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Reproduction of the Ethiopian Mountain Adder, *Bitis parviocula* Böhme, 1976 (Reptilia: Viperidae), at ZSL London Zoo

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The Ethiopian Mountain Adder, *Bitis parviocula* Böhme, 1976, is an endangered Ethiopian endemic, confirmed to occur in the montane forest of the southwest (Böhme, 1977; Spawls, 2021), with reported collection localities ranging from 1700–2800 m in elevation (Largen and Rasmussen, 1993). Its body colours and patterns are highly contrasting and likely facilitate camouflage (Spinner et al., 2013) in the richly structured ground habitat this species is presumed to inhabit. Limited numbers of museum specimens and minimal reliable field data are available for reference (e.g., Gower et al., 2016; Petrilla et al., 2021), and little is known about the ecology of *B. parviocula.* The species has been kept in captivity since at least 2007 (Sánchez et al., 2011) and captive reproduction has been briefly summarised (Maritz et al., 2013), but detailed information regarding captive management, reproductive biology, and breeding is lacking. The present work describes the successful reproduction of *B. parviocula* at ZSL London Zoo, with detail on husbandry and animal behaviour throughout the preceding years.

Materials and Methods

Origins of animals. Two *B. parviocula* were donated to ZSL London Zoo in August 2016. These snakes were reportedly F2 captive bred in the private sector and approximately one year of age at the time of arrival. The origin of the founder animals is unknown. The male weighed 110 g and the female weighed 90 g at acquisition.

Captive habitat. From August 2019 the snakes were housed in individual vivaria measuring 1800 x 900 x 900 mm (L x W x H), creating a ground area of 1.62 m^2. Vivaria were enriched with organic substrate (primarily peat-free compost and chipped bark), live plants (*Asplenium nidus*; *Calathea* sp.; *Elettaria cardamomum*; *Nephrolepis* sp.; *Scindapsus aureum*), rocks, and branches to create structure, dappled shade, and hiding places across thermal, visible, and UV light gradients.

Lighting. Ambient lighting on a 12 h:12 h cycle consisted of T8 warm white room lighting and, over each vivarium, eight 550 mm 24-W T5 lamps including one Arcadia T5 D3 14% and various brands of cool-white non UVb-emitting T5 lamps, all within reflector units (LightWave T5 LW24-HO). A single Osram UltraVitalux 300-W mercury vapour lamp within a reflector dome was positioned to create a bask zone. Across the vivarium, the lighting scheme created an ambient UV index (UVi) of 0.5–1 at ground level, increasing to 4.7 in the bask zone, and with areas beneath cover of UVi 0, measured using a Solarmeter 6.5 UV meter.

Temperature and water provisions. Room temperature was controlled via air conditioning and, after April 2020, provision of vivarium photoperiod, bask zone availability, ambient temperature, and precipitation intensity and frequency were informed by data from the highlands of southwest Ethiopia (Table 1; www.meteoblue.com). There, mean maxima range from 18.0–24.0°C and mean minima range from 12.0–15.0°C (Dinerstein et al., 2017). Between August 2020 and July 2021 daytime ambient temperatures in each vivarium ranged from approximately 20–28°C. A bask zone large enough for each snake when coiled was available and surface temperatures ranged from 30–36°C, as measured with Precision Gold N85FR non-contact thermometer. During the same time period, average night-time ambient temperature ranged from 18.5–22.5°C. Vivaria were sprayed with reverse osmosis water from a hand-pump sprayer. A bowl suitable for drinking water, but not large enough for animals to entirely fit into, was continuously available to the animals.

Feeding. Snakes were offered defrosted, commercially bred rodents and birds, ranging in size from quail chicks and large mice to large degus and small adult rats, on a fluctuating timescale of once every 7–28 days to provide less predictability for the snakes. Food item size was less

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Table 1. Selected husbandry parameters for *Bitis parviocula* at ZSL London Zoo. Daily high and low ambient temperatures were recorded directly from the cool zone of the female snake's vivarium. Bask zone duration and approximate daily spray duration were timed to replicate climate data measurements from the native geographic range.

Month	Average daily high temperature (°C)	Average daily low temperature (°C)	Bask zone daily availability (h)	Approximate daily spray duration (min)
January	23.2	18.5	4	< 1
February	23.1	19.2	5	< 1
March	24.3	19.5	7	< 1
April	23.9	19.9	8	1–2
May	23.6	19.3	8	1–3
June	25.8	19.0	8	1–4
July	23.7	18.7	8	2–6
August	25.2	19.5	8	2–6
September	27.1	21.5	7	1–3
October	27.9	22.7	7	< 1
November	27.0	22.9	5	< 1
December	26.1	22.5	4	< 1

than twice the width of a snake's head. For the male and female snake, respectively, weight was 592 g and 1260 g, as measured using a digital balance (Salter 1160BKDR) in April 2020. At that time, total length, measured using a scaled digital photograph in ImageJ 1.8.0 (Schneider et al., 2012), was 967 mm for the male and 1002 mm for the female.

Results

Initial cohousing and courtship. Between March and May 2020, the male and female cohabited in the female's vivarium (Fig. 1A). At this time ambient temperatures ranged from 20–26°C, with a bask zone temperature of 30–32°C. On the first introduction, at 1 pm on 12 March 2020, both snakes appeared agitated, and the female raised her tail vertically, waved it sinuously and everted a small (ca. 1–2 mm) length of soft tissue from the proximal side of the cloaca (Fig. 1B–E). After approximately 2 h together, the male was noted to be courting the female, directing chin-jerking and rapid tongue flicking along the length of the female's body (Phelps, 2010). During courtship, the male occasionally moved his tail under the female's tail to align cloacae and attempt intromission. Three hours after the initial introduction, the male was removed from the female's vivarium and housed individually, being moved back into the female's vivarium several days later to facilitate natural behaviour. The male was introduced to the female on four separate occasions during the following 51 days, for a total of 18 days of cohabitation. The duration of cohousing was based on the behaviour of both individuals. When housed together, courtship was frequently observed and lasted up to 3 h before the pair naturally separated themselves; confirmed intromission was not observed. Especially toward the latter period of cohousing, the male was noted to spend much time apparently guarding the female, resting on or beside her. After the male consistently showed no further interest in courting the female, both animals were housed individually for the remainder of the non-breeding period.

From June 2020 the female was noted to increase basking duration and, additionally, the distal two thirds of the body appeared larger and fuller than usual despite normal feeding and defecating. The female was radiographed at the zoo's veterinary hospital 82 days after the first introduction of the male to assess development of any potential offspring. None were apparent and in the following several months, the female returned to a normal body appearance. Feeding was consistent throughout this time and mass increased from 1260 g to 1280 g between April and July 2020.

Cohousing and reproduction. The husbandry of the snakes continued to be based on climate data from the natural geographic range, and in December 2020 the male began fasting and became hyperactive. At this time, his mass was 744 g. The male was introduced to the female's vivarium on 8 December 2020 for a total of 47 out of the next 66 days. This represented cohabitation for 71% of the reproductive period, compared to 35% of the reproductive period terminating in May 2020. Courtship behaviour was immediately seen and noted throughout much of the duration of cohousing. Following

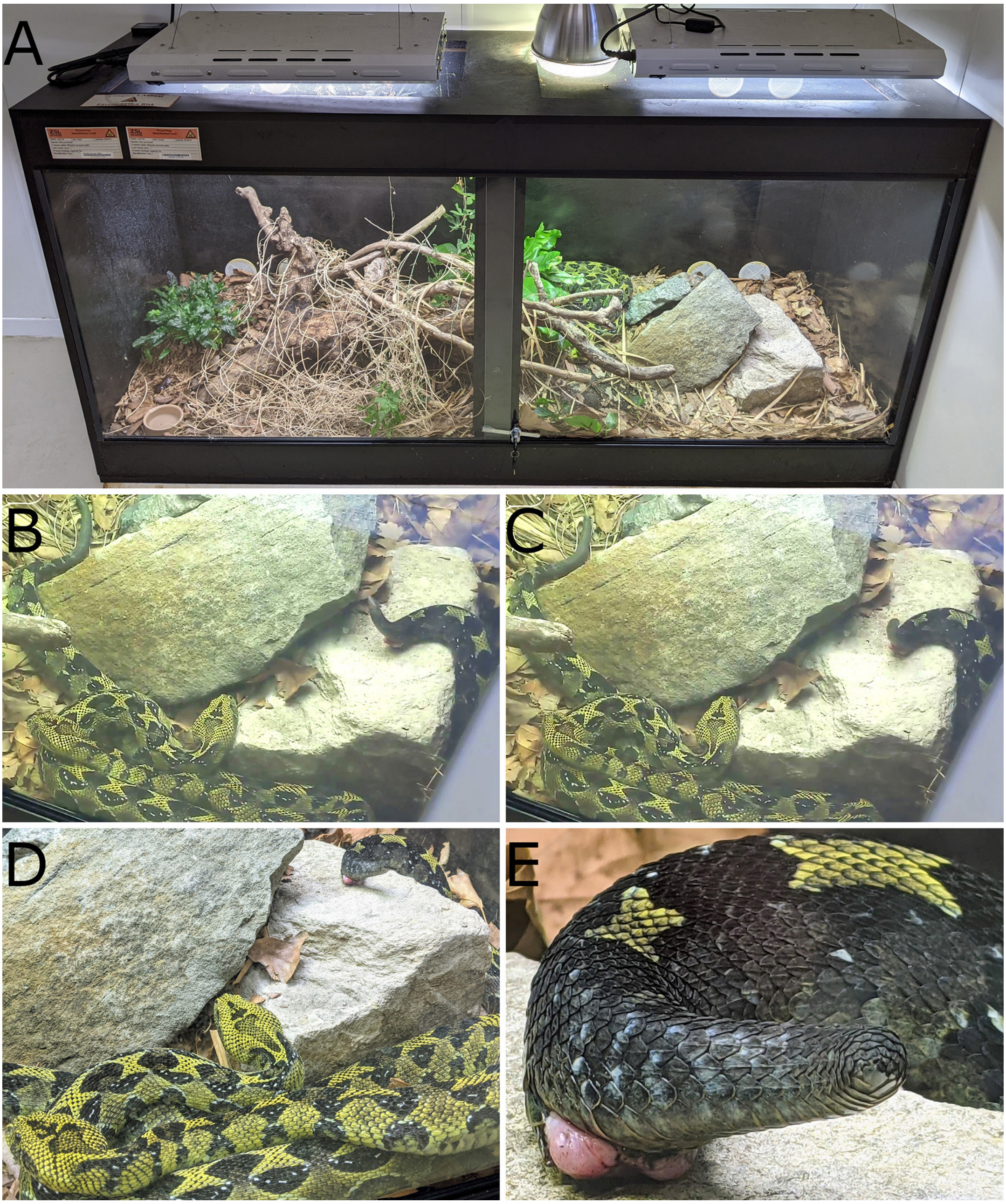

Figure 1. (A) The vivarium where *Bitis parviocula*, visible centre-right, reproduced at ZSL London Zoo. (B, C) Tail held vertical while undulating from side to side, demonstrated by female *B. parviocula* while being courted by male conspecific. (B-D) depict the tail-waving behavior. (D, E) Closer views of male courting female, the latter with partially raised tail (D). (E) Tissue partially everted from the cloaca of the female. Photos by Daniel Kane.

separation, the female began basking more than usual and increased in mass from 1116 g in January to 1289 g in April. On 16 June 2021, 125–191 days after being cohoused, the female gave birth overnight. A total of 13 young (Table 2) were removed from the vivarium over the course of two days (Fig. 2). It is unknown whether all young were born during the same event or if there was an interval between the first and last births. One young was stillborn and four presented with deformities of variable severity, including severe scoliosis and kyphosis of the spine and externalised viscera. The deformed snakes were euthanised on welfare grounds and the cause of these deformities remains unknown. The remaining eight snakes were all physically healthy.

Pre- and post-parturition body weights for the female were 1289 and 916 g, respectively, representing a relative litter weight of 29% of the female's pre-parturition weight. The female consumed a meal 35 days before parturition and refused to feed on two subsequent occasions prior to parturition; food was accepted six days post-parturition. This snake sloughed 288 days prior to birthing and again 34 days after parturition.

Discussion

The captive reproduction of *B. parviocula* represents a rare occurrence and highlights the importance of thorough record keeping and dissemination of data from observations of poorly known species under controlled conditions (Maritz et al., 2016; Kane et al., 2021). Aside from novel data on husbandry and reproduction, novel tail-waving behaviour from the female is reported for the first time. The tail was held nearly vertical and undulated from side to side for several seconds at a time. This behaviour was regarded as an indicator of stress as prior to initial cohabiting of the male and female, it

Figure 2. *Bitis parviocula* juvenile at ZSL London Zoo, August 2021. Photo by Daniel Kane.

was only observed in association with disturbance from outside the vivarium, or during necessary handling, and never in response to potential prey. Furthermore, this was often accompanied by coiling, hissing, and occasionally striking at perceived threats. Caudal luring is identified in *B. arietans* (Merrem, 1820) by horizontal or vertical tail-waving (Glaudas and Alexander, 2017). The male *B. parviocula* as well as male *B. arietans* previously maintained at ZSL London Zoo were, on occasion, observed to hold the tail horizontally and wave it from side to side during presentation of food (DK, pers. obs.).

The reproductive event described herein produced eight male and five female young. Snakes were sexed visually by relative tail length, with males having proportionally longer tails than females (Tomović et al., 2010; Maritz and Alexander, 2011). When published *B. parviocula* reproductions (Maritz et al., 2013; present study) are combined, the overall sex ratio of 1:1.07 is not substantially different from equal which suggests similar birth rates for each sex. The data presented by Maritz et al. (2013) do not include a discussion on neonate health, so a clutch-to-clutch comparison is not possible. Bonnet et al. (2001a) reported a high incidence of stillborn *B. gabonica* (Duméril et al., 1854) in captive reproduction, hypothesised to be a result of inbreeding. Pedigree of the breeding pair of *B. parviocula* from ZSL is unknown, therefore relatedness cannot be ruled out as a contributing factor to the deformities observed in some of the neonates.

The available data suggest that the gestation period of different *Bitis* species in captivity are variable, which may be unsurprising considering the adult size difference within this diverse genus. Available data do not allow investigation into the effect of same-species adult female size on gestation period. Additional potential influences on gestation period include environmental or nutritional factors (Bonnet et al., 2001a,b; Lourdais et al., 2004). Sperm storage (Seigel and Ford, 1987) may enable variation in time from mating to subsequent birth and in recent years has been demonstrated in an increasing number of viperids (Devan-Song et al., 2017; Silva et al., 2020). The precise date of conception in the present study is not known, however 66 days elapsed between first and final cohabiting of the reproductive pair in 2021. Possible sperm storage notwithstanding, the potential range in duration of gestation represents 35–52% of the total gestation period and is comparable to some *B. arietans* records (Rosi, 1988b; Haagner, 1990). With additional documented breeding of *B. parviocula* the potential mechanisms influencing gestation period may become better known.

Table 2. Selected reproductive data for some *Bitis* species. *Bitis parviocula* is most closely related to *B. gabonica*, *B. heraldica*, *B. nasicornis*, and *B. rhinoceros* (Ceríaco et al., 2020); *B. heraldica* (Bocage, 1889) is not included due to a lack of reproductive data. *Bitis arietans* is included, however, due to available reproduction data. Values are presented as mean (range) and unavailable data are denoted with an asterisk.

***Bitis* Species**	**Weight of Reproductive Female (g)**	**Total Length of Reproductive Female (mm)**	**Litter Size**	**Sex Ratio (M : F)**	**Gestation Length (days)`**	**Infertile Ova / Stillborn Young**	**Neonate Weight (g)**	**Neonate Total Length (mm)**	**Observation Type**	**Reference**
arietans	2400	1100	41	0.95:1	159	18 infertile ova	(14–25)	(235–255)	Captive	Rosi, 1988b
arietans	1950	980	30	0.86:1	136	13 infertile ova	(15–19)	*	Captive	Rosi, 1988b
gabonica	*	1045 (630–1330)	18 (5–32)	*	*	*	*	*	Wild	Angelici et al., 1998
nasicornis	*	7930 (630–1181)	25 (13–42)	*	*	*	*	*	Wild	Angelici et al., 1998
parviocula	1116	1091	13	1:0.63	(125–191)	1 stillborn young; 4 severely deformed	18.1 (16–20)	259 (239–279)	Captive	This study
parviocula	*	780	11	1:0.57	*	*	(8.2–24.0)	(170–240)	Captive	Maritz et al., 2013
parviocula	*	900	12	1:1.4	*	*	(8.2–24.0)	(170–240)	Captive	Maritz et al., 2013
parviocula	*	1050	16	1:1.7	*	*	(8.2–24.0)	(170–240)	Captive	Maritz et al., 2013
rhinoceros	4620	1400	49	1:0.96	198	1 stillborn young; 1 died during birth	(34–40)	(295–315)	Captive	Rosi, 1988a
rhinoceros	*	*	≤ 60	*	≤ 365	*	*	(250–350)	Captive	Slegers, 1996

The reproductive events described above and by Maritz et al. (2013) do not indicate whether metabolic costs related to reproduction are met via stored fat reserves (Glaudas et al., 2020), facultative feeding (Winne et al., 2006), or a combination of both (Pleguezuelos et al., 2007). Captive female *B. parviocula* (present study) and *B. arietans* (Hartmann and Steiner, 1984) continued to feed throughout most of gestation, contrary to wild gravid *B. nasicornis* (Shaw & Nodder, 1792) and *B. gabonica* (Luiselli and Akani, 2003). Therefore, it is unknown whether the feeding ecology of *B. parviocula* is different from congeners or if the observed difference is an effect of captivity. A captive *B. parviocula* female reproduced in two consecutive years (Maritz et al., 2013), but long-term data for comparison with other individuals are lacking.

According to captive data, mating and gestation in the wild are likely to occur during the warmest period of the year, avoiding the typical June–August rains in the highlands of the Ethiopian southwest. This phenology may be considered adaptive in *B. parviocula*, whose ectotherm metabolism is affected by external temperature. The gravid female *B. parviocula* in this study basked extensively during pregnancy, likely maintaining a higher preferred body temperature than during the non-reproductive period. However, measuring core body temperature was outside the scope of this work. Parturition in the rainy season (Angelici et al., 1998) may synchronise the appearance of young vipers with the period of greatest prey availability, presumably including amphibians and small mammals - known dietary components of at least four congeneric species (Bonnet et al., 2001a; Luiselli and Akani 2003; Glaudas et al., 2017).

Rearing of young *B. parviocula* in ZSL was uneventful and not dissimilar to rearing other young *Bitis*. New-born mice, reported to form part of the natural diet in subadult *B. gabonica* and *B. nasicornis* (Luiselli and Akani, 2003), were consumed by all individuals the first time they were provided, 20–22 days post-birth, with little hesitation. The young snakes sloughed their skin immediately following birth and again 60–88 days later. Between mean body weight on Day 0 (18.125 g) and Day 132 (49.625 g), the mean increase in body weight of the eight young *B. parviocula* was 31.5 g.

The present work provides new data on successful captive management and reproduction of *B. parviocula*, as well as a description of a novel behaviour we believe to be a stress response. Novel information on natural history is especially important to gather for poorly known species, including *B. parviocula*. These data may directly inform conservation actions such as population viability analyses. The authors encourage further efforts to increase knowledge of poorly known snake species, both in controlled conditions and in the field.

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