Most mammals and approximately 20% of squamates (lizards and snakes) are viviparous, whereas all crocodilians, 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 *Lubahya*) 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. 6.1.1 [18]. We supplemented new sequence data with sequences from GenBank for three species. To minimize missing data, we included chimeric sequences for Caledonisinctus austrolecodonius, Cryptoblepharus vanenckedi 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 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 equally high degree of maternal investment [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 placenta 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 placenta 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

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), MCM1 (663 bp), MVAR (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. Dasis is weakly supported as sister to Eutropis, together forming an Asian Mabuyinae clade recovered as sister to the Afro-Neotropical clade.

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 placenta [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).

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(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 Villanueva University on 13 May 2016, project number AS14-07.

Data accessibility. DNA sequences are available in GenBank (electronic supplementary material, table S1); *beast* input data 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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**Appendix A**

**Lubuya**—Revised diagnosis: trunk elongate, tail length greater than twice snout–vent length; 32–35 presacral vertebrae; limbs short, pentadactyl; subdigital lamellae keeled; 32 mid-body scale rows; dorsal-most eight scale rows tricarinate, lateral and ventral scale smooth; nostrils dorsally positioned; nasal scales in broad contact; postnasal scale much higher than wide; supranasal scales in broad contact behind nares; three suprarocular scales; lower eyelid with transparent disc; fronto-nasal scale undivided, separated from frontal by prefrontal scales; prefrontals in contact; seven supralabials, first four anterior to third subocular; third (last) subocular largest and contacts lip; three pointed ear lobules; dorsal colour brown to olive, ventral cream to pale blue; greater than or equal to five pale longitudinal body stripes, sometimes bordered by black[28,30]. Content: Lubuya iversii (Bocage, 1879). Distribution: scattered localities from central Angola to eastern Zambia and southern Democratic Republic of the Congo[16,29,30].

**References**


