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Reproductive characteristics of *Afrixalus septentrionalis* Schiøtz 1974 and *Hyperolius viridiflavus ngorongoriensis* Schiøtz 1975 (Anura: Hyperoliidae) from a miombo seasonal wetland in northern Tanzania

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The family Hyperoliidae Laurent 1943 is a large clade of reed frogs endemic to sub-Saharan Africa, Seychelles, and Madagascar. Many characteristics of this diverse clade have recently been investigated, such as the extensive variation in colour and sexual dimorphism/dichromatism (Veith et al., 2009; Portik, 2015), yet many details of species-specific natural history remain unknown. Natural history characteristics, such as egg descriptions and clutch size are useful not only for identifying species, but as variables that have major implications for reproductive strategies across this diverse group. This study details egg clutch characteristics for two species: *Afrixalus septentrionalis* Schiøtz 1974, and *Hyperolius viridiflavus ngorongoriensis* Schiøtz 1975 (following the descriptions from Schiøtz (1999), Pickersgill et al. (2004), and lineages identified in Portik (2015)), that are heretofore unknown and compares these characteristics to those of closely related lineages from the literature. Clutch and/or egg characteristics of both species represent records for their respective genera.

Amphibian surveys were conducted during the evening of 9 December 2017 (approx. 20:00h) in northern Tanzania approximately 20 km southwest of Lake Manyara (-3.965577967, 35.658988, 1171m a.s.l.). The seasonal wetland is part of a miombo woodland habitat mosaic (Fig. 1e). Pairs of *Hyperolius* and *Afrixalus* frogs engaged in amplexus were caught by hand and deposited in a plastic bag overnight. Confining fertilised females to a plastic bag is necessary for accurate egg counts as many hyperoliid species lay eggs in multiple smaller clutches which can confound total egg counts. Only those species lacking recorded breeding information were evaluated for egg characteristics: *Hyperolius v. ngorongoriensis* and *Afrixalus septentrionalis*. Both of these lineages (*H. v. ngorongoriensis* and *A. septentrionalis*) are members of species complexes with unclear taxonomic status, therefore we evaluated appearance, morphology, call, and geographic ranges for all potential identifications.

In the morning, eggs released in the bags were counted and the diameter of five eggs (excluding egg capsules) per species were measured with digital callipers (Mitutoyo Corp., Takatsu-ku, Japan). This method was chosen to ensure that total fecundity for the individual was recorded instead of collecting egg deposits in the water which might represent a fraction of the eggs laid by a female in a single night. In order to add these data to a larger test of a correlation between egg counts and female body size (Snout-Urostyle-Length (SUL)) to examine a potential size-fecundity relationship, we combined all available data on total egg clutches, egg diameters, and characteristics for other species of *Afrixalus* and *Hyperolius* (Grafe and Linsenmair, 1989; Schiøtz, 1999; Rödel, 2000; Channing, 2001; Channing and Howell,

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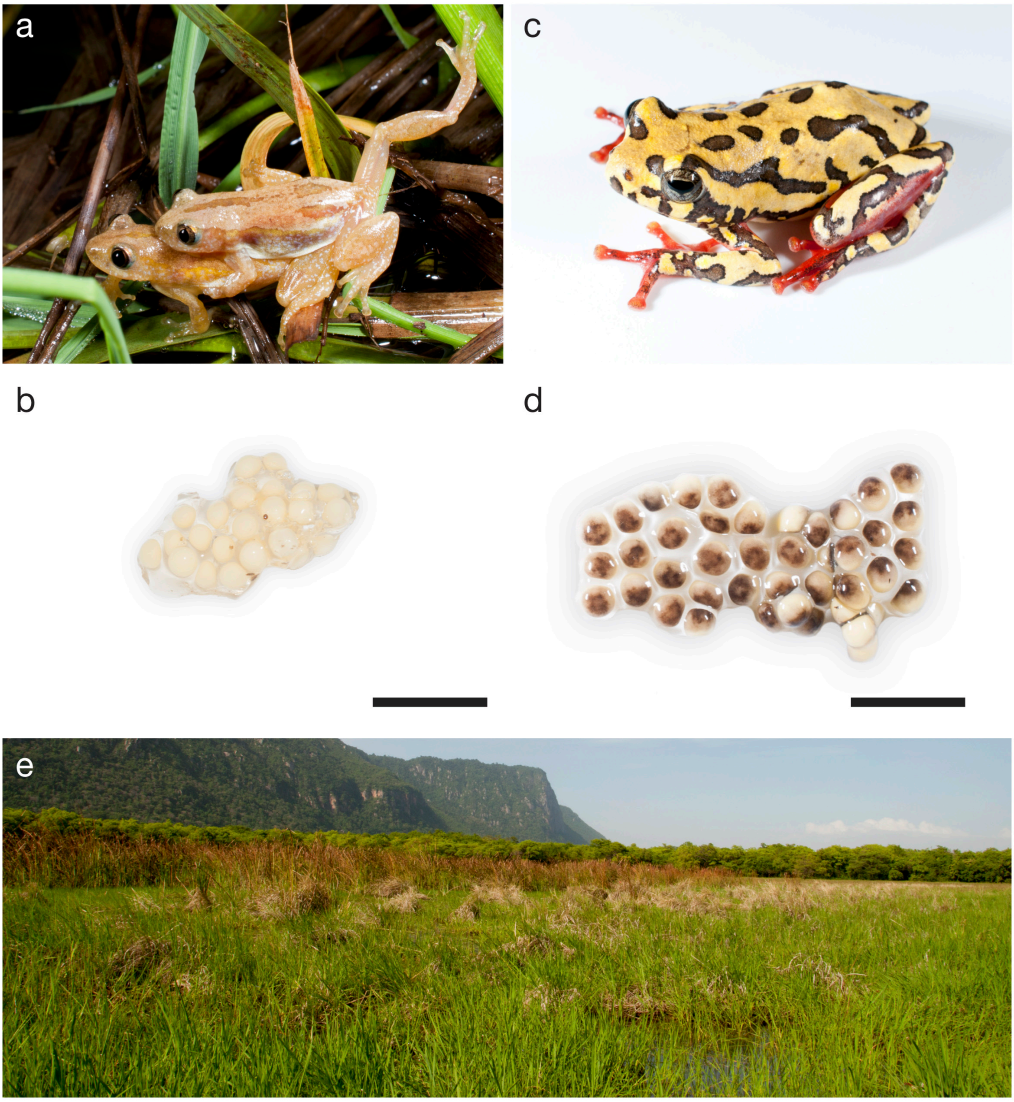

Figure 1. Specimen and site photos. a) *Afrixalus septentrionalis* in amplexus. b) *Afrixalus septentrionalis* eggs (scale bar 5 mm). c) *Hyperolius viridiflavus ngorongoriensis* female. d) *Hyperolius viridiflavus ngorongoriensis* eggs (scale bar 5 mm). e) Photo of collection site; seasonal wetland in Northern Tanzania.

2006; Pickersgill, 2007; Kouamé et al., 2015). As many of these values are listed as ranges, we used the largest recorded number of eggs and largest body size. When conflicting accounts were present in the literature, the characteristics described in AmphibiaWeb (2018) were used. No published comprehensive phylogenetic tree of *Hyperolius* species containing these species exists, so correlations of body size and egg counts could not be corrected for phylogeny. However, based on very preliminary analyses, the represented *Hyperolius* species with egg clutch data are a small sampling from across the radiation, and thus phylogenetic signal is expected to play a small role in potential correlations (Wieczorek et al., 2000, 2001; Pyron and Wiens, 2011).

The *Afrixalus* lineage represented by this study population is assigned to *A. septentrionalis*. This lineage is currently recognised on AmphibiaWeb (2018), and our population has the same morphology (small size with a

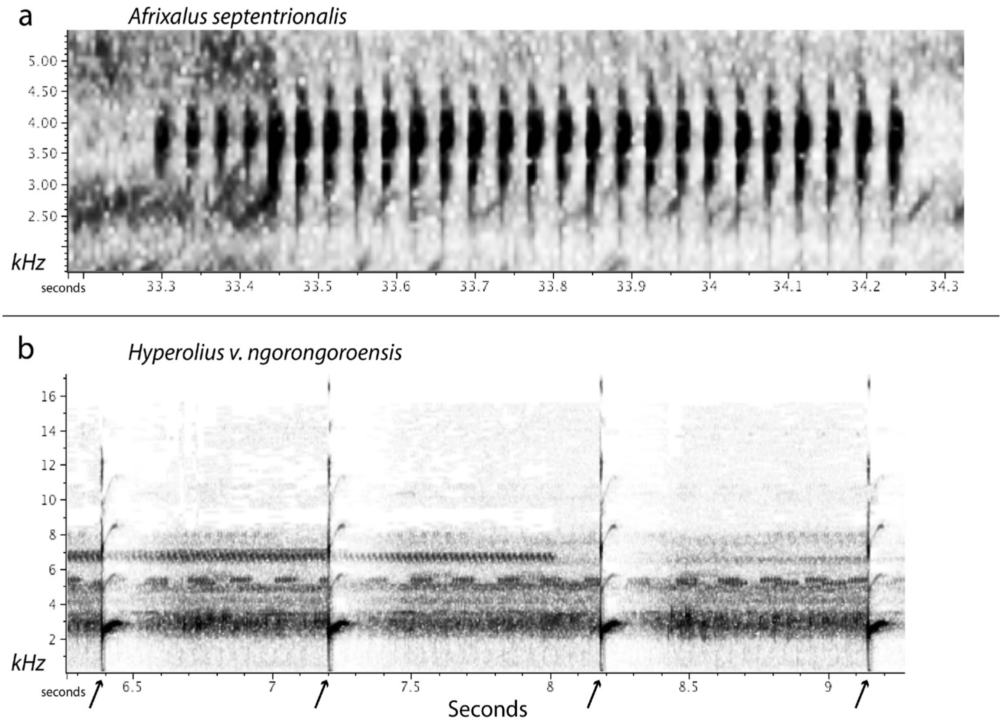

Figure 2. Voices of *Hyperolius viridiflavus ngorongoriensis* and *Afrixalus septentrionalis*. a) The spectrogram of *A. septentrionalis* focuses on a single vocalization to highlight the pulses in the trill; b) The spectrogram of *H. v. ngorongoriensis* shows multiple vocalizations from one individual. Each note is identified by an arrow to distinguish from the background chorus of trills.

single dark middorsal stripe, see below) as published accounts and was found in the small, restricted range of this species. According to one authority (Frost 2018), *A. septentrionalis* is considered to be synonymised with *A. stuhlmanni*. However, this cannot be valid as *A. stuhlmanni* has two dorsal stripes or a mottled dorsum. Our population does not match the call of *A. stuhlmanni,* which is a "short, high-pitched chatter" with pulse per second (pps) rates of 6–14 (Pickersgill, 2007). *Afrixalus septentrionalis* has a "long, even buzz" (Schiøtz, 1999). The spectrogram in Schiøtz (1999) shows 29 pps. Our call recordings match the voice of *A. septentrionalis* in Schiøtz (1999) (Fig. 2) with each buzz lasting approximately 0.8–1.2 seconds with 20–32 pulses with a rate of ~26 pps.

The identification to *Hyperolius viridiflavus ngorongoriensis* is potentially more complex as two *viridiflavus* species (*H. v. ngorongoriensis* and *H. v. goetzei*) have a very similar colouration to this population (Fig. 3) and distributions that are potentially adjacent (Schiøtz, 1999). Some sources have collapsed the clade that contains these lineages into *H. v. glandicolour*. However, recent work (Portik, 2015) has identified the *H. v. ngorongoriensis* lineage is paraphyletic with respect to *H. v. glandicolour*, though the relationship to the lineage known as *H. v. goetzei* is unclear. *Hyperolius v. goetzei sensu stricto* is found in the Southern Highlands of Tanzania (Pickersgill, 2007), but a similar lineage with the same designation is also known from the vicinity of Lake Victoria. Our call recordings are similar to those from the Lake Victoria population of *H. v. goetzei*, but we were unable to find any recordings or description of voice for *H. v. ngorongoriensis.* As this population is near the exact locality of the individual represented in (Portik, 2015), we maintain the use of *H. v. ngorongoriensis* for continuity.

The *Afrixalus septentrionalis* pair was observed in axillary amplexus on grasses close to the water surface (Fig. 1a). The female was larger than the male (23.3 mm and 21.1 mm respectively) and the pair laid 197 white eggs in small clumps (Fig. 1b). This number is higher than has been recorded for other *Afrixalus* species

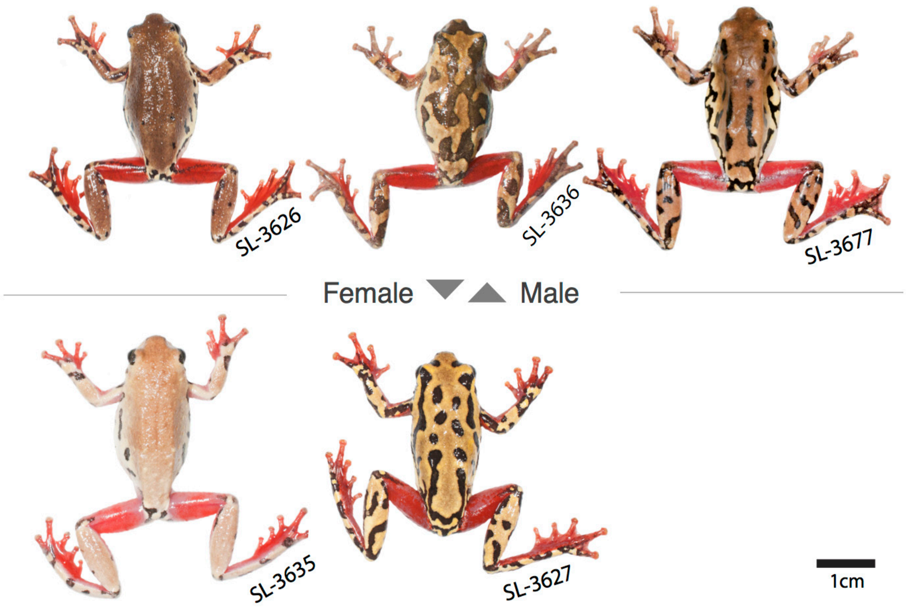

Figure 3. Plate showing colour variation of male (above) and female (below) *Hyperolius viridiflavus ngorongoriensis* from the study site. Individuals are roughly to scale and labelled with specimens numbers.

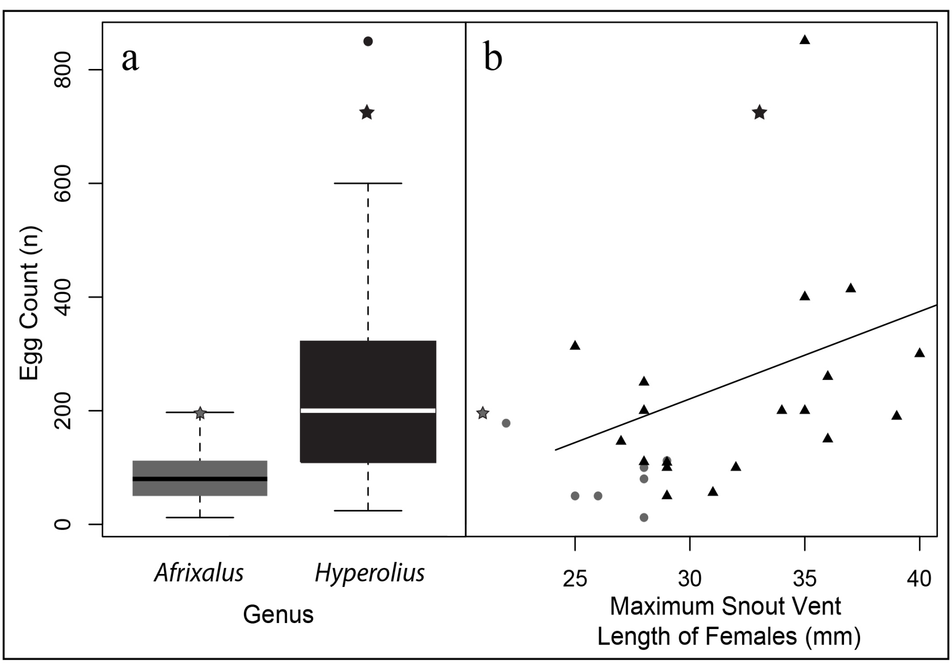

Figure 4. Egg Counts from this study and from the available literature. a) Egg counts for species of *Afrixalus* and *Hyperolius* compiled from the literature (grey = *Afrixalus*, black = *Hyperolius*). b) Egg counts in *Hyperolius* (black triangles) and *Afrixalus* (grey circles) species compared to maximum female snout vent length. *Hyperolius* regression line shown. The two species in this study (*Afrixalus septentrionalis* and *Hyperolius viridiflavus ngorongoriensis*) are shown as stars on both graphs.

Table 1. Life history characteristics including body (snout vent length; SVL), clutch and egg size for the genera *Hyperolius* and *Afrixalus* collected from the literature.

Genus	Ssp.	Species	Egg Clutch (n)	Diamete r Eggs (mm)	male SVL (mm)	female SVL (mm)	Both SVL (mm)	Max F. SVL (mm)	Max clutch (n)
Afrixalus		*aureus*	50	1	17–24	19–26		26	50
Afrixalus		*crotalus*		1	17-22	19-24		24	
Afrixalus		*delicatus*		1.3	15—22	16-24		24	
Afrixalus		*dorsalis*	100-112	1.7-1.9	25–28	26–29		29	112
Afrixalus		*dorsimaculatus*		2.2	21-24				
Afrixalus		*fornasini*	~80				30-40		80
Afrixalus		*fulvovittatus*	10-80		23–27	25–28		28	80
Afrixalus		*knysnae*		1			25	25	
Afrixalus		*septentrionalis**	197	1.23			19–21	21	197
Afrixalus		*spinifrons*	10-50	1.2	<20	<25		25	50
Afrixalus		*uluguruensis*	10-12		21-25	24-28		28	12
Afrixalus		*vittiger*	100	1.6-1.7	23-27	25-28		28	100
Afrixalus		*weidholzi*	83-178	1.0-1.7	18-21	21-22		22	178
Hyperolius	*vir.*	*angolensis*	200						200
Hyperolius	*vir.*	*ngorongoriensis* *	725	1.35	30-33	30-33		33	725
Hyperolius	*vir.*	*mariae*	330	1.2			<25		330
Hyperolius	*vir.*	*nitidulus*	183-850	1.4-2.2	23-30	27-35		35	850
Hyperolius	*vir.*	*nyassae*		1.5					
Hyperolius	*vir.*	*ommatostictus*	>512						512
Hyperolius	*vir.*	*verrucosus*	600	1.3					600
Hyperolius		*acuticeps*	60-292	0.7 - 1.2					292
Hyperolius		*argus*	200	1			27-34	34	200
Hyperolius		*bopeleti*	~100		19–22				100
Hyperolius		*castaneus*	100-150		22–26	25–36		36	150
Hyperolius		*cinnamomeoventris*	146		19–28	19–27		27	146
Hyperolius		*concolour*	200-300	1.9-2.0	24-31	30-40		40	300
Hyperolius		*cystocandicaus*	120-260		23-28	30-36		36	260
Hyperolius		*frontalis*	24		25–29				24
Hyperolius		*fusciventris*	250		18-23	23-28		28	250
Hyperolius		*guttulatus*	259-414		27–35	29–37		37	414
Hyperolius		*horstockii*		2			<43	43	
Hyperolius		*kivuensis*	190	1.5	22–33	33–39		39	190
Hyperolius		*langi*	56	1.6	20–24	26–31		31	56
Hyperolius		*lateralis*	109	1.8	18–26	21–29		29	109
Hyperolius		*mitchelli*	50-100	1.7	23-27	25-32		32	100
Hyperolius		*parkeri*	36-110		21–24		21-28	28	110
Hyperolius		*pickersgilli*	50	1	<22	<29		29	50
Hyperolius		*pictus*	60-100	2			23-29	29	100
Hyperolius		*poweri*	200	1	16-20				200
Hyperolius		*pusillus*	216-313	0.8 - 1.3			<25	25	313
Hyperolius		*semidiscus*	200	1			<35	35	200
Hyperolius		*spinigularis*	150-200	1.8 - 2	18-24	<28		28	200
Hyperolius		*substriatus*	130	1.8 - 2.5	21-29	30-37		37	130
Hyperolius		*tuberilinguis*	236-400	1.5	25-33	30-35		35	400

(mean = 95.44, SD = 60.20, range = 10–178; Fig. 4a), though very few species have documented egg counts (n = 9), and those that do might be confounding total egg counts with small sub-clutches. The average egg diameter for *Afrixalus septentrionalis* was 1.23 mm (SD = 0.04). No clutches were observed in the field and therefore the oviposition site could not be determined, but it is common in this genus to attach eggs to leaves (sometimes folded and glued) overhanging water.

The *H. v. ngorongoriensis* pair was observed in axillary amplexus on reeds >30 cm above the surface of the water. The female was larger than the male (30.8 mm and 27.9 mm respectively; Fig. 1c). The pair laid 725 eggs with dark and light poles and an average diameter of 1.35 mm (SD = 0.02; Fig. 1d). *Hyperolius* species typically lay between 100–200 eggs (Table 1), though members of the *Hyperolius viridiflavus* superspecies complex, to which *H. v. ngorongoriensis* belongs, may lay more (overall *Hyperolius* mean = 263.61, SD = 202.61; *Hyperolius viridiflavus* superspecies members mean = 536.17; Fig. 4). No clutches were encountered in situ, but observations from Kenya state that in this species, eggs are deposited directly in water (Malonza et al., 2010).

The relationship between body size and egg counts was evaluated in the *Afrixalus* and *Hyperolius* species with available clutch size information. No significant relationships were found (*Afrixalus* $F_{(1,6)} = 5.505$, P = 0.057, adjusted $R^2 = 0.392$; *Hyperolius* $F_{(1,18)} = 1.981$, P = 0.1763, adjusted $R^2 = 0.049$; Fig. 4b).

Life history traits, such as egg counts and descriptions, are valuable as both diagnostic characteristics when encountering egg masses in the field and as traits for studying evolutionary reproductive strategies for each species (Gomez-Mestre et al., 2012; Liedtke et al., 2016). The members of the *Hyperolius viridiflavus* superspecies complex appear to have a very different strategy from other *Hyperolius* species with very large egg counts compared to most other species. The reason for these large clutches is not known. Body size was hypothesised to play a role in the clutch size of each species, but no correlation was found in this preliminary dataset. While members of the *Hyperolius viridiflavus* superspecies are considered “large” for the genus (*viridiflavus* complex vary from small to large: 20–40 mm; Pickersgill, 2007), other “large” species did not have particularly large clutch sizes. One possibility for the lack of correlation could be that some species invest in a smaller number of larger eggs, such as the very large *Afrixalus lacteus* and *A. laevis* which lay several clutches that only contain 4-10 eggs of exceptionally large diameter. In this study, these two lineages have relatively high egg counts and relatively small eggs, supporting this alternative view. Thus, body size may instead correlate to “investment” (egg diameter x egg number) instead of simply egg number. As more data are compiled on egg counts and characteristics (such as diameter), and more comprehensive phylogenetic datasets become available, the role of fecundity investment in diversification within the family Hyperoliidae will become clearer.

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