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Reproductive Biology of the Fossorial Snake *Apostolepis gaboi* (Elapomorphini): A Threatened and Poorly Known Species from the Caatinga Region

**Henrique B. Braz**¹, **Karina N. Kasperoviczus**¹, **Thaís B. Guedes**²³

**Abstract.** Knowledge of reproductive biology is essential to understand the intrinsic traits of any species and to elaborate effective conservation strategies, particularly for threatened species. Despite recent advances, knowledge on the reproductive biology of snakes remains deficient for most species, especially for tropical fossorial taxa. *Apostolepis gaboi* is a psammophilous, fossorial, and threatened snake endemic of the Quaternary Sand Dunes of the São Francisco River, Caatinga domain. Here, we present information on its reproductive biology based on macroscopic and microscopic data obtained from the examination of all specimens preserved in Brazilian museums. Specifically, we addressed sexual maturity, sexual dimorphism, clutch size, timing of gametogenesis, activity of the sexual segment of the kidney (SSK), and female sperm storage. *Apostolepis gaboi* is one of the smallest species of the genus. Females attain sexual maturity at larger body sizes and grow larger than males. However, adult females have relatively shorter tails than males. We found no sexual dimorphism in body circumference and head size. Clutch size is small and averages 4.5 eggs. All males from the wet season showed testes in spermiogenesis, ductus deferentia packed with sperm, and SSK hypertrophied and secretory. Secondary vitellogenesis also occurs in the wet season. Moreover, females in early vitellogenesis from the wet season showed sperm stored in the posterior infundibulum. Mating occurs in the second half of the wet season, as suggested by the finding of aggregations of sperm in the uterine lumen of a female in early secondary vitellogenesis. Mating is associated with gonadal activity in males. Sperm storage confers flexibility for females to ovulate at the most suitable time in an unpredictable habitat such as the Caatinga. *Apostolepis gaboi* exhibits similarities (association between mating and spermiogenesis, small clutch) and differences (absence of sexual dimorphism in head size and stoutness) compared with another Elapomorphini species. Lastly, we argue that some of the intrinsic traits of *A. gaboi* (small clutch and small body size) may increase its vulnerability to extinction, raising additional concerns to its conservation.

**Keywords.** Clutch size; Conservation; Morphology; Reproductive cycles; Sexual dimorphism; Spermatogenesis; Sperm storage; Vitellogenesis.

**Resumo.** O conhecimento sobre biologia reprodutiva é essencial para entender as características intrínsecas de qualquer espécie e para elaborar estratégias de conservação efetivas, particularmente para espécies ameaçadas. Apesar dos avanços recentes, o conhecimento sobre a biologia reprodutiva de serpentes permanece deficiente para a maioria das espécies, especialmente para espécies fossórias tropicais. *Apostolepis gaboi* é uma serpente psamófila, fossória e ameaçada das Dunas do Rio São Francisco, no domínio da Caatinga. Nós apresentamos informações sobre sua biologia reprodutiva com base em dados macroscópicos e microscópicos obtidos a partir do exame de todos os espécimes preservados em coleções brasileiras. Especificamente, abordamos a maturidade sexual, dimorfismo sexual, tamanho da ninhada, época da gametogênese, atividade do segmento sexual renal (SSR) e estocagem de esperma em fêmeas. *Apostolepis gaboi* é uma das menores espécies do gênero. Fêmeas maturam com maiores tamanhos de corpo e são maiores que machos. Entretanto, fêmeas adultas têm caudas relativamente menores do que machos. Não encontramos dimorfismo sexual na circunferência corporal e no tamanho da cabeça. O tamanho da ninhada é pequeno (média = 4,5 ovos). Todos os machos da estação chuvosa apresentaram testículos em espermiogênese, ductos deferentes cheios de esperma e SSR hipertrofiado e secretor. A vitelogênese secundária também ocorre na estação chuvosa. Além disso, fêmeas em início de vitelogênese da estação chuvosa apresentaram esperma estocado no infundíbulo posterior. O acasalamento ocorre na segunda metade da estação chuvosa, como sugerido pelo encontro de agregações de esperma no lúmen uterino de uma fêmea em início de vitelogênese. O acasalamento é associado com a atividade gonadal em machos. A estocagem de esperma confere flexibilidade às fêmeas para ovular no momento mais adequado em um habitat imprevisível como a Caatinga. *Apostolepis gaboi* exibe similaridades (associação entre cópula e espermatogênese, ninhada pequena) e diferenças (ausência de dimorfismo sexual no tamanho da cabeça e robustez) em comparação com outro Elapomorphini. Por último, argumentamos que algumas das características intrínsecas de *A. gaboi* (ninhada e tamanho corporal pequenos) podem aumentar sua vulnerabilidade à extinção, levantando preocupações adicionais para sua conservação.

INTRODUCTION

Since reproduction is the way species transmit their genes to the next generation, a comprehensive understanding of reproductive biology is essential to design and implement effective conservation strategies, particularly for threatened species (Shine and Bonnet, 2009). Knowledge of snake reproduction has significantly increased over the years, but detailed information remains deficient for most species (Shine and Bonnet, 2009; Mathies, 2011). This issue has been highlighted in conservation studies worldwide, but the deficiency of reproductive data is most noticeable in tropical regions, particularly for fossorial or semi-fossorial snakes (Böhm et al., 2013). For most tropical fossorial snakes, data on reproductive biology are fragmented and based on fortuitous observations (e.g., Funk-Pontes and Di-Bernardo, 1988; Ferrarezi et al., 2005; Barbo et al., 2011). Few studies have addressed the reproductive biology of Neotropical fossorial snakes comprehensively (Marques, 1996; Marques and Puerto, 1998; Balestrin and Di-Bernardo, 2005; Braz et al., 2014; de Resende and Nascimento, 2015). Consequently, the lack of reproductive data for fossorial snakes impairs robust evaluations of their conservation status.

Fossoriality can impose major challenges to morphology, thermoregulation, and life history (Pough, 1980; Pizzatto et al., 2007), ultimately constraining potential reproductive output. For example, fossorial or semi-fossorial snake species often have small clutches (Marques, 1996; Marques and Puerto, 1998; Willson and Dorcas, 2004; Balestrin and Di-Bernardo, 2005). Thus, in addition to their relevance for conservation, studies on the reproduction of fossorial snakes are also of interest for a complete understanding of the reproductive strategies of snakes.

Elapomorphini Jan, 1862 snakes are fossorial and share several morphological specializations for this lifestyle (Savitzky, 1983; Ferrarezi, 1993; Harvey, 1999). The tribe comprises nearly 51 species allocated to three genera (Apostolepis Cope, 1862, Elapomorphus Wiegmann, 1843, and Phalotris Cope, 1862) that are widely distributed in cis-Andean South America (Wallach et al., 2014; Uetz et al., 2018; Guedes et al., 2018a, b). Because of their secretive fossorial habits, little is known of their reproductive biology. Only one species (Phalotris lativittatus Ferrarezi, 1993) has been studied in detail (Braz et al., 2014). For Apostolepis, the most speciose genus of Elapomorphini (Wallach et al., 2014; Guedes et al., 2018a, b; Uetz et al., 2018), reproductive information is limited to meager records on the number of vitellogenic follicles or clutch size of four species (Ferrarezi et al., 2005; Barbo et al., 2011; Nogueira et al., 2012; Braz et al., 2015). Five species of Apostolepis are listed as endangered in the Brazilian Red List of Threatened Species, and another four species lack sufficient data to assess their extinction risk (ICMBio, 2018). Consequently, data on reproductive traits of the genus are essential for conservation.

Apostolepis gaboi Rodrigues, 1992 is a psammophilius, fossorial snake endemic to the Caatinga and is restricted to two localities along the left bank of the São Francisco River, state of Bahia, Brazil (Rodrigues, 1992; Guedes et al., 2014; Guedes et al., 2018ab). The species has been recorded only in bushy and herbaceous caatinga on dunes and sandy soil (Rodrigues, 1992; Guedes et al., 2014), an area subject to severe anthropic threats and degradation (ICMBio, 2018). Accordingly, a recent assessment of the extinction risk of the Brazilian fauna has considered A. gaboi as an endangered species (ICMBio, 2018). Apart from meager information on microhabitat and prey items (Rodrigues, 1992; Damasceno, 2002; Guedes et al., 2014; Guedes et al., 2018b), no biological information is available for the species. Therefore, conservation strategies will benefit from basic biological research, particularly on its reproductive biology.

Here, we provide the first data on the reproductive biology of Apostolepis gaboi on the basis of the examination of all preserved specimens available in Brazilian museums. Specifically, we provide information on sexual maturity, sexual dimorphism, clutch size, timing of gametogenesis, activity of the sexual segment of the kidney, and sperm storage. We also explore whether A. gaboi exhibits a reproductive pattern similar to that reported for another Elapomorphini species (e.g., associated reproductive tactics, small clutch size) and how the intrinsic traits of the species may influence its vulnerability to extinction.

MATERIALS AND METHODS

Study area

Specimens were collected in the municipalities of Barra (n = 33) and Queimadas (n = 2), both in the state of Bahia, in the Caatinga domain (Fig. 1A–B). The area comprises a strip of depositional sandy soil along the São Francisco River (from 250–500 m elevation) and is covered by bushy and herbaceous caatinga (Rodrigues, 1992; Guedes et al., 2014; Fig. 1C). The climate is hot semi-arid (BSh type in the Köppen-Geiger climate classification; Peel et al., 2007). Mean temperatures are high throughout the year (average 26°C), but a higher thermal amplitude is traceable between May and October (Fig. 1D). Rainfall is scarce (ca. 640 mm per year) and irregular among years; consequently, extreme droughts are frequent, but flooding can also occur (Prado, 2005). Despite this unpredictability, the highest amount of rainfall occurs on average between November and March (hereafter wet season; Fig. 1D) when more than 80% of the annual rainfall occurs.
Data collection

We examined all available preserved specimens of *Apostolepis gaboi* (*n* = 35) housed in Brazilian scientific collections of the Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu de Zoologia da Universidade Federal da Bahia (MZUFBA), and Museu de Zoologia da Universidade Estadual de Feira de Santana (MZUEFS). All specimens were collected in the following localities in Bahia state: Barra, Ibiraba, MZUFBA 1673–1704; Barra, Icatú, MZUEFS 981; Queimadas, MZUSP 10290, 10457. For each specimen, we measured snout–vent length (SVL), tail length, and body circumference (at the middle of the body) using a tape measure. We also measured head length and head width using a digital caliper (to the nearest 0.01 mm). Next, we made a mid-ventral incision in

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**Figure 1.** Geographic distribution of *Apostolepis gaboi* and climatic pattern for the location of collection of the specimens examined in this study. (A) Map showing the positioning of the Caatinga region in northeastern Brazil. (B) Location of collection of the specimens of *A. gaboi* examined. (C) View of the sand dunes of the São Francisco River, state of Bahia (photo by Leonardo Ribeiro). (D) Monthly temperature and rainfall in the municipality of Barra (village of Ibiraba), state of Bahia, based on a dataset for climate conditions from 1960–1990 obtained from WorldClim, version 1.4 (Hijmans et al., 2005) with spatial resolution of 5 minutes.
most specimens and recorded the sex (after gonadal inspection). For females, we recorded the general aspect of the oviduct (straight vs. folded), the number of ovarian follicles, and the diameter of the largest ovarian follicle. We could not dissect only 3 of the 35 specimens examined (including the holotype).

For a subset of specimens, we collected samples of the right side of the reproductive tract for histological analyses. For males (n = 17), we collected the distal portion of the ductus deferens and anterior region of the testis and kidney. For females (n = 8), we collected the vaginal pouch, glandular uterus, and posterior infundibulum (sensu Siegel et al., 2011). Tissue samples were processed by the standard paraffin method for light microscopy. Histological sections were cut at 5 μm and stained with hematoxylin and eosin. Sections of the testes were analyzed to identify spermatogenic stages. Sections of the ductus deferentia were evaluated for the presence of spermatozoa, and sections of the kidneys were examined qualitatively to assess hypertrophy/atrophy and secretory activity of the sexual segment of the kidney (SSK). Sections of the vaginal pouches, glandular uteri, and posterior infundibula were examined for the presence of sperm.

The specimens examined histologically were collected during the wet seasons of 1996 (March, September, and December) and 2004 (January, February, and December). Histological sections were photographed with an Olympus Q-Color5™ digital camera mounted on an Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan). The software Image-Pro Express, version 5 (Media Cybernetics Inc., 2004) was used for image capture.

Analyses

We scored females as sexually mature (i.e., adult) if they had vitellogenic follicles, oviductal eggs, folded oviducts, or sperm in the reproductive tract. Because our sampling was limited to the wet season, we were unable to determine the annual variation in follicular diameter, and consequently the minimum size of ovarian follicles at the beginning of secondary vitellogenesis (Almeida-Santos et al., 2014). Thus, small whitish follicles were classified as primary vitellogenesis, whereas yellowish enlarged follicles were classified as secondary vitellogenesis. The presence of hypertrophied uterine shell glands (which occur only during secondary vitellogenesis; Braz et al., 2018) was used as an auxiliary criterion to determine the vitellogenic stage. Males were considered adults if they had spermatozoa in the reproductive tract (Braz et al., 2014). To increase the sample size for the analysis of sexual dimorphism, we estimated the sexual maturity of the three non-dissected specimens based on their SVL and the results obtained for the dissected specimens. Because we found no gravid females (see Results), we estimated clutch size by counting the number of secondary follicles.

We calculated a Sexual Size Dimorphism (SSD) index according to Gibbons and Lovich (1990): 
\[
1 - \left( \frac{\text{mean adult SVL of the larger sex}}{\text{mean adult SVL of the smaller sex}} \right)
\]

This index is arbitrarily expressed as positive if females are larger and negative if males are larger (Gibbons and Lovich, 1990; Shine, 1994). Raw data were tested for normality using Kolmogorov-Smirnov tests and homogeneity of variances using Levene’s tests. We tested for sexual differences in SVL using a Welch t-test and body shape (tail length, body circumference, head length, and head width) using ANCOVA with SVL as the covariate. Because the variances were heterogeneous for most variables, we rank-transformed the data to meet the requirements of ANCOVA (Conover and Iman, 1982). Prior to ANCOVAs, we tested for the assumption of nonsignificant interactions of the covariate with class variables, and a separate-slopes model ANCOVA was used when these interactions were significant. Values are presented in the text as \(\bar{X} \pm SD\), and differences were considered significant when \(P < 0.05\). Statistical tests were performed using Statistica version 7.0 (StatSoft Inc., 2004).

RESULTS

Morphology and sexual dimorphism

A total of 5 out of 32 dissected specimens were sexually immature (3 females and 2 males). The smallest specimen examined was an immature male measuring 210 mm SVL. The smallest adult female measured 258 mm SVL. The

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**Table 1.** Morphological traits and sexual dimorphism in adult *Apostolepis gaboi*. Statistical tests are Welch t-test (for sexual size dimorphism) and ANCOVA with SVL as the covariate (for body shape). Significant differences are indicated in bold. Sample sizes are n = 12 for females and n = 17 for males, except for SVL comparisons, in which sample size for males is n = 18.

<table>
<thead>
<tr>
<th>Trait (mm)</th>
<th>Female</th>
<th>Male</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{X} \pm SD)</td>
<td>Range</td>
<td>(\bar{X} \pm SD)</td>
</tr>
<tr>
<td>Snout-vent length</td>
<td>345.5 ± 86.5</td>
<td>258–494</td>
<td>273.4 ± 33.0</td>
</tr>
<tr>
<td>Tail length</td>
<td>27.8 ± 5.2</td>
<td>21–35</td>
<td>30.5 ± 4.7</td>
</tr>
<tr>
<td>Body circumference</td>
<td>17.1 ± 5.0</td>
<td>10–25</td>
<td>13.5 ± 1.7</td>
</tr>
<tr>
<td>Head length</td>
<td>7.57 ± 1.82</td>
<td>5.38–10.88</td>
<td>6.38 ± 0.43</td>
</tr>
<tr>
<td>Head width</td>
<td>3.51 ± 1.23</td>
<td>2.15–5.63</td>
<td>2.79 ± 0.28</td>
</tr>
</tbody>
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smallest adult male measured 223 mm SVL, but one male larger than that was sexually immature (SVL = 277 mm). Among the three non-dissected specimens, one female (MZUSP 10290; 217 mm SVL) was assumed to be immature because it was smaller than the smallest adult female examined. The other two specimens were assumed to be adults since the male specimen (MZUSP 10547) was the largest male examined (SVL = 386 mm) and the female (MZUEFS 981; 270 mm SVL) was larger than the smallest adult of its sex. Adult females were larger but had relatively shorter tails than adult males (Table 1, Fig. 2A). Body circumference and head size were similar between the sexes (Table 1, Fig. 2B–D). The SSD index was 0.35.

Reproduction

Adult females were collected in January (n = 7), February (n = 3), and March (n = 2). Follicular diameter ranged from 2.23–19.03 mm. Females with ovarian follicles larger than 5.0 mm were categorized as secondary vitellogenesis. These females also showed well-developed uterine shell glands (Fig. 3A). Females in secondary vitellogenesis were collected in January (n = 4), February (n = 2), and March (n = 1), but enlarged follicles (12.3–19.0 mm) were found in females from late January to mid-February. The number of secondary follicles ranged from 2–9 (X = 4.5 ± 2.9; n = 6). We found no gravid female.
Three adult females had sperm in the reproductive tract. These females were collected in January, February, and March and had early secondary follicles (5.5, 9.3, and 8.4 mm, respectively). Sperm were found in numerous sperm storage receptacles in the posterior infundibulum (Fig. 3B). In two females (February and March), spermatozoa were aligned parallelly with their heads oriented to the epithelium of the receptacles (Fig. 3B–C). In one female (January), spermatozoa were arranged unorderedly in a receptacle, and a spermatozoon was observed at the opening of this receptacle, presumably while entering this structure (Fig. 3D). This female also showed aggregations of spermatozoa in the lumen of the glandular uterus (Fig. 3A).

Adult males were collected in October (n = 1), December (n = 5), January (n = 9), and February (n = 3). Except for the individual from October (which was not examined histologically), all adult males showed testes in maximum spermiogenesis. Seminiferous tubules showed all spermatogenic stages (i.e., spermatogonia, spermocytes, and spermatids; Fig. 4A). Mature spermatozoa were abundant in the testicular lumens and ductus deferentia of all males (Fig. 4A–B). All males showed SSKs noticeably hypertrophied and distinguished from the other tubules in the kidney (Fig. 4C). The SSK tubules were characterized by eosinophilic columnar epithelia filled with large secretory granules (Fig. 4C). These granules often filled the lumens of the SSK tubules. The characteristics exhibited by the SSKs indicate secretory activity.

**DISCUSSION**

**Morphology and sexual dimorphism**

Among the *Apostolepis* with sufficient sampling of adult body sizes, *A. gaboi* is smaller than *A. ammodites*.
Ferrarezi et al., 2005, A. assimilis Reinhardt, 1861, A. cea‑rensis Gomes, 1915, A. dimidiata Jan, 1862, A. nigrolineata Peters, 1869, and Apostolepis sp. from the Caatinga high‑lands (Giraudo and Scrocchi, 1998; Ferrarezi et al., 2005; Sawaya et al., 2008; Barbo et al., 2011; Curcio et al., 2011; T.B. Guedes, pers. obs.) but larger than A. albicolaris Lema, 2002 only (Nogueira et al., 2012). Therefore, A. gaboi seems to be one of the smallest species of the genus.

Female‑biased SSD is common in snakes and often related to the lack of male–male combat (Shine, 1994). The SSD index of Apostolepis gaboi (= 0.35) is similar to that reported for many snakes lacking male combat (Shine, 1994), including another Elapomorphini species (Phalotris lativittatus = 0.33; Braz et al., 2014), suggesting that male combat is absent in A. gaboi. When male combat is lacking, females are usually larger than males due to selection for increased fecundity, since clutch size typically increases with body size in snakes (Shine, 1994). We could not test for such a relationship due to the small sample size; however, this probably occurs in A. gaboi since the smallest females (SVL = 258–350 mm) had the smallest clutches (2 vitellogenic follicles) and the largest females (SVL = 340–453 mm) had the largest clutches (6–9 vitellogenic follicles). Selection for higher fecundity might also explain sexual maturation at larger body sizes in females than in males. By delaying sexual maturity, females start reproducing at larger body sizes, thus producing larger clutches (Shine, 1994).

Longer tails in males is recurrent in snakes and might reflect sexual selection. Because the hemipenes are housed inside the tail base, longer tails might reflect longer hemipenes. Moreover, longer tails might enhance male mating success (King, 1989; Shine et al., 1999). The lack of sex divergence in head size and body circumference (stoutness) in Apostolepis gaboi contrasts with results for Phalotris lativittatus in which males have larger heads and are stouter than females (Braz et al., 2014). Thus, the lack of head size dimorphism might reflect dietary similarity between the sexes. Apostolepis gaboi is reported to prey on Amphibolophis hastata Vanzolini, 1991 (Damasceno, 2002), which is a small amphibiaenid (SVL < 149 mm; Vanzolini, 1991). Other potential prey are also small limbless or elongate vertebrates, including Amphibolophis arenaria Vanzolini, 1991 (SVL ≤ 245 mm; Teixeira et al., 2016) and the fossorial or semi‑fossorial lizards Notobachia abeleph‑ara Rodrigues, 1984 (SVL < 63 mm; Ramiro et al., 2017), Calypptommatus leiolepis Rodrigues, 1991 (SVL ≤ 79 mm; Ramiro et al., 2017), C. nicterus Rodrigues, 1991 (SVL ≤ 69 mm; Rodrigues et al., 2001), and Microablepharus maximiliani (Reinhartd and Lütken, 1862) (SVL < 40 mm; Dal Vechio et al., 2014). Therefore, reliance on small prey by both sexes may explain the lack of sexual head size dimorphism in A. gaboi (see also Marques et al., 2006).

Figure 4. Histological sections of the testis, distal ductus deferens, and kidney of male Apostolepis gaboi during the wet season. (A) Seminiferous tubules during spermiogenesis showing an active epithelium and many spermatozoa in the lumen. (B) Ductus deferens with many spermatozoa in the lumen. (C) Sexual segment of the kidney hypertrophied during the secretory phase. Ep: epithelium, Lu: lumen, M: muscle, Pct: proximal convoluted tubule, Sc: spermatocyte, Sd: spermatid, Sg: spermatogonia, Sz: spermatozoa, Ssk: sexual segment of the kidney. Hematoxylin‑eosin. Scale bar = 100 μm.
Reproduction

Female Apostolepis gaboi produce small clutches. Estimating clutch size by counting secondary follicles rather than oviductal or oviposited eggs (as we did here) can overestimate clutch size in some species because not all secondary follicles may indeed be ovulated (Almeida-Santos et al., 2014). Thus, the clutch size of A. gaboii may be even smaller than we estimated. Small clutches (but elongate eggs) seem to be characteristic of Elapomorphini (1–8 eggs, Braz et al., 2014; see also Braz et al., 2015) and several unrelated fossorial or semi-fossorial snakes (Marques, 1996; Marques and Puorto, 1998; Willson and Dorcas, 2004; Balestrin and Di-Bernardo, 2005; Shine et al., 2006). Morphological constraints imposed by fossoriality and the costs associated with the underground locomotion by gravid females might play a major role in determining the small clutch size in fossorial snakes (Pizzatto et al., 2007; Braz et al., 2014).

Infundibular sperm storage has been widely reported in many viperids, colubrids, and natricids from North America (Siegel et al., 2011) but only recently in Neotropical snakes (Philodryas patagoniensis [Girard, 1858]; Rojas et al., 2015 and Erythrolamprus miliaris Linnaeus, 1758; Rojas et al., 2019). Our observations expand infundibular sperm storage to a third dipsadid lineage and indicate that this feature is likely a widespread but overlooked phenomenon in Neotropical snakes.

Shortly after mating, sperm migrate to the posterior infundibulum and fill sperm storage receptacles (Fox, 1956; Siegel and Sever, 2008). As sperm enter receptacles, they become more ordered, remaining parallelly aligned during the sperm storage period (Fox, 1956; Siegel and Sever, 2008). Sperm release is believed to occur only once eggs enter the infundibulum through the mechanical pressure exerted by the moving egg (Fox, 1956; Siegel et al., 2011). Thus, our observation of a female with a single spermatozoon oriented towards the opening of a receptacle filled with unordered spermatozoa indicates that the spermatozoon was entering the receptacle. This finding, together with the sperm aggregations observed in the uterine lumen of the same female, strongly suggests that this female had mated recently. Furthermore, the SSKs of male Apostolepis gaboi are secretory during the wet season. In most snakes, hypertrophy and secretory activity of the SSK are associated with mating periods (Aldridge et al., 2011). Collectively, the above observations suggest that mating in A. gaboii occurs at least in January but possibly from December to March.

Because our sample was restricted to the wet season, we could not assess cyclic variations in gametogenesis. However, our data provide useful insights into the phenology of Apostolepis gaboi. Both males and females are reproductively active during the wet season. Males undergo spermiogenesis between at least December and February. The fact that all males were at the same spermatogenic stage suggests that testicular activity is synchronized among individuals. Additionally, the concomitance of mating and spermiogenesis indicates an associated reproductive tactic in males (Crews, 1984), a pattern shared with another Elapomorphini species (Phalotris lativittatus: Braz et al., 2014).

Determining the female reproductive tactic is a complicated task with the information available. Among the three females exhibiting infundibular sperm storage, the one interpreted as recently mated was in early secondary vitellogenesis (January) and therefore follicles were not ready for ovulation. In this particular case, mating is certainly dissociated from ovulation, and fertilization is ensured by the sperm stored in the infundibulum. This pattern differs from that suggested for Phalotris lativittatus (Braz et al., 2014). The other two females exhibiting infundibular sperm storage were also in early secondary vitellogenesis. As these two females were also from the inferred mating period (February and March; late wet season), the sperm stored in the infundibulum can also be the result of recent mating. However, sperm can remain in sperm receptacles for several months after ovulation or a few months after parturition, but it seemingly disappears before next mating season (reviewed by Siegel et al., 2011). Thus, sperm stored by these two females could alternatively reflect remnants from other periods.

Timing of ovulation remains unknown for the species. Gravidity and oviposition occur during the dry season in many oviparous xenodontines inhabiting other areas of the Caatinga (Vitt, 1983; Vitt and Vangilder, 1983). Gravidity and oviposition during the dryer periods of the year may be disadvantageous for a species inhabiting sandy soil areas as Apostolepis gaboi. For example, potential nest sites with suitable hydric conditions for incubation are presumably scarce during the dry season. However, mothers and offspring may exhibit adaptive responses (e.g., nest-site selection, embryo tolerance to extreme climates) to deal with unpredictable environments (Shine and Brown, 2008). In many snake species, sperm can be stored within sperm receptacles for months until ovulation (Siegel and Sever, 2008). Nevertheless, sperm storage certainly confers flexibility for female A. gaboii to ovulate at the most suitable time in an unpredictable habitat such as the Caatinga.

Implications for conservation

Apostolepis gaboi was recently listed as endangered in the Brazilian Red List of Threatened Species based on its restricted distribution and growing habitat loss (Criterion B; ICMBio, 2018). We raise additional concerns about the conservation of A. gaboii on the basis of some intrinsic traits of the species that may contribute to an increasing
extinction risk. Species exhibiting "slow" life histories (slow growth, late maturation, and small clutches) might be more vulnerable to extinction since they would have reduced ability to recover from population declines after adverse events (Purvis et al., 2000; Webb et al., 2002). Although small clutches are only one component of slow life histories that could increase vulnerability to population declines, small clutches per se might be a good predictor of extinction risk in mainland squamates (Siliceo and Diaz, 2010). One could argue that female A. gaboi could compensate for their small clutches by producing multiple clutches per reproductive season. However, we suggest this is unlikely to occur given the unpredictability of the Caatinga rainfall and the lack of robust evidence that tropical snakes indeed produce multiple clutches within a reproductive season (Almeida-Santos et al., 2014). Furthermore, a recent study has found that extinction risk is greater in the largest and smallest vertebrates and the probability of being threatened is greater in species with restricted geographic ranges (Ripple et al., 2017). In particular, the smallest species are more likely to have restricted range and be threatened by habitat loss and anthropogenic activities (Ripple et al., 2017). As a small species occupying a small area (ca. 628 km²) subject to anthropogenic pressures (Guedes et al., 2014; ICMBio, 2018), A. gaboi is thus at great risk of extinction. Habitat protection is central to curb threats and ensure the persistence of the species.

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