approved by Villanova University on 13 May 2016, project number AS14-07.

Data accessibility. DNA sequences are available in GenBank (electronic supplementary material, table S1); *BEAST input file available in Dryad: <http://dx.doi.org/10.5061/dryad.gv047> [21].

Authors' contributions. B.R.K. and M.M. collected data; J.L.W., M.M. and T.R.J. ran analyses; A.S., W.C. and P.W. collected tissues; all authors contributed to the design of the study, wrote the manuscript, agreed to be held accountable for the content therein and approved the final version of the manuscript.

Competing interests. We have no competing interests.

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