The functional significance of residual yolk in lizards

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Abstract

Residual yolk is assumed to be an important source of energy and nutrients during early life in nonmammalian amniotes. Available data show that the mean size of residual yolk is far smaller in lizards than in turtles, snakes, crocodiles, and birds, raising a question of whether residual yolk is of functional significance in lizards. Here, we compared data from 26 lizard species with those from other nonmammalian amniotes to test the hypothesis that residual yolk is functionally less significant in species producing more fully developed offspring. In our sample, species mean offspring water contents ranged from 73% to 84% of body wet mass; species mean proportions of carcass dry mass, fat-body dry mass, and residual yolk dry mass to offspring dry mass ranged from 84% to 99%, 0% to 5.0%, and 0% to 14.4%, respectively. Lizards are, on average, more fully developed at hatching or birth than snakes, as revealed by the fact that the mean proportion of carcass dry mass to body dry mass and offspring water contents were both higher in lizards than in snakes. We conclude that the functional significance of residual yolk during early life is generally less evident in lizards. Even in the lizards where residual yolk is of potential functional significance, this portion of yolk contributes little, if any, to postembryonic growth. Future work could usefully collect data across a wider spectrum of reptile taxa to establish a precocial–altricial continuum and test the hypothesis that species with a smaller amount of residual yolk are closer to the precocial end of the continuum.

Key words: carcass, fat body, lizards, offspring, postembryonic growth, residual yolk

Lecithotrophic embryos complete development often without exhausting yolk deposited by their mother in individual eggs prior to ovulation (Kaplan 1980; Kamler et al. 1998; Lee et al. 2007; Koláčková et al. 2015; Wu et al. 2017). Towards the end of embryonic development, the unutilized yolk, namely residual yolk, is internalized into the abdominal cavity (Pezaro et al. 2013). Residual yolk provides immediately available energy and nutrients for maintenance metabolism, activity, specific dynamic action, and/or somatic tissue growth during early life (Van Dyke et al. 2011; Panda et al. 2015; Wu et al. 2017; Murphy et al. 2020; see also Murakami et al. 1992; Radder et al. 2007; Giordano et al. 2014). Residual yolk is essential to the species whose foraging, immune, and digestive abilities are not well developed at hatching or birth (Troyer 1984; Panda et al. 2015). Residual yolk is especially important for the species where hatchlings overwinter in the nest, dig out of nests and/or disperse over a long distance (Kraemer and Bennett 1981; Nagle et al. 1998; Tucker et al. 1998; Willette et al. 2005; Spencer and Janzen 2014). So far, 9 small oviparous lizard species of the families Gekkonidae (*Hemidactylus turcicus*), Lacertidae (*Mesalina guttulata*, *M. olivieri* and *Podarcis sicula*), and Scincidae (*Lampropholis delicate*, *L. guichenoti*, *Menetia greyii*, *Morethia adelaidensis*

and *M. boulengeri*) have been reported to exhaust yolk prior to hatching (Table S1).

The size of residual yolk varies among taxa or species, among populations of the same species, among clutches of the same population or family, and even between the sexes of the same clutch (Spencer and Janzen 2014; Koláčková et al. 2015; Wu et al. 2017). Environmental factors experienced during embryogenesis such as temperature and moisture also affect the size of residual yolk. Earlier studies on a wide range of vertebrate taxa including turtles (Gutzke et al. 1987; Booth and Astill 2001; Booth 2002), lizards (Ji and Braña 1999; Ji et al. 2002; Warner et al. 2012), snakes (Ji and Du 2001; Lin et al. 2005; Gao et al. 2010), crocodiles (Manolis et al. 1987; Allsteadt and Lang 1995; Wang and Zhou 2000), and birds (Yalçın et al. 2008; Eiby and Booth 2009; Koláčková et al. 2015) consistently show that high incubation or gestation temperatures or dry substrates result in less developed and thus smaller offspring with more residual yolks. The partitioning of yolk between embryonic (the yolk supporting differentiation and growth of embryos) and postembryonic (the yolk supporting maintenance and growth during early life) stages is species specific or phylogenetically related (Wu et al., 2017; Qu et al. 2019), as revealed by the following

2 facts. First, the mean quantity of residual yolk dry mass (YDM) relative to body dry mass varies little between different studies on the same species, and this is especially true when embryos complete development under the same or similar conditions (Table S1). Second, within reptiles, the taxon mean quantity of residual YDM relative to body dry mass is the lowest in lizards (~6%) and the highest in alligators and crocodiles (~33%), with turtles (~17%) and snakes (~17%) in between (Table S1). Within birds, the size of residual yolk also varies with hatchling maturity, with the mean size being smaller in altricial species (~15%) than in precocial species (~25%) (Table S1). The far smaller taxon mean relative size of residual yolk raises a question of whether this portion of the yolk is functionally significant for hatchling lizards during their early posthatching activities and/or growth.

Here, we reported data collected from 26 (23 oviparous and 3 viviparous) species of lizards, including 7 oviparous species of agamid (*Calotes versicolor*), gekkonid (*Gekko japonicus*), lacertid (*Eremias argus*, *Eremias brenchleyi*, *Podarcis muralis*, and *Takydromus wolteri*), and scincid (*Plestiodon chinensis*) species with published data on the ratio of residual YDM to total hatchling dry mass (Table S1). We compared these data with those reported previously for other nonmammalian amniotes (snakes in particular) to address the above question. In particular, we tested the hypothesis that residual yolk is functionally less significant in species that produce more fully developed offspring.

Materials and Methods

Adult lizards were collected from various localities in China (16 species of the families Agamidae, Gekkonidae, Lacertidae, and Scincidae) and Spain (10 species of the family Lacertidae) between 1987 and 2019 (Table 1). Detailed procedures for the maintenance of lizards and collection of eggs and hatchlings or neonates were described elsewhere (Agamidae: Ji et al. 2002; Lin et al. 2007; Qu et al. 2011; Gekkonidae: Ji et al. 1991, Ding et al. 2012; Lacertidae: Ji and Braña 1999; Hao et al. 2006; Ma et al. 2019b; Scincidae: Ji et al. 2007; Lu et al. 2021). Only hatchlings or neonates completing embryonic development under the thermal (ranging from 25 °C to 29 °C for both oviparous and viviparous species) and/or hydric (ranging from −220 to −12 kPa substrate water potentials for oviparous species) conditions not differentially affecting the size of residual yolk were used in this study. More specifically, 1,026 hatchlings (3–134 of each oviparous species) and 70 neonates (7–54 of each viviparous species) with none from the same clutch or litter were weighed, measured for snout–vent length (SVL) and tail length, and then euthanized by freezing at −20 °C on the day of hatching or birth. Frozen individuals were later thawed, dissected, and separated into residual yolk, fat bodies, and carcass. The 3 body components were dried to a constant mass in an oven at 60 °C for 48 h to obtain carcass dry mass (CDM), fat-body dry mass (FDM), and residual YDM for each dissected individual.

We performed all statistical analyses using R 3.6.1 (R Development Core Team 2020). We used ordinary least squares (OLS) and phylogenetic generalized least squares (PGLS) regressions with RMS 5.1-4 (Harrell 2019) and CAPER 1.0.1 (Orme et al. 2018) to test whether the relationship between each selected pair of offspring components was significant. We used Akaike's Information Criterion (AIC) and likelihood-ratio test (Warne and Charnov 2008) to assess the adequacy of the models tested. We performed phylogenetic ANOVA and PGLS to account for the nonindependence of data due to the shared evolutionary history of species. To do that, we estimated divergence time for the 26 species of lizards using BEAST 1.10 under uncorrelated lognormal relaxed clock model and priori Yule model with 13 mitochondrial protein-coding genes and two ribosomal RNA (rRNA) genes, and we used Eremias fossil calibrations, which is from Qinling Mountains in China (Table S2, Figure 1; Li et al. 2004; Drummond and Rambaut 2007). Phylogenetic signal was measured by Pagel's (1999) lambda (λ), which indicates the strength of the phylogenetic relationship. Lambda values of or near 0 indicate phylogenetic independence, and values of or near 1 indicate that the variable is fully explained by evolutionary history and thus shows the maximal strength of phylogenetic signal. Throughout this paper, descriptive statistics are expressed as mean ± *SE* and range, and the significance level is set at $\alpha = 0.05$.

Results

Table 1 reports descriptive statistics for body size (SVL and tail length), mass, and 3 main components (carcass, fat bodies, and residual yolk) of newly hatched or newborn offspring. Species mean values for offspring water contents ranged from 73% in *G. japonicus* and *Eremias multiocellata* to 84% in *Psammodromus hispanicus* of body wet mass, with an overall mean of 80% (Figure 1). Species mean proportions of CDM to offspring dry mass ranged from 84% in *P. chinensis* to 99% in *P. hispanicus* and *Takydromus septentrionalis*, with an overall mean of 94% (Figure 1). Fat bodies were absent in 7 species (Table 1); in the other 19 species, species mean proportions of FDM to offspring dry mass ranged from 0.08% in *Eremias przewalskii* to 5.0% in *Eutropis multifasciata*, with an overall mean of 1.5% (Figure 1). Residual yolk was absent only in *E. multifasciata* (Table 1); in the other 25 species, species mean proportions of YDM to offspring dry mass ranged from 0.7% in *Zootoca vivipara* to 14.4% in *P. chinensis*, with an overall mean of 5.1% (Figure 1).

The species mean proportion of YDM to body dry mass was 9.2% in *C. versicolor*, 2.7% in *G. japonicus*, 4.3% in *E. argus*, 6.7% in *E. brenchleyi*, 0.9% in *P. muralis*, 1.9% in *T. wolteri*, and 14.4% in *P. chinensis* (Table 1). These values were nearly the same or very close to those reported previously for these 7 species (Table S1). Only in 9 (*Podarcis pityusensis* [6.6%], *Goniurosaurus hainanensis* [6.8%], *E. brenchleyi* [6.7%], *C. versicolor* [9.2%], *Phrynocephalus przewalskii* [9.6%], *E. przewalskii* [9.9%], *Acanthodactylus erythrurus* [10.1%], *Leiolepis reevesii* [12.2%], and *P. chinensis* [14.4%]) or ~35% of the 26 species did YDM account for higher than 6% of body dry mass (Table 1), an overall mean proportion for 30 lizard species studied thus far (Table S1). Increasing the number of study species from 30 to 49 and the number of study genera from 18 to 26 only slightly altered the taxon mean proportion of YDM to body dry mass reported previously for lizards (reducing from 6.4% to 5.7%), and the difference was not statistically significant ($t = 0.389$, $df = 79$, $P = 0.689$).

The 3 offspring body components were positively related to each other, but the rate (regression slope) at which FDM varied with YDM did not differ significantly from 0 in either the OLS or the PGLS regression model (Table 2). The relationship

Table 1. Descriptive statistics, expressed as mean ± *SE* and range, for size (SVL and tail length), mass, and 3 main body components of newly hatched (oviparous species, O) and newborn (viviparous species, V) lizards

Species	Pwarity mode	N	SVL (mm)	Tail length (mm)	Wet body mass (mg)	Dry body mass (mg)	Carcass dry mass (mg)	Fat-body dry mass (mg)	Residual yolk dry mass (mg)
Calotes versicolor[1]	O	44	24.6 ± 0.2 22.4–27.1	46.4 ± 1.0 30.2–59.5	555.9 ± 10.7 398.1–723.5	106.5 ± 2.4 80.7–148.4	96.6 ± 2.3 63.2–140.1	0	9.8 ± 0.7 1.9–22.5
Leiolepis reevesii[1]	O	43	38.2 ± 0.3 32.8–44.2	73.0 ± 1.0 57.1–83.5	1681.3 ± 34.8 1228.4–2353.0	283.3 ± 9.4 170.8–390.3	247.6 ± 7.5 143.0–347.0	1.0 ± 0.3 0–8.1	34.7 ± 4.7 0–110.7
Phrynocephalus przewalskii[1]	O	43	24.5 ± 0.2 21.4–27.7	29.8 ± 0.4 23.2–35.1	648.1 ± 14.2 443.0–897.0	109.9 ± 2.9 80.0–174.0	99.3 ± 2.6 66.0–151.0	0	10.5 ± 1.5 0–55.0
Phrynocephalus versicolor[1]	O	36	23.6 ± 0.2 20.5–25.9	27.5 ± 0.3 22.7–32.5	584.3 ± 11.8 386.0–713.0	95.4 ± 2.3 56.0–119.7	90.4 ± 2.1 56.0–119.0	0	5.0 ± 0.9 0–28.0
Gekko japonicus[2]	O	38	26.1 ± 0.3 21.8–29.9	24.2 ± 0.4 19.4–29.9	446.9 ± 14.7 254.0–616.0	120.6 ± 3.4 73.6–163.3	115.5 ± 3.3 70.6–159.7	1.7 ± 0.2 0.5–7.2	3.3 ± 0.4 1.1–14.0
Goniurosaurus hainanensis[2]	O	43	40.0 ± 0.4 31.6–43.7	30.9 ± 0.5 24.0–39.0	1480.9 ± 41.9 819.6–2015.1	318.5 ± 9.7 168.0–435.0	282.1 ± 7.8 166.3–371.5	14.9 ± 1.1 0.8–34.0	21.5 ± 1.9 0–54.7
Acanthodactylus erythrurus[3]	O	3	31.6 ± 0.7 30.5–32.9	45.7 ± 2.2 42.7–50.1	817.1 ± 59.3 724.5–927.6	155.0 ± 14.7 128.8–179.8	137.8 ± 12.7 114.5–158.3	1.5 ± 0.5 0.5–2.5	15.6 ± 1.7 13.5–19.0
Eremias argus[3]	O	91	26.3 ± 0.2 21.8–30.7	30.7 ± 0.4 23.0–39.1	498.8 ± 8.2 293.5–714.1	93.9 ± 1.8 57.1–141.0	87.9 ± 1.5 55.5–126.6	2.0 ± 0.1 0–6.7	4.0 ± 0.5 0.1–30.0
Eremias brenchleyi[3]	O	127	26.1 ± 0.2 19.7–30.4	35.9 ± 0.4 20.5–46.0	459.5 ± 5.9 278.0–617.8	86.2 ± 1.4 53.5–169.0	80.4 ± 1.4 39.2–166.6	0	5.8 ± 0.4 0–27.5
Eremias multiocellata[3]	V	9	29.2 ± 0.4 27.7–31.4	40.7 ± 0.8 38.0–46.0	527.8 ± 8.3 500.3–584.9	142.5 ± 20.3 83.4–248.3	135.9 ± 19.9 81.3–247.6	0.15 ± 0.07 0–0.62	6.5 ± 2.6 0.15–21.3
Eremias przewalskii[3]	V	7	29.7 ± 0.8 27.8–34.3	34.2 ± 2.0 31.0–46.0	671.5 ± 29.1 621.0–843.8	114.6 ± 11.7 85.1–167.1	103.1 ± 10.5 78.7–153.8	0.09 ± 0.08 0–0.56	11.3 ± 3.1 4.1–28.3
Iberolacerta monticola[3]	O	14	26.3 ± 0.2 24.9–27.7	37.6 ± 0.7 30.0–39.4	409.0 ± 9.4 331.2–475.5	87.5 ± 2.0 69.3–95.4	84.2 ± 1.8 68.0–92.4	1.7 ± 0.1 0.4–2.4	1.5 ± 0.3 0.1–5.4
Lacerta schreiberi[3]	O	4	32.5 ± 0.5 31.4–33.3	51.3 ± 0.8 49.3–53.3	851.6 ± 35.5 768.3–940.0	188.6 ± 5.2 173.7–197.5	182.5 ± 5.6 166.6–192.0	3.2 ± 0.5 2.6–4.7	2.9 ± 0.8 0.8–4.4
Podarcis atrata[3]	O	20	28.3 ± 0.3 25.3–30.5	47.3 ± 0.9 40.3–51.8	536.7 ± 17.2 387.0–680.9	116.0 ± 4.6 81.8–148.6	105.9 ± 3.9 74.4–135.1	3.8 ± 0.3 1.5–5.8	6.4 ± 0.9 0.5–14.5
Podarcis bocagei[3]	O	14	25.6 ± 2.0 24.4–26.8	36.0 ± 0.6 31.9–40.3	377.8 ± 8.1 328.0–441.1	71.3 ± 2.2 59.5–88.0	69.3 ± 2.0 58.2–83.1	0.84 ± 0.13 0.1–1.6	1.2 ± 0.2 0–3.3
Podarcis muralis[3]	O	67	25.3 ± 0.2 21.2–28.5	37.7 ± 0.4 25.8–44.6	350.5 ± 4.5 259.6–438.2	69.1 ± 0.9 47.3–94.8	67.6 ± 0.9 46.6–91.1	0.84 ± 0.10 0–4.4	0.6 ± 0.07 0–2.3
Podarcis pityusensis[3]	O	48	30.3 ± 0.2 26.1–32.9	52.5 ± 0.6 36.4–60.4	632.5 ± 10.9 512.5–819.7	145.0 ± 2.9 114.6–198.3	130.1 ± 2.5 88.1–173.0	3.8 ± 0.3 1.7–19.6	9.5 ± 1.3 0–55.2
Psammodromus algirus[3]	O	25	25.8 ± 0.3 22.4–28.0	42.9 ± 1.5 28.0–56.7	380.4 ± 13.3 257.4–480.0	73.6 ± 3.3 38.8–97.8	70.7 ± 3.2 38.7–94.5	0.57 ± 0.06 0.1–1.3	2.3 ± 0.3 0–7.1
Psammodromus hispanicus[3]	O	6	25.0 ± 0.7 23.5–28.0	32.2 ± 1.5 28.0–38.1	326.8 ± 26.4 257.4–448.5	52.8 ± 5.5 0.65–1.39	52.1 ± 5.4 38.7–77.2	0.17 ± 0.03 0.1–0.3	0.52 ± 0.17 0–1.0
Takydromus amurensis[3]	O	33	23.3 ± 0.2 19.7–27.0	37.6 ± 0.6 30.8–44.5	309.6 ± 5.7 231.0–368.0	56.2 ± 0.8 45.1–64.2	55.1 ± 0.9 43.6–63.9	0.16 ± 0.09 0–2.8	0.94 ± 0.29 0–7.3
Takydromus septentrionalis[3]	O	134	24.7 ± 0.2 19.8–29.1	50.2 ± 0.6 30.7–67.0	333.9 ± 6.1 196.0–495.0	67.0 ± 1.3 32.6–102.9	66.3 ± 1.3 32.5–102.8	0	0.74 ± 0.13 0.1–9.6
Takydromus sexlineatus[3]	O	39	20.0 ± 0.2 17.6–22.3	45.8 ± 0.6 38.0–58.0	203.7 ± 5.1 154.2–268.7	36.4 ± 0.8 27.8–49.8	35.1 ± 0.8 27.6–49.8	0	1.3 ± 0.3 0–7.7
Takydromus wolteri[3]	O	34	22.0 ± 0.2 18.3–24.7	39.2 ± 0.6 29.8–45.6	232.1 ± 4.9 173.1–293.0	40.9 ± 0.8 33.5–50.1	40.2 ± 0.8 28.0–49.1	0	0.77 ± 0.22 0.1–5.8
Zootoca vivipara[3]	O	34	22.4 ± 0.2 19.9–24.0	25.0 ± 0.3 21.3–28.7	241.4 ± 3.6 207.2–299.0	48.4 ± 0.7 42.5–60.2	47.5 ± 0.7 41.7–59.7	0.58 ± 0.04 0.2–1.0	0.33 ± 0.06 0–1.4
Eutropis multifasciata[4]	V	54	34.2 ± 0.3 29.4–38.0	44.4 ± 0.5 36.2–52.4	1008.2 ± 12.7 778.2–1211.4	208.2 ± 3.8 138.1–295.1	197.7 ± 3.3 132.9–248.5	10.5 ± 1.4 1.5–77.9	0
Plestiodon chinensis[4]	O	86	28.9 ± 0.3 22.6–33.3	35.1 ± 0.4 25.8–43.6	639.3 ± 11.3 432.8–943.2	135.9 ± 2.6 72.4–209.0	115.4 ± 2.7 67.0–183.1	0.13 ± 0.02 0–1.1	19.6 ± 1.2 5.4–56.2

Species of the same family share a numeric superscript. 1: Agamidae; 2: Gekkonidae; 3: Lacertidae; 4: Scincidae. *N*: the number of newly hatched or newborn lizards.

between CDM and FDM exhibited the strongest phylogenetic signal; the other 2 relationships were both phylogenetically independent (Table 2). PGLS regression models were better than OLS regression models in all cases. YDM explained ~45% variation in CDM, and FDM explained ~48% variation in CDM (Table 2).
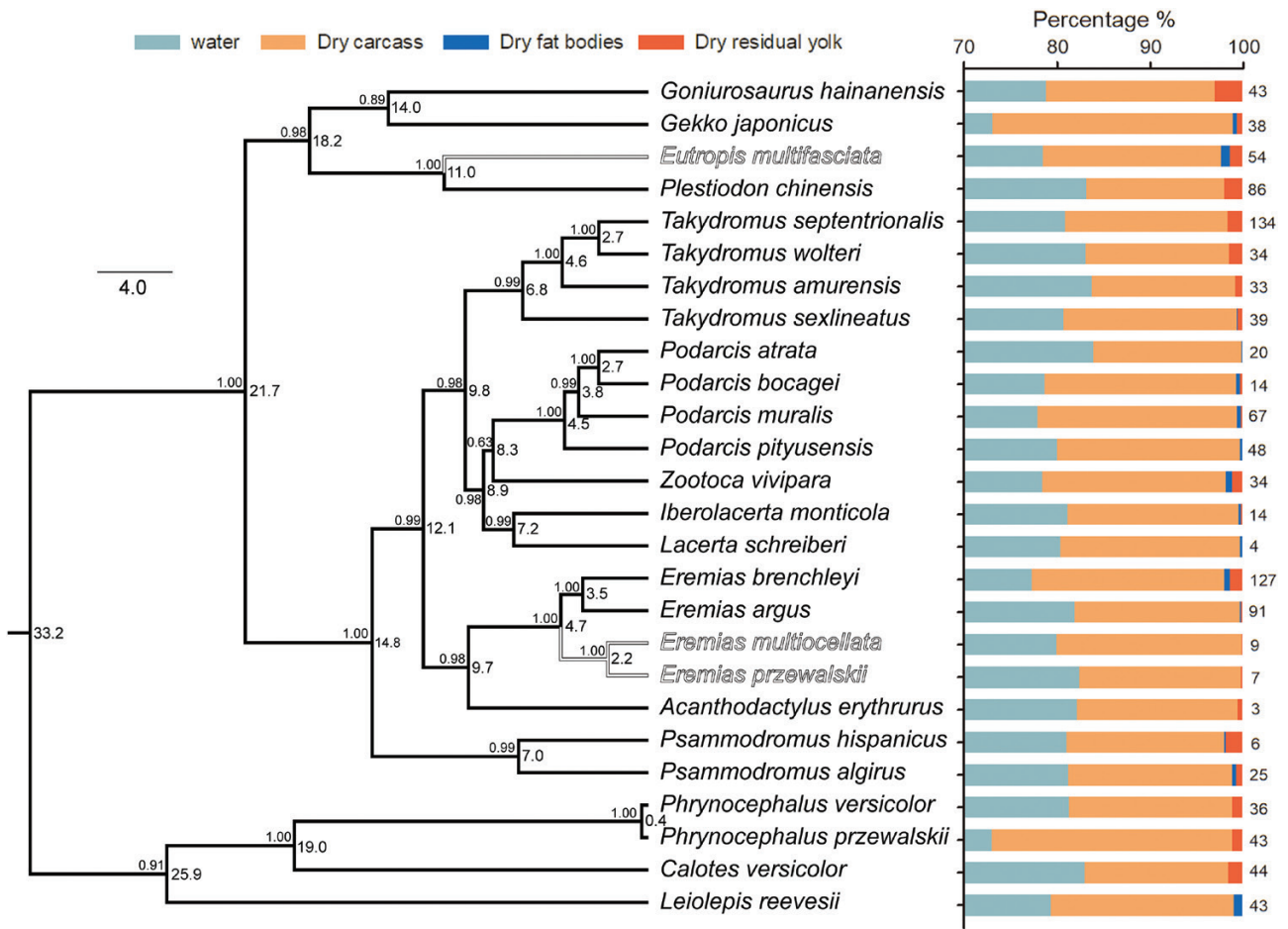
Figure 1. The phylogeny of the 26 species of lizards obtained from Bayesian Inference analysis based on 13 concatenated mitochondrial protein-coding genes and 2 rRNA genes (left), and percentages of 4 body components in newly hatched or newborn lizards (right). Numbers on each node of the tree are posterior probability (above the branch) and divergence time (millions of years ago). Each color indicates a body component. Numbers on the right edge of the figure are sample sizes. Oviparous species are in solid font, and viviparous species in hollow font.

Table 2. Parameters of regressions between each pair of 3 body components (carcass, residual yolk, and fat bodies in dry mass) using ordinary least squares (OLS) and phylogenetic generalized least squares (PGLS) regression models

Models	N	Slope	Intercept	r^2	ln likelihood	AIC	λ	$F_{1, 24}$	P value
OLS regression model									
Carcass vs. Residual yolk	26	5.17 ± 1.08	72.42 ± 11.44	0.488	−136.66	275.31		17.40	<0.001
Fat bodies vs. Residual yolk	26	0.087 ± 0.084	1.24 ± 0.88	0.043	−70.08	142.17		1.15	0.284
Carcass vs. Fat bodies	26	12.87 ± 2.48	83.98 ± 9.56	0.529	−135.57	273.13		19.58	<0.001
PGLS regression model									
Carcass vs. Residual yolk2	26	3.15 ± 1.18	128.11 ± 30.35	0.230	−134.20[a]	272.39	0.00	25.43	<0.001
Fat bodies vs. Residual yolk3	26	0.057 ± 0.076	4.83 ± 1.94	0.023	−62.71[a]	129.41	0.00	0.57	0.457
Carcass vs. Fat bodies1	26	12.61 ± 2.04	92.70 ± 25.81	0.515	−128.24[a]	260.48	1.00	7.17	0.013

N: the number of species studied. Models with a superscript of [a] are significantly better than their alternate regression models.

Discussion

We added a viviparous skink *E. multifasciata* to the list of lizard species where embryos exhaust all of their yolk prior to hatching or birth (Table 1). That the species mean proportions of YDM to body dry mass in *C. versicolor*, *G. japonicus*, *E. argus*, *E. brenchleyi*, *P. muralis*, *T. wolteri*, and *P. chinensis* were very close to those reported previously for these species substantiated an earlier conclusion that the relative quantity of residual yolk is a species-specific trait in reptiles (Wu et al. 2017). The 3 offspring body components were positively related to each other in snakes, with all these relationships exhibiting strong (λ values ranging from 0.54 to 0.84) phylogenetic signals (Wu et al. 2017). Here, we found that the positive relationship between FDM and YDM was not significant and that only the relationship between CDM and FDM exhibited a strong (λ = 1.00) phylogenetic signal (Table 2).

Increasing the number of study species from 30 to 49 and the number of study genera from 20 to 28 only slightly, but not significantly altered the taxon mean proportion of YDM to body dry mass ever reported for lizards. This is primarily because all newly sampled species are insectivores in which the mean size of residual yolk is far smaller than in omnivorous or herbivorous species (Troyer 1983; Werner 1988; Packard et al. 1992; Ar et al. 2004). For example, the mean size of residual YDM relative to hatchling dry mass is ~29% in the rough-tailed rock lizard *Laudakia stellio*, ~31% in the green iguana *Iguana iguana*, and ~32% in the Egyptian spiny-tailed lizard *Uromastyx aegyptius* (Table S1). *Laudakia stellio* is omnivorous (Lo Cascio et al. 2001; Ibrahim and El-Naggar 2013; Qashqaei and Ahmadzadeh 2015), both *I. iguana* (Troyer 1984) and *U. aegyptius* (Foley et al. 1992; Castilla et al. 2011) are herbivorous, and all other species studied thus far are basically insectivorous. The finding that the taxon mean size of residual yolk is far smaller in lizards (insectivorous species in particular) than in other nonmammalian amniotes provides the following 3 inferences regarding the functional significance of residual yolk in lizards.

First, the functional significance of residual yolk as an endogenous source of energy and nutrients during early life is generally less evident in lizards than in other nonmammalian amniotes, as evidenced by its relatively small size in lizards. In none of the 26 lizard species did we find that the mean proportion of YDM to body dry mass was higher than the taxon mean values reported for turtles (~17%), snakes (~17%), crocodiles (~33%), and birds (~19%). Moreover, only in 9 (*P. pityusensis*, *G. hainanensis*, *E. brenchleyi*, *C. versicolor*, *P. przewalskii*, *E. przewalskii*, *A. erythrurus*, *L. reevesii*, and *P. chinensis*) or ~35% of the 26 species did we find that YDM accounted for higher than 6% of body dry mass (Table 1), which is the taxon mean proportion previously reported for lizards (Table S1). In snakes, YDM accounting for lower than 6% of body dry mass only occurs in viviparous species (Wu et al. 2017). It is worth noting that viviparous snakes on average have smaller amounts of residual yolk not because they invest less in the yolk but because they invest more in the yolk as a maternal investment in embryogenesis to produce more fully developed offspring that are longer and heavier due to metabolic water production accompanied by yolk depletion (Wu et al. 2017). In the Jacky dragon (*Amphibolurus muricatus*) where residual yolk wet mass accounts for ~6% of hatchling wet mass and hatchlings often start feeding within 24-h posthatching, residual yolk contributes very little, if any, to their energy budgets during early life and is of trivial functional significance (Radder et al. 2007). As in birds (~76% vs. ~59% mean values [Arieli 1983; Ar et al. 1987]), water contents are much lower in the yolk than in the hatchling in reptiles (~78% vs. ~59% mean values [Du et al. 2001; Ar et al. 2004; Cai et al. 2007; Ma et al. 2019a]). Accordingly, YDM should be at least a bit higher than 6% of hatchling dry mass in *A. muricatus*. Based on the conclusion drawn in *A. muricatus* and including 3 species (*P. pityusensis*, *G. hainanensis*, and *E. brenchleyi*) where YDM accounted for lower than 7% of body dry mass, we conclude that the role of residual yolk is of trivial significance in at least 20 or ~77% of the 26 lizard species studied herein.

Second, residual yolk is of functional significance in several lizard species covering a diverse range of taxa. Although there is a common assumption that residual yolk is an important source of energy during early life in lizards, this idea has been empirically tested only in 2 species, *I. iguana* (Troyer 1984) and *C. versicolor* (Radder et al., 2004; Pandav et al. 2006). YDM accounts for 31% of hatchling dry mass in *I. iguana* (Troyer 1983; Werner 1988; Packard et al. 1992). Unlike hatchling Jacky dragons that start feeding soon after hatching (Radder et al. 2007), hatchling green iguanas do not eat before ingesting feces from other conspecifics to obtain the gut microbes necessary to digest their herbivorous diet (Troyer 1984). Residual yolk is of functional significance in *I. iguana* because it can be mobilized to support maintenance metabolism, growth, and other activities during early life before hatchlings are capable of digesting exogenous plant food (Troyer 1983, 1984). *Calotes versicolor* was 1 of the 6 species studied herein where YDM accounted for higher than 9% of body dry mass (Table 1). In *C. versicolor*, residual yolk is an important part of the energy budget set aside by developing embryos, and as such, selection favors the maximization of yolk reserves at hatching for posthatching needs (Radder et al. 2004). In this study, we found 5 species (*P. przewalskii*, *E. przewalskii*, *A. erythrurus*, *L. reevesii*, and *P. chinensis*) where species mean proportions of YDM to body dry mass ranged from 9.6% to 14.4%, with the mean size of residual yolk in each species being larger than in *C. versicolor*. Hence, because of the relatively large residual yolk in these 5 species, the residual yolk is likely to play a significant role in hatchling survival immediately posthatch, but studies of posthatching biology are needed to confirm this hypothesis.

Third, even in the lizards where residual yolk is of potential functional significance, this portion of yolk contributes little, if any, to postembryonic growth. Reptiles of different species or taxa share a common pattern of embryonic growth or yolk depletion during embryogenesis (Fischer et al. 1991; Du et al. 2001; Lu et al. 2009; Ma et al. 2019a). Natural selection for a given size of residual yolk at hatching or birth is determined by the feeding ability of newly hatched or newborn offspring, or by the time period when they have a negative energy balance (Lance and Morafka 2001; Nagle et al. 2003; Pezaro et al. 2013; Spencer and Janzen 2014). More fully developed offspring typically have larger carcasses (and thus larger linear sizes) but smaller residual yolks and hold more water largely due to metabolic water gain accompanied by yolk depletion (Wu et al. 2017). Within squamate reptiles, lizards are, on average, far more fully developed at hatching or birth than snakes, as revealed by the following 3 facts. First, the taxon mean proportion of CDM to body dry mass was much higher in lizards (~94% in all 26 species combined, and ~89% in the 6 species where YDM accounted for higher 9% of body dry mass; Table 1) than in snakes (~65% in oviparous species, and ~80% in viviparous species; Wu et al. 2017). Second, taxonomic group rather than reproductive mode is an important source of variation in CDM at hatching or birth, which is significantly greater in lizards than in snakes after accounting for YDM (Table 3). Third, the taxon mean offspring water content was much higher in lizards (~82% in all 26 species combined, and ~80% in the 6 species with YDM accounting for higher 9% of body dry mass; Table 1) than in snakes (~74% in oviparous species, and ~78% in viviparous species; Wu et al. 2017). Residual yolk does have a role in supporting postembryonic growth in a diverse array of ectothermic vertebrates including the aforementioned 2 lizard species, *I. iguana* (Troyer 1984) and *C. versicolor* (Pandav et al. 2006). Within squamate reptiles, such a role is evident in oviparous snakes where YDM accounts for up to 34% of hatchling dry mass

Table 3. Summary table of the conventional (nonphylogenetic) ANOVA and phylogenetic ANOVA (PANOVA) examining the effects of reproductive mode (oviparity vs. viviparity) and animal taxa (lizards vs. snakes) on carcass dry mass with the same amount of yolk dry mass

	ANOVA		PANOVA	
	$F_{1, 42}$	P value	$F_{1, 43}$	P value
Reproductive mode	0.163	0.689	2.373	0.131
Animal taxa	17.655	<0.001; L > S	10.988	<0.01; L > S
R × A interaction	0.575	0.452	12.783	< 0.01

R: reproductive mode; A: animal taxa; L: lizards; S: snakes.

([Wu et al. 2017](#)). In the present study, the maximal size of residual yolk and the minimal size of carcass both occurred in *P. chinensis* where YDM and CDM accounted for ~14% and ~84% of hatchling dry mass, respectively ([Table 1](#)). Thus, all else being equal, residual yolk would be more likely to support postembryonic growth in *P. chinensis* than in other lizard species. However, compared to snakes (oviparous snakes in particular), *P. chinensis* is developmentally more mature at hatching and can be therefore defined as a precocial species where residual yolk contributes little, if any, to postembryonic activities and/or growth.

Our experience with numerous species of insectivorous lizards including *P. chinensis* is that hatchlings or neonates start feeding less than 2-day posthatching. Unlike lizards, oviparous snakes, turtles, and crocodiles often do not eat but become more fully developed in the first 1–5 weeks of life as most, if not all, of early growth is achieved by the transfer of nutrients from residual yolk to carcass ([Burger 1989](#); [Lee et al. 2007](#); [Sirsat et al. 2016](#); [Rowe et al. 2017](#); [Wu et al. 2017](#)). In this context, oviparous snakes, turtles, and crocodiles are more likely to be altricial. Future work could usefully collect data from species across a more ecologically and phylogenetically diverse array of taxa to establish a precocial–altricial continuum in reptiles and test the hypothesis that species or taxa with a smaller amount of residual yolk are closer to the precocial end of the continuum.

Author Contributions

X.J., F.B., and Y.-F.Q. conceived the study. All authors collected the data. K.G., Y.-F.Q., and X.J. analyzed the data. X.J., K.G., and F.B. wrote the first version of the manuscript. All authors participated in writing and rewriting multiple versions and approved the final manuscript.

Conflict of Interest

The authors have no competing interests.

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Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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