

RESEARCH ARTICLE

Reproductive cycles of the arboreal and viviparous snake *Corallus hortulana* (Serpentes, Boidae) from the Brazilian Amazon

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Abstract

Although the reproduction of some Boidae snakes has been studied through the macroscopic anatomy of the gonads, without microscopic morphology, there is little precision in describing reproductive cycles, especially for males. The relationship between the reproductive cycle—gametogenesis and reproductive seasonality throughout the year—has yet to be detailed for many Boidae species. We present macroscopic and histological data on the reproductive tracts of both male and viviparous female *Corallus hortulana* (Linnaeus 1758) (Amazon Tree Boa) from the Brazilian Amazon rainforest. Males present spermiogenesis and spermiation mainly during January and June (wet season to beginning-dry season). Contrary to common assumption, we speculate that sperm production in *C. hortulana*, even in the tropical Amazon, is likely energetically demanding, influenced by rainfall, given that testicular involution occurred between May and November (most of the driest months of the year). Females present ovarian follicles in processes of vitellogenesis from December to August (wet season and part of the dry season). However, newborns are found in the wild mainly in the wet season, characterizing the cycle of female *C. hortulana* as seasonal. Unprecedentedly, we present the first microscopic description of the pouch and non-glandular uterus in a Boidae species. The cells that compose the epithelial tissue of the non-glandular uterus have been suggested for maintaining and supporting spermatozoa (sperm storage) in snakes, but we found no evidence that sperm storage occurs in this portion of the oviduct in *C. hortulana*. This study not only improves our understanding of reproductive biology in a boid, but because several aspects of reproductive cycles are conservative in the family (e.g., season of birth of newborn snakes), it aids in the development of effective conservation policies for endangered species such as *Corallus cropanii*, the rarest Boidae in the Americas.

KEYWORDS

Amazon tree boa, histology, oviduct, reproduction, testes

1 | INTRODUCTION

In Squamata (the lineage of snakes and lizards), species exhibiting continuous reproduction—such as females that are gravid or give birth throughout the year and males that maintain peak spermatogenesis year-round—are rare, and predominantly found in tropical rainforests (Fitch, 1970; Mathies, 2011; Siqueira et al., 2022). However, even in the Amazon rainforest, several snake species present seasonal reproductive cycles. For example, females of the live-bearing pit viper *Bothrops atrox* (Viperidae) and tree boa in the *Corallus caninus* complex (including *C. caninus* and *Corallus batesii*) give birth primarily in the dry and the wet seasons, respectively (Pizzatto & Marques, 2007; Silva, et al., 2019). Similarly, male *B. atrox* also displays a seasonal pattern with testicular regression occurring during the dry season (Silva et al., 2019), but no data is available for males of *C. caninus*. Such non-seasonality suggests that other factors, beyond weather, such as prey availability and life history, also influence reproductive cycles (Krohmer & Lutterschmidt, 2011; Lutterschmidt & Mason, 2009; Saint-Girons, 1982).

Few studies have been published on species of Boidae using histological and tissue morphology evidence (Bento et al. 2024a, 2024b; Pinheiro et al., 2020). Most existing studies have focused solely on reproductive aspects using macroscopic data (Bertona & Chiaraviglio, 2003; Garcia & Almeida-Santos, 2021; Pizzatto & Marques, 2007). Similarly, species of Pythonidae, which include large constrictor snakes, often exhibit convergent biological traits with Boidae (Murphy & Henderson, 1997; Reed & Rodda, 2009; Reynolds et al., 2014), are equally underrepresented in studies involving microscopic analyses of their reproductive aspects (Currylow et al., 2022; Shine et al., 1998, 1999).

Corallus hortulana (Linnaeus 1758) is widely distributed across Brazil (see the full distribution at Nogueira et al., 2019), including the Amazon rainforest, Atlantic Forest, Caatinga, Pantanal, and the northern part of the Cerrado, primarily inhabiting low-elevation areas (Colston et al., 2013). It is a medium-sized, viviparous species with a generalist diet composed of birds, bats, frogs, lizards, and small mammals (Carvalho et al., 2019; Martins & Oliveira, 1998; Pizzatto et al., 2007, 2009; Yáñez-Muñoz et al., 2017). Additionally, this snake exhibits both external (e.g., laterally compressed body) and internal (e.g., reproductive organ topography) morphological adaptations that support its arboreal lifestyle (Pizzatto et al., 2007). So far, current descriptions of the reproductive traits of *C. hortulana* are still insufficient to establish a precise reproductive pattern for the species and determine whether its reproductive cycle is

influenced by geographic range (Pizzatto & Marques, 2007). For instance, considering a pooled sample of females from a broad range of climates and different Biomes (Amazon, Atlantic Forest, Caatinga, and Cerrado), *C. hortulana* birth season occurs during the summer (wet season) and autumn (dry season) (Pizzatto & Marques, 2007). In contrast, Bernarde and Machado (2010) observed a female and four newborns in the Amazon rainforest in November (spring/wet season), possibly suggesting year-round reproduction or geographic variation in the reproductive timing for the species. More information on births is needed to fully understand potential geographic variation in the female reproductive cycles of *C. hortulana*.

In other neotropical tree boas, such as the *C. caninus* complex, *Corallus cookii*, and *Corallus ruschembergii*, newborns have primarily been found in the wet season (Henderson, 2015; Pizzatto & Marques, 2007). Given that many reproductive traits are highly conservative among boids (Pizzatto & Marques, 2007), studies on the reproduction of neotropical tree boas, at both macroscopic and microscopic levels, can offer new insights into the reproductive strategies of the genus. Such insights might help conservation policies for the rarest boid in the Americas: the semi-arboreal, IUCN endangered-listed *Corallus cropanii*, for which there is no reproductive information (Machado-Filho et al., 2011; Silveira et al., 2021; Stafford & Henderson, 1996).

We collected macroscopic and histological data on the reproductive tracts of both male and female *C. hortulana* from the Brazilian Amazon rainforest to describe the species reproductive cycle, sexual maturity, and litter size. We compare our findings for *C. hortulana* with the reproductive patterns reported for other boid species.

2 | MATERIALS AND METHODS

2.1 | Specimens and study area

We examined 153 specimens of *C. hortulana* collected throughout the Amazon rainforest and preserved in scientific collections (Appendix A, Figure 1). Our sample consisted of 75 females (51 adults, 10 subadults, and 14 newborns), 75 males (61 adults, 12 subadults, and 2 newborns), and three unsexed newborn individuals. Because the largest newborn in captivity (record in literature) measured 520 mm snout-vent length (SVL), we classified any preserved specimens with SVL less than this value as newborn (Bernarde & Machado, 2010; Pizzatto & Marques, 2007; Viana, 2017).

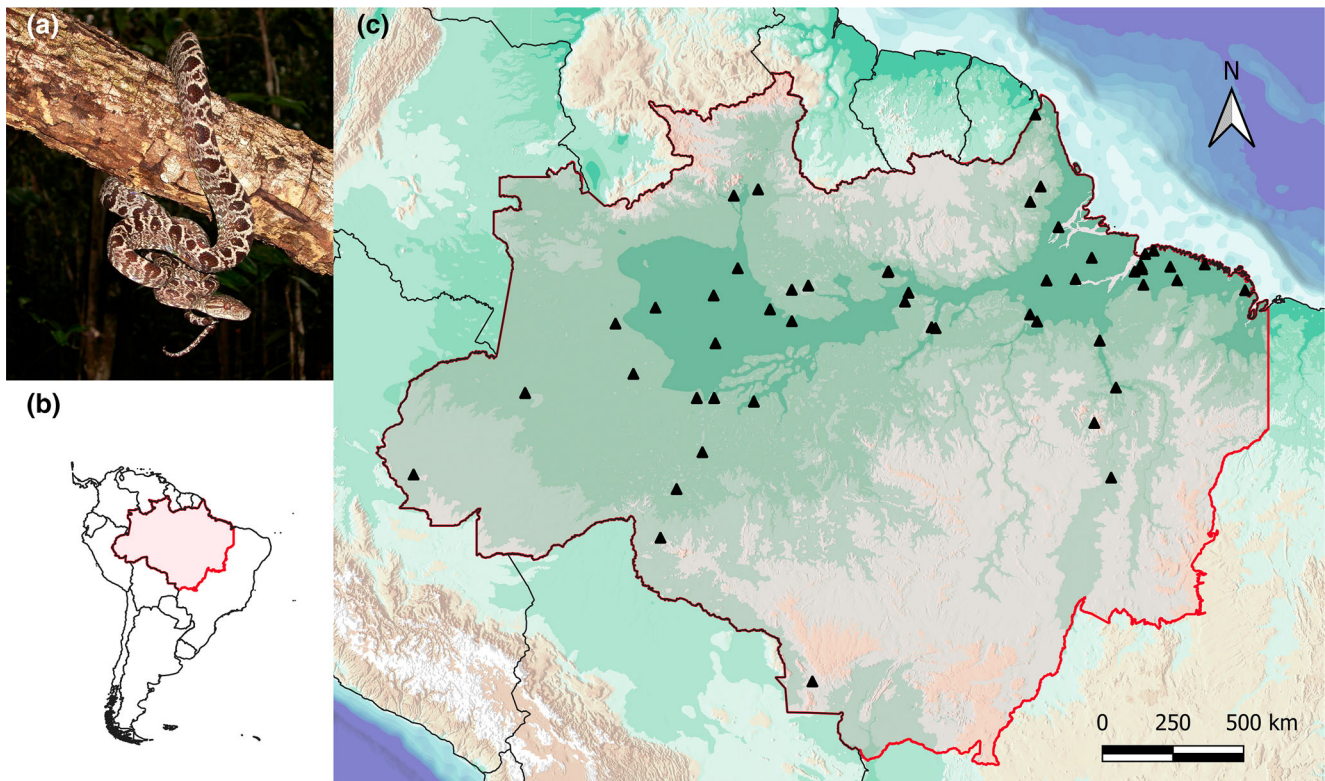


FIGURE 1 Distribution map of sampled *Corallus hortulana*. (a) *Corallus hortulana* specimen photographed in Viruá National Park, Roraima, Brazil. (b) Overview of the South American continent. (c) Collection sites (triangles) of *C. hortulana* within the Brazilian Amazon rainforest (highlighted in red) used in this study.

In general, areas within the Amazon rainforest have an equatorial climate with relatively constant mean temperatures throughout the year and well-marked seasonal rainfall (Marengo & Fisch, 2021; Nobre et al., 2009). Therefore, we compared samples between dry (May to October) and wet seasons (November to April).

2.2 | Gross morphology

Before performing dissections, we measured SVL and tail length (TL) of each specimen. Females were considered sexually mature (adult) if they had ovarian follicles bigger than 10 mm in length (in vitellogenesis processes), corpora lutea, or folded oviducts (indicative of recent egg-laying) (Pizzatto & Marques, 2007; Vieira et al., 2010). Males were considered adults if they had convoluted ductus deferentia or/and spermatozoa in the reproductive tract (Pizzatto & Marques, 2007; Shine, 1977a).

To describe seasonal variation in the reproductive cycle, we analyzed the sizes of the ductus deferentia, testes, and seminiferous tubules, and the size and number of ovarian follicles. In adult females, we recorded the number of ovarian follicles >10 mm as

an indication of litter size (Shine, 1977b; Silva, Braz, et al., 2020) and the diameter of the largest ovarian follicle. In adult males, we recorded the diameter of the right distal ductus deferentia (DDD; between the kidney and the cloaca) and the length and width of the right testis (± 0.1 mm). For macroscopic measurements, we utilized a digital caliper (± 0.1 mm). We estimated testis volume (TV) in each season using the cylinder volume formula: $TV = \pi r^2 h$, where r = width of the right testis and h = length of the right testis. We measured the height of the seminiferous epithelium height (SEH) and seminiferous tubule diameter (STD) using ImageJ software v1.46 (Abramoff et al., 2004) on photomicrographs of those structures (see Section 2.4).

2.3 | Morphological data analyses

All morphological variables were evaluated and tested for normality through quantile–quantile plots (q–q plots; Supplementary material 1) and Shapiro–Wilk, and homogeneity of variances was assessed using Levene’s test. We tested the correlation between SVL and the males reproductive structures (DDD, TV, STD, and SHE) using linear

regression. We then used the Mann–Whitney U test for all variables that were uncorrelated to SVL, or permutational ANCOVA (PerANCOVA) for the TV, as it was significantly correlated to SVL.

We used linear regression to determine the relationship between litter size and maternal SVL (Supplementary material 2). All analyses were performed using R 4.0.2 (R Core Team, 2021).

2.4 | Histology

In adult females ($n = 12$, states of Amazonas, Amapá, and Pará), we excised the posterior portions of the right non-glandular uterus and pouch (see Siegel et al., 2011 for the nomenclature of the oviduct sections) to check for the presence of spermatozoa. In adult males ($n = 16$, Brazilian states of Amazonas, Amapá, and Pará), we collected the mid-region of the testes and the distal ductus deferentia (the portion between the kidney and the cloaca). Tissue samples were stored in 70% ethanol, dehydrated, and embedded in historesin. We used a rotary microtome to produce transverse sections ($5\ \mu\text{m}$) and stained the slides with toluidine blue-fuchsin (Junqueira and Junqueira & Carneiro, 2023). Testis and ductus deferens sections were assessed for sperm production and presence, respectively. We classified spermatogenesis according to the six-cell stages presented by Silva, Braz, et al. (2020). However, we only observed the following three stages: (1) spermiogenesis (differentiation in sperm cells), (2) spermiation (mature spermatozoa in

the lumen), and (3) quiescence (reduced germinal epithelium, with few spermatogonia). All photomicrographs were taken at a magnification of $200\times$ and $400\times$, using a Leica DM500 optical microscope with an integrated Leica ICC50 W camera (Leica Microsystems-Wetzlar, Germany).

3 | RESULTS

3.1 | Female reproductive cycle and litter size

Adult females averaged 1354 ± 124 mm SVL (range 1130–1750 mm), and their tails measured 334 ± 23 mm (range 296–397 mm). Females in early vitellogenesis and advanced vitellogenesis had ovarian follicles measuring between 10 and 26 mm length (Figure 2). The number of vitellogenic ovarian follicles (>10 mm) in each female averaged 8 ± 4 follicles (range = 2–18, $n = 12$) and was not correlated with female SVL ($R^2 = 0.2$, $p = 0.13$). A pregnant female measuring 1371 mm SVL (voucher: IB47223; location: Tucuruí, Pará, Brazil) was found with embryos ($n = 5$, but some embryos were removed from the uterus before this study) measuring between 345 and 369 mm SVL during end-wet season (April). We recorded 19 newborn individuals in the wild measuring 476 ± 19 mm SVL (range: 443–507 mm), and tails 121 ± 11 mm (range: 106–152 mm). Sixteen newborn individuals preserved in the collections were collected during the wet season,

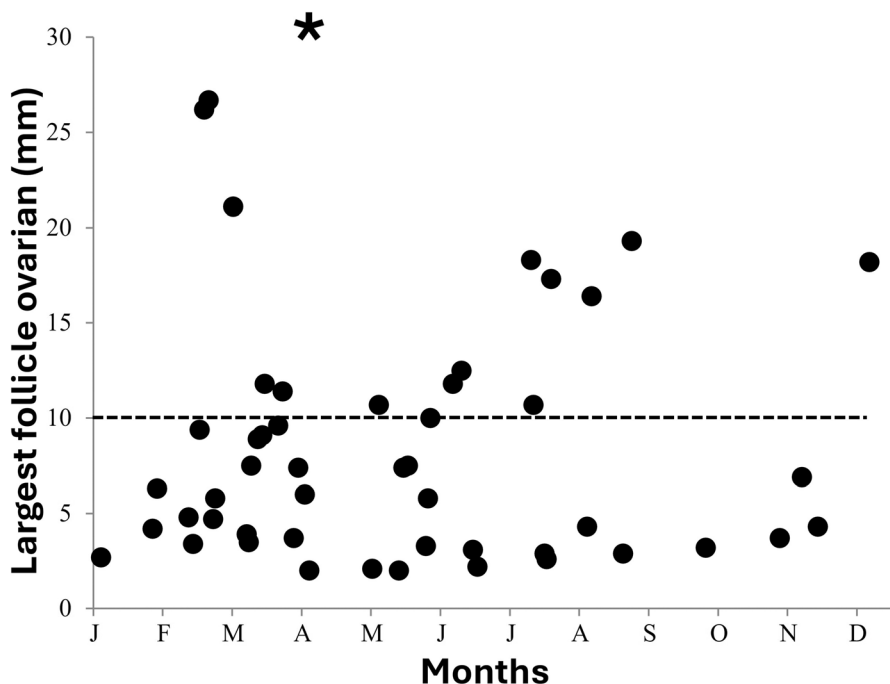


FIGURE 2 Ovarian cycle of *Corallus hortulana* in the Brazilian Amazon. Circles represent ovarian follicles; the dashed line separates previtellogenic follicles from follicles in the process of vitellogenesis; * represents a record of a pregnant female.

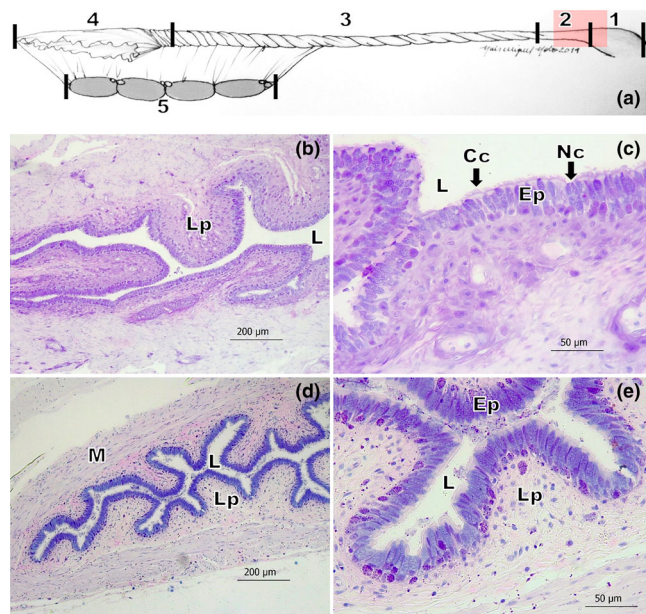


FIGURE 3 Female reproductive tract of *Corallus hortulana*. (a) Sections of oviduct and right ovary (schematic drawing by Luís Miguel Lobo). (b) The non-glandular uterus. (c) The non-glandular uterus highlighting the presence of ciliated and non-ciliated cells in a vitellogenic female (18 mm ovarian follicle length). (d) The pouch in vitellogenic female (19 mm ovarian follicle length). (e) Higher magnification of the pouch. Cc, ciliated cell; Ep, luminal epithelium; L, lumen; Lp, lamina propria; M, muscular tissue; Nc, non-ciliated cell. 1, pouch; 2, non-glandular uterus; 3, glandular uterus; 4, infundibulum; 5, ovary. Square shaded in red indicates the location of the oviduct that was collected for this study.

in November ($n = 4$), December ($n = 1$), January ($n = 1$), February ($n = 2$), March ($n = 5$), and April ($n = 3$), and only three during the dry season, in June ($n = 1$) and August ($n = 2$).

The non-glandular uterus and the pouch (Figure 3a) were characterized by a pseudostratified epithelium composed of ciliated and non-ciliated cells, a thick lamina propria, and external musculature (Figure 3b–e). No female had sperm in the non-glandular uterus (Figure 3b,c) or in the pouch (Figure 3d,e).

3.2 | Male reproductive cycle

Adult males averaged 1260 ± 108 mm SVL (range 1065–1555 mm), and their tails measured 317 ± 29 mm (range 260–379 mm). Different testicular stages were observed throughout the year. Testicular quiescence ($n = 7$) was observed from May to November (mainly in dry season; Figure 4a,b). Spermiogenesis ($n = 3$) was observed from February to May (wet season and transition to dry season; Figure 4c). Spermiation

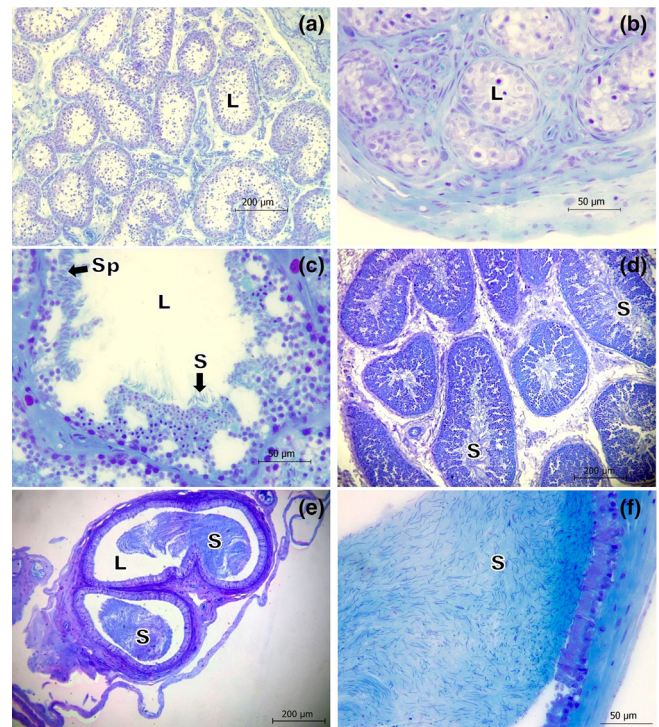


FIGURE 4 Photomicrography of the testes and ductus deferentia of *Corallus hortulana*. (a) Seminiferous tubules in complete regression or involution (Voucher: MPEG17393 with 154 μ m of seminiferous tubule diameter (STD) and 1325 mm of snout-vent length-SVL; this male had ductus deferens packed with sperm). (b) Higher magnification of the seminiferous tubules in involution in an adult male, which also had ductus deferens filled with sperm from prior testicular activity (Voucher: MPEG20691 with 66 μ m STD and 1065 mm SVL). (c) Spermiogenesis: many spermatids in differentiation (Voucher: MPEG21548 with 270 μ m STD and 1195 mm SVL). (d) Spermiation: peak of cellular division and mature spermatozoa abundant in lumen (Voucher: MPEG24621 with 269 μ m STD and 1270 mm SVL). (e) Ductus deferens packed with sperm in lumen. (f) Higher magnification of the ductus deferens packed with sperm in lumen. L, lumen; Sp, spermatid; S, spermatozoa.

($n = 5$; Figure 4d) was observed from January to June (mid-wet season to beginning-dry season) and during the late dry season (September, $n = 1$). Sperms were observed in the ductus deferentia throughout the year, even among males with testicular quiescence (Figure 4d,e).

The seminiferous tubule diameter was greater in the wet season than in the dry season ($U = 11$, $p = 0.04$; Figure 5a). The seminiferous epithelium height ($U = 12$, $p = 0.06$; Figure 5b), the testicular volume ($R^2 = 0.02$, $p = 0.23$; Figure 5c), and the diameter of the ductus deferentia ($U = 714$, $p = 0.23$; Figure 5d) were similar between seasons.

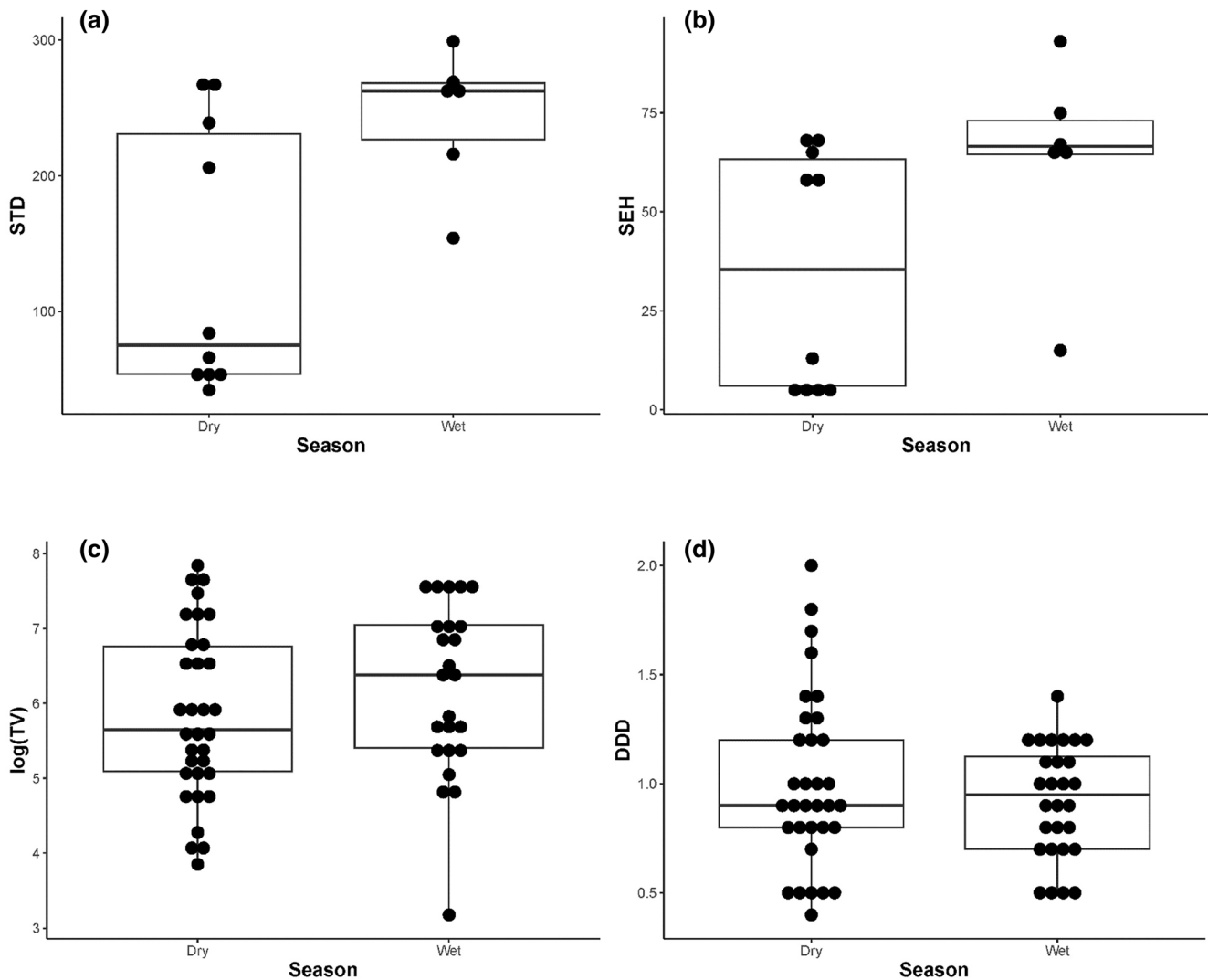


FIGURE 5 Seasonal variation in the male reproductive tract of *Corallus hortulana*. (a) Seminiferous tubule diameter (μm). (b) Seminiferous epithelial height (μm). (c) Testes volume (mm^3). (d) Ductus deferentia diameter (mm). The median line represents the median value of the data; boxes show standard errors; whiskers represent the minimum and maximum values.

4 | DISCUSSION

4.1 | Reproductive cycles

Among Boidae snakes, male reproductive cycles of a few species (*Boa constrictor*, *Epicrates cenchria*, and *C. hortulana*) have thus far been studied only through macroscopic measurements of the testes (Pizzatto & Marques, 2007) or by histology with very limited sample sizes (2–5 individuals per species) from restricted times of the year (Bento et al. 2024a, 2024b). Most boids, including *C. hortulana*, have been inferred to have a continuous testicular cycle when analyses were solely macroscopic (Pizzatto & Marques, 2007). However, at the cellular level, we show that testicular involution occurs in *C. hortulana*, primarily during the dry season, indicating a discontinuous reproductive cycle of

individual males (Figure 6). At the population level, the spermatogenic cycle of *C. hortulana* is seasonal semi-synchronous, with spermiogenesis and spermiation mainly during the wet season to beginning-dry season (for cycle classification see Mathies, 2011). The presence of sperm in the ductus deferentia of males with involuted testes was essential for distinguishing non-reproductive adult males from immature individuals. Therefore, we recommend that collecting multiple organs from the male reproductive system can be essential to gain a clearer understanding of reproductive strategies and the interactions between reproductive organs (Almeida-Santos et al., 2014; Silva et al., 2023).

Given the seasonality of male cycles, we speculate that sperm production in *C. hortulana* is likely energetically demanding (Olsson et al., 1997), even in the tropical Amazon. During the wet season, males may benefit from

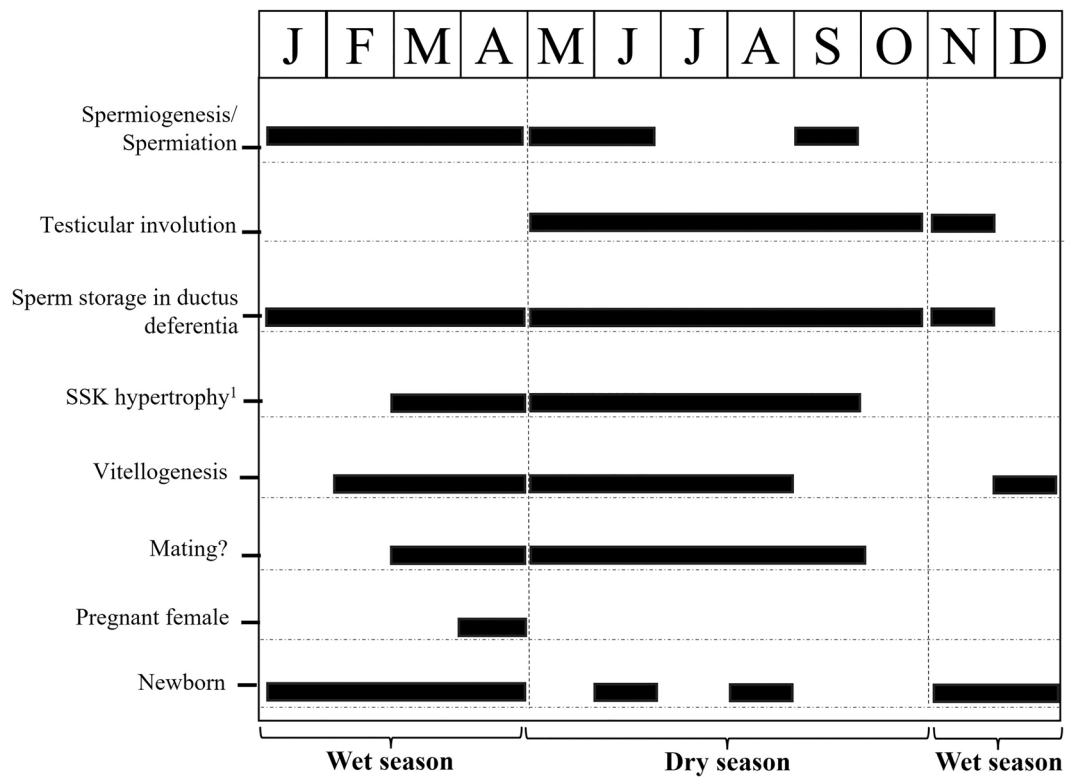


FIGURE 6 Overview of the reproductive events in male and female *Corallus hortulana* from the Brazilian Amazon examined in this study. 1, sexual segment of the kidney (Pinheiro et al., 2020).

favorable biotic and abiotic conditions that stimulate spermatogenesis (Saint-Girons, 1982), and subsequent hypertrophy of the sexual segment of the kidney (SSK; male reproductive accessory organ), which is androgen-dependent and regulated by testosterone (see Krohmer et al., 2004 for details on SSK). Therefore, integrated studies that combine anatomical and physiological analyses of reproduction with assessments of environmental factors, such as prey availability and climate, could enhance our understanding of snake reproductive strategies. For instance, temperature and rainfall appear to be key abiotic factors influencing the seasonality of the male reproductive cycle (Aldridge, 1975; Lutterschmidt & Mason, 2009). Although temperatures remain high throughout the year in the Amazon (Marengo & Fisch, 2021), rainfall seems to significantly impact the reproductive activity of the male *C. hortulana*.

Using the same males we used in this study for the histology of the testes and ductus deferentia, Pinheiro et al. (2020) analyzed the SSK cycle for *C. hortulana*, showing hypertrophy of SSK epithelial cells from March to September. Therefore, there is a synchronization between spermatogenesis and SSK hypertrophy (Figure 6), with SSK hypertrophy beginning in March, the wet season, and ending in September at the end of the dry season (for climate see Nobre et al., 2009). This

pattern indicates that mating for Amazonian species likely occurs during this period of SSK hypertrophy. In support, matings have been recorded in free-ranging snakes in June and September (Batatas Island located east of the Brazilian Amazon) and in May for snakes maintained in captivity in the Amazon region (da Costa Silva & Henderson, 2014; Viana, 2017). The synchronization of mating periods, testicular activity, and SSK hypertrophy is, indeed, common among squamates (Resende & Avelar, 2021; Rojas et al., 2013; Schuett et al., 1997, 2002). Secretions produced by SSK cells are passed to the female during copulation and are generally associated with the formation of the copulatory plug (Friesen et al., 2013). However, in some snake species, testicular regression can occur well before the complete regression of the SSK (Silva, Braz, et al., 2020; Silva et al., 2022). Consequently, in those cases, because sperm can be stored in the ductus deferentia and SSK hypertrophy lasts longer than spermatogenesis, the mating period may be extended (Silva, Braz, et al., 2020; Silva et al., 2022).

The vitellogenesis period in *C. hortulana* females, as revealed by macroscopical analyses, is extended and coincides with SSK hypertrophy and sperm storage in the ductus deferens (Figure 6). Furthermore, matings are often linked to the processes of vitellogenesis and estrus/receptivity to courtship (Aldridge & Duvall, 2002;

Almeida-Santos & Salomão, 2002; Denardo & Taylor, 2011). During periods of vitellogenesis and mating, female snakes often have sperm stored in specialized sites within the oviduct, such as the non-glandular uterus (Braz & Almeida-Santos, 2022; Jurkfitz et al., 2023; Loebens et al., 2018; Rojas et al., 2015; Silva, Barros, et al., 2020). Although we did not find sperm in the pouch or non-glandular uterus of *C. hortulana*, we present the first detailed description of the morphology of these oviductal regions in Boidae snakes. The microscopic morphology of the pouch and non-glandular uterus in *C. hortulana* closely resembles that observed in other snake families (Jurkfitz et al., 2023; Rojas et al., 2015). However, additional studies on the female reproductive tract of Boidae snakes are needed, particularly to explore the morphological changes (e.g., histochemistry) associated with reproductive (vitellogenesis) and non-reproductive periods.

Pregnant females of the Boidae family are rarely encountered in the wild, as reflected by the limited representation of these animals in scientific collections (Pizzatto & Marques, 2007) and confirmed in this study, which recorded only one pregnant *C. hortulana* in April. However, our data for the Amazon areas show parturition follows a seasonal pattern, with most newborns recorded during the wet season (Figure 6). It is suggested that a wet season with favorable temperature and humidity, along with an abundance of prey, may enhance newborn Squamata survival (Fitch, 1970). However, very little is known about the ecology and habits of newborn snakes, including *C. hortulana*. Thus, prey and predator population dynamic studies can be decisive in understanding the reproductive and survival strategies of this viviparous and arboreal species. Overall, available data indicate that for many Boidae species, including *C. caninus* species complex, *Epicrates* spp. and *Boa constrictor*, births predominantly occur during spring and summer (wet season) (Pizzatto & Marques, 2007).

4.2 | Sexual maturity and litter size

Female Boidae, including *C. hortulana*, are generally larger and reach sexual maturity at a greater body size compared to males (Bertona & Chiaraviglio, 2003; Pizzatto & Marques, 2007; Rivas & Burghardt, 2001). For *C. hortulana* distributed in the Amazon rainforest and analyzed in this study, the smallest adult female has 650 mm SVL larger than the smallest adult male. Larger body sizes and later sexual maturity in females are advantageous as they produce larger litters (Shine, 1994). Conversely, the larger size of males may be associated with male–male combat (Shine, 1994). On the other hand, in

some Boidae species, such as *C. caninus*, *E. cenchria*, and *Epicrates assisi*, where females reach larger body sizes than males, male combat can still occur (Guedes et al., 2019; Pizzatto & Marques, 2007). In addition, male combat has also been reported for *C. hortulana* in the Atlantic Forest biome (Santos et al., 2020).

In our study, we estimated litters ranging from 2 to 18 newborns across the Brazilian Amazon, which is within the range recorded for a pooled sample from various areas (Pizzatto & Marques, 2007). Despite the fact Pizzatto and Marques (2007) found a correlation between litter size and maternal SVL in *C. hortulana*, we did not, when considering only specimens from the Amazon. Litter size varies among snake species depending on their microhabitat, decreasing with increased arboreality (Pizzatto et al., 2007). *Corallus hortulana* has a litter size similar to that of the sister arboreal species *C. caninus* (3–14 newborn) (Pizzatto & Marques, 2007). In contrast, the non-arboreal and larger *B. constrictor* and *Eunectes* spp. have significantly larger litter sizes (5–41 and 49–82 newborns, respectively: Pizzatto & Marques, 2007).

Newborn SVL of *C. hortulana* from the Amazon rainforest ranged from 345 to 507 mm SVL (Bernarde & Machado, 2010; Viana, 2017; this study). In comparison to other Boidae species, newborn SVL is relatively consistent, with *Epicrates* spp. ranging from 312 to 400 mm SVL and *C. caninus* ranging from 360 to 390 mm SVL (Pizzatto & Marques, 2007). Meanwhile, species in the genus *Eunectes* can give birth to newborns measuring 685 mm SVL (Pizzatto & Marques, 2007).

5 | CONCLUSION

The reproductive cycles of male *C. hortulana* in the Brazilian Amazon appear to be influenced by rainfall, with testicular involution occurring during the dry season. Females exhibit an extended vitellogenic cycle, with parturition occurring mainly in the wet season; however, records of pregnant females were scarce. We present the first microscopic description of the pouch and non-glandular uterus in a Boidae snake. Microscopic analyses of the non-glandular uterus in vitellogenic females revealed a tissue morphology (e.g., external musculature, lamina propria, and pseudostratified epithelium), like what has been observed in other snakes that store sperm in this portion of the oviduct. However, we found no evidence of sperm storage in the individuals analyzed, nor any references in the published literature for any other Boidae storing sperm in the oviduct. Finally, given the conservatism of reproductive traits within Boidae (Pizzatto & Marques, 2007), this study may contribute to developing effective conservation policies for endangered

species such as *C. cropanii*, the rarest Boidae in the Americas.

AUTHOR CONTRIBUTIONS

Karina M. P. Silva: Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; formal analysis; data curation. **Lígia Pizzatto:** Investigation; methodology; writing – review and editing; data curation. **Luciana Frazão:** Investigation; methodology; writing – review and editing; data curation. **Selma M. Almeida-Santos:** Investigation; methodology; writing – review and editing. **Maria Ermelinda Oliveira:** Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; formal analysis; data curation.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Voucher numbers of the specimens of *Corallus hortulana* from the collections of the Instituto Butantan (IBSP), Instituto Nacional de Pesquisas da Amazônia

(INPA), Museu Paraense Emílio Goeldi (MPEG), and Universidade Federal do Amazonas (CZPB-UFAM) examined in this study. Asterisk (*) represents the animals for which histological analyses were performed.

State	Voucher numbers
Acre	MPEG20401, CZPB962.
Amazonas	IB52190, IB64249, IB43436, IB24163, IB50199, IB52192, INPA1453, INPA1493, INPA1513, INPA1492, INPA17239, INPA27273, INPA33854, INPA1455, INPA1457, INPA1504, INPA8570, INPA-H31676, INPA27274, INPA30450, INPA29491, MPEG17393*, MPEG20691*, MPEG20848*, MPEG22203*, MPEG23627, MPEG24371*, MPEG 23567, MPEG22201*, MPEG17385, MPEG17455*, MPEG20845*, MPEG17398, MPEG17392, MPEG23768, CZPB871, CZPB770, CZPB872.
Amapá	MPEG26622*, MPEG19935*, MPEG19678, IB24809, IB25398, IB25947, IB26573, IB25399.
Maranhão	IB41085, MPEG15696.
Mato Grosso	IB49532, IB49533, IB49563, IB50051, IB41498.
Pará	CZPB760, CZPB928, CZPB764, CZPB771, CZPB763, CZPB768, CZPB932, CZPB767, CZPB766, CZPB762, CZPB857, CZPB831, IB46892, IB47092, IB47606, IB47626, IB14622, IB27017, IB32025, IB37586, IB46234, IB46661, IB46881, IB46894, IB47091, IB47223, IB46875, IB46888, MPEG16724, MPEG10834, MPEG14866, MPEG1744, MPEG4489, MPEG4490, MPEG6588, MPEG18801*, MPEG19336, MPEG19383, MPEG19934*, MPEG19936, MPEG19937, MPEG21548*, MPEG21773*, MPEG21774*, MPEG21775, MPEG21776*, MPEG21784*, MPEG21785*, MPEG21787, MPEG21793, MPEG22675, MPEG23244*, MPEG23245, MPEG24621*, MPEG19026*, MPEG3346, MPEG4488, MPEG6902, MPEG8556, MPEG20614*, MPEG19985*, MPEG8556, MPEG6902, MPEG19701*, MPEG3973*, MPEG3346, MPEG21777*, MPEG 21790, MPEG22673, MPEG22674*, MPEG18921*, MPEG10978, MPEG11720, MPEG15520, MPEG16575, MPEG25593, MPEG21792, MPEG23440.
Rondônia	IB53870, MPEG19526, INPA1156, INPA34179, INPA32226, INPA32225, INPA32283, INPA1150.
Roraima	CZPB980, CZPB979, CZPB981, CZPB962, CZPB967, CZPB969, INPA19250, INPA19252, INPA19255.