revista de ciências da saúde NOVA ESPERANÇA VOLUME 23 - NÚMERO 3 ISSN ELETRÔNICO 2317-7160

SPERMATOGENESIS IN Lithobates palmipes (Spix, 1824) IN A FRAGMENT OF NORTHEASTERN ATLANTIC FOREST.

ESPERMATOGÊNESES EM Lithobates palmipes (Spix, 1824) EM UM FRAGMENTO DE MATA ATLÂNTICA NORDESTINA.

Vanessa Alexandre Lourenço [I*] , Caio Ian Delfino Oliveira [II] , Marcela Meira Ramos Abrantes [III] , Márcio Frazão Chaves [IV] , Stephenson Hallison Formiga Abrantes [V] , Artur da Nóbrega Carreiro [VI]

Abstract. *Lithobates palmipes* (Spix, 1824) is a species in the family *Ranidae*, primarily associated with lowland tropical rainforests. It is widely distributed in the Amazon but also occurs separately in northeastern Brazil, within the Atlantic Forest. Amphibians, in general, have reproductive cycles that can be influenced by climatic factors such as temperature, humidity, and precipitation. The present study investigated the histological aspects of the tests of Lithobates palmipes (Spix, 1824) with the aim of understanding its reproductive cycle and how the species responds to environmental variations. The analyzed species exhibits a continuous gametogenic cycle, allowing for the production of sperm throughout the year, regardless of rainy or dry seasons. The research was based on testicular samples from individuals collected between September 2011 and June 2012, in a fragment of the Atlantic Forest known as Mata do Buraquinho, located in Paraíba, Brazil. Histological analyses revealed that the tests of Lithobates palmipes (Spix, 1824) exhibit cystic spermatogenesis, with germ cells organized in lobules and associated with Sertoli cells. During the study period, different stages of spermatogenesis were identified, with a predominance of spermatogonia in some months and the presence of spermatocytes and spermatids in others. These findings indicate that the species maintains continuous reproduction regardless of seasonal rainfall variations, which can be explained by the presence of permanent bodies of water in the studied area. The study underscores the importance of histological analysis for understanding the reproductive biology of tropical anurans and its relevance to conservation strategies for species inhabiting areas impacted by urbanization. Furthermore, these results can contribute to environmental management and the protection of species that rely on fragile ecosystems.

Keywords: amphibians; cells; histology; testes; reproduction.

Resumo. *Lithobates palmipes* (Spix, 1824) é uma espécie da família *Ranidae*, associada principalmente a florestas tropicais úmidas de terras baixas, sendo amplamente distribuída na Amazônia, mas ocorrendo também de forma disjunta no Nordeste brasileiro, na Mata Atlântica. Os anfíbios, em geral, possuem ciclos reprodutivos que podem ser influenciados por fatores climáticos, como temperatura, umidade e precipitação. O estudo realizado investigou os aspectos histológicos dos testículos de Lithobates palmipes (Spix, 1824), com o objetivo de compreender seu ciclo reprodutivo e as respostas dessa espécie às variações ambientais. A espécie analisada apresenta um ciclo gametogênico do tipo contínuo, o que permite a produção de espermatozoides ao longo do ano, sem depender das estações chuvosas ou secas. A pesquisa foi realizada a partir de amostras de testículos de indivíduos coletados entre setembro de 2011 e junho de 2012, em um fragmento da Mata Atlântica, na Mata do Buraquinho, Paraíba. As análises histológicas revelaram que os testículos de Lithobates palmipes (Spix, 1824) apresentam espermatogênese cística, com as células germinativas organizadas em lóculos, associadas às células de Sertoli. Durante o período de estudo, foram identificados diferentes estágios da espermatogênese, com predominância de espermatogônias em alguns meses e presença de espermatócitos e espermátides em outros. Esses achados indicam que a espécie mantém uma reprodução contínua, independentemente das variações sazonais de chuvas, o que pode ser explicado pela presença de corpos d'água permanentes na área estudada. O estudo reforça a importância da análise histológica para entender a biologia reprodutiva dos anuros tropicais e sua relevância para as estratégias de conservação de espécies que habitam ambientes impactados pela urbanização. Além disso, esses resultados podem contribuir para a gestão ambiental e para a proteção de espécies que dependem de ecossistemas frágeis.

Palavras-chave: anfíbios; células; histologia; testículos; reprodução.

[I*]Graduanda em Medicina Veterinária, Faculdade Nova Esperança (FACENE), João Pessoa, Paraíba, Brasil
vanessaalexandre015@gmail.com
https://orcid.org/0009-0000-5904-6079

[II] Graduando em Medicina Veterinária, Faculdade Nova Esperança (FACENE), João Pessoa, Paraíba, Brasil
https://orcid.org/0009-0001-3534-3105

[III] Graduação em ciências biológicas UEPB, Tecnóloga em Gestão Ambiental Unicesumar, Doutora em Biociência Animal UFRPE, Professora UNIFIP, Patos, Paraíba, Brasil,
https://orcid.org/0000-0001-6786-0425

[IV] Licenciatura e Bacharelado em Ciências Biológicas UFPB, Doutor em Ciência Animal Tropical UFRPE, Lotado em Unidade Acadêmica de Biologia, Centro de Educação e Saúde UFCG, Cuité, Paraíba, Brasil
https://orcid.org/0000-0002-0341-2882

[V] Biólogo, Doutor em Ciência Animal UFCG, Unidade Acadêmica de Ciências Biológicas, Patos, Paraíba, Brasil,
https://orcid.org/0000-0001-5727-8781

[VI] Médico Veterinário formado pela UFCG, Mestre e Doutor em Ciência e Saúde pela UFCG na área de Morfofisiologia Animal, Clínico de Animais Silvestres e Exóticos, Lotado na Faculdade Nova Esperança, João Pessoa, Paraíba, Brasil,
https://orcid.org/0000-0002-2131-7432

Submissão:12/12/2025 Aceite: 12/12/2025
DOI: 10.17695/rcsne.vol23.n3.p211-218

INTRODUCTION

The order Anura is the most diverse and representative among amphibians, with approximately 7,568 species distributed across virtually all continents, except for frozen regions[1]. Their populations are highly vulnerable to environmental variations due to their metabolic and reproductive physiology, with a wide diversity of reproductive modes, each dependent on optimal and specific environmental conditions[1]. About 41% of anuran species are threatened with extinction, which reinforces the need for studies on this group[2]. They are considered excellent bioindicators, as they can provide information on the conditions of terrestrial and aquatic ecosystems. In addition, their permeable skin makes most anurans dependent on wetlands, thus limiting the group's mobility and dispersal or migration routes, making them even more susceptible to environmental changes than animals with greater mobility and autonomy to migrate, disperse, or even escape[2].

The interface between the terrestrial and aquatic environments gives amphibians a reproductive behavior that is unique among vertebrates: the need for adequate locations for amplexus, external fertilization, and oviposition, as well as their reproductive physiology being subject to extrinsic (environmental) and intrinsic (hormonal cycles) factors[3]. Temperature and photoperiod are the main environmental factors that control seasonal reproductive cycles in amphibians. Environmental changes in these factors can interfere with spermatogenesis and establish continuous, discontinuous, or potentially continuous gametogenic cycles[4]. Discontinuous types are commonly found in species from temperate zones and generally have a discrete breeding season with pronounced changes in gonad size, gamete production, and accessory sexual structures. In the potentially continuous type, there is a partial interruption in gametogenic activity during some seasons of the year, but primary spermatogonia in males remain sensitive to gonadotropic stimulation. Species inhabiting tropical regions usually have a continuous reproductive cycle[4]. In fact, the characteristics of thermal and water balance are fundamental in the evolutionary history of amphibians, making water balance and thermal tolerance inseparable[5].

However, the reproductive period can also occur during the dry season when suitable environments such as permanent water bodies are available[6]. In these regions, the highest peaks of sex hormone production coincide with physiological preparation for the reproductive period, where the highest rates of androgen production determine the development of secondary sexual characteristics, the maintenance of sexual behavior, and the start of gametogenesis in anurans[6].

The former genus Rana (Linnaeus, 1758), previously considered a single taxon encompassing all members of the family Ranidae, is no longer considered to constitute a monophyletic group[7]. Current recommendations divide the Ranidae family, which comprises 437 species, into 27 genera, with the genus Rana being retained for a restricted group of 58 species from Eurasia and North America. The genus Lithobates currently comprises 51 species from North, Central, and South America to southern Brazil[7]. Lithobates palmipes (Spix, 1824) is a species of the Ranidae family, mainly associated with lowland tropical rainforests, widely distributed in the Amazon, but also occurring disjointedly in northeastern Brazil, in the Atlantic Forest. However, data on Lithobates palmipes (Spix, 1824) are scarce, particularly for individuals collected in the Brazilian Atlantic Forest[8]. Therefore, this study aimed to evaluate possible changes in the histological aspects of the testes in males of this species in a fragment of the northeastern Atlantic Forest, providing additional data on their biology.

MATERIALS AND METHODS

Study area and sampling.

To verify the reproductive activity of *Lithobates palmipes*(Spix, 1824) males, testicles provided by the Herpetology Collection of the Federal University of Paraíba were analyzed. Therefore, ECUA (Ethics Committee on the Use of Animals) documentation was not necessary, since the animals were seized. This material consisted of twenty testicles from animals collected from September to December 2011 and January to June 2012 in the Mata do Buraquinho Permanent Preservation Area (7º08'42"S and 34º51'54"W), located southwest of the municipality of João Pessoa, State of Paraíba. This is a 515-hectare remnant of Atlantic Forest within the urban matrix[9]. With a hot and humid climate, the study area has autumn-winter rains, with average annual temperatures of approximately 25°C and total rainfall varying between 1500 and 1700 mm, and relative humidity around 80%. The period of highest rainfall occurs between March and August. The dry season is considered short, lasting between one and three months[9].

Sampling and collection of individuals

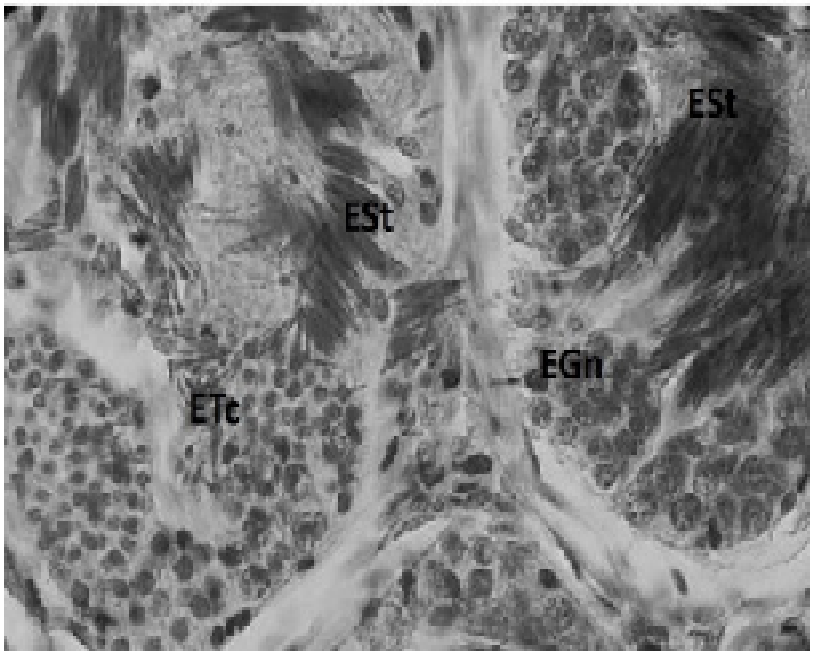
After opening the coelomic cavity of the specimens, the testes were identified and analyzed. Each testis analyzed was previously fixed in 10% buffered formalin for routine histological processing. These were then dehydrated in a series of increasing alcohol concentrations. Inclusion was performed with paraffin and 5µm cross sections to obtain the cuts that were later stained with Hematoxylin and Eosin-H/E for the preparation of histological slides for light microscopy. The reproductive cells were evaluated for their morphology, arrangement, and numerical proportion, identifying the correlation between reproductive periods with various environmental factors such as precipitation, temperature, and humidity. The germ cells were identified and organized into five spermatogenic stages, considering the stage at which they were found within the seminiferous tubule. Stage 1 – Seminiferous epithelium containing only spermatogonia and seminiferous tubule without a defined lumen; Stage 2 – Primary and secondary spermatocytes at the margin of the lumen; Stage 3 – Spermatids at different stages of differentiation. Data on rainfall indices, relative humidity, and average temperature were provided by the National Institute of Meteorology (INMET, 2016).

RESULTS AND DISCUSSION

Males of *Lithobates palmipes*(Spix, 1824) reach about 7 to 8 centimeters in rostrum-cloacal length. The testes are located in the coelomic cavity near the kidneys and connected to the dorsal wall of the abdomen by the mesorchium. They are described as paired, rounded, compact organs, slightly yellowish, whitish, or milky white in color. Anatomical variations can be observed during the reproductive period.

Through the analysis of histological sections, it was possible to observe that the structural units of the testes of Lithobates palmipes(Spix, 1824), called seminiferous tubules, house germline cells and Sertoli cells inside them. The germ cells, in turn, are grouped into cysts characterized by cytoplasmic extensions of Sertoli cells. The cysts are at various stages of cell differentiation, characterizing cystic spermatogenesis (Figure 1).

FIGURE 1 - Seminiferous tubules of *Lithobates palmipesstained* with HE in the reproductive phase. EGn: Spermatogonia; ETc: Spermatocytes; ESt: Spermatids.



In all slides analyzed, the seminiferous tubules were well defined and the cell stages well demarcated (Figure 1), with three cell types easily identified in the testes of Lithobates palmipes(Spix, 1824): spermatogonia, spermatocytes, and elongated spermatids, with each locule presenting several cysts of spermatogenic cells (Figure 1).

Spermatogonia were characterized as the largest cells within the locules, with clearly visible cytoplasm, located at the locular periphery, with an ovoid shape and granular chromatin (Figure 1). They are surrounded by connective tissue called follicular cells or Sertoli cells, producing several cell copies within these cysts, undergoing various cell maturation processes.

Primary spermatocytes, originating from the last mitotic divisions, had a shape similar to spermatogonia, but were smaller due to the decrease in cytoplasmic volume and had a slightly fusiform shape in association with Sertoli cells. Their chromatin is initially diffuse, varying during the prophase of the first meiotic division.
Spermatids, resulting from the second meiotic division, have sparse cytoplasm and are characterized by the elongated shape of the nucleus (Figure 1). These cells group into compact bundles, oriented in the same direction as they mature, breaking up the cystic arrangement.

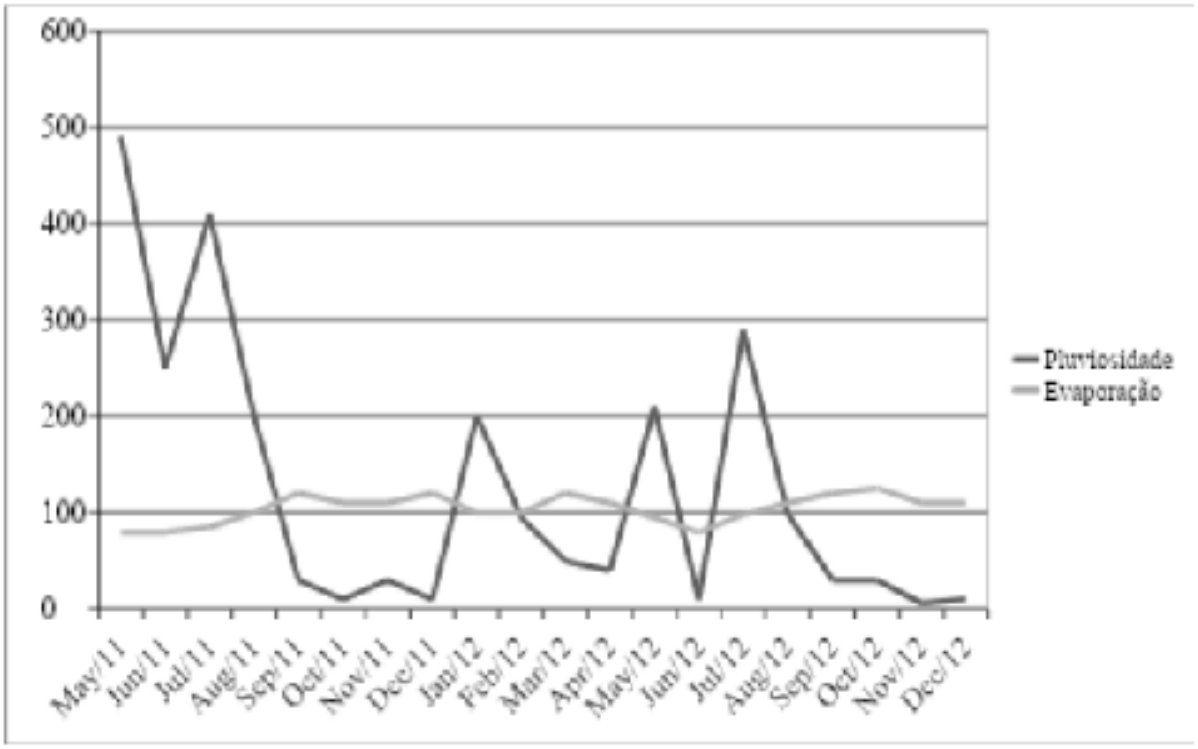
Of the twenty testes analyzed, seven belonged to juvenile individuals and thirteen were from adult individuals. The juveniles were captured during the rainy season (January, February, and March). The testes of these individuals exhibited the first stage of spermatogenesis, presenting only spermatogonia (Table 1).

Title: Germinative seasonality in the testis of Lithobates palmipes

	Set 2011	Nov 2011	Dec 2011	Jan 2012	Feb 2012	Mar 2012	Apr 2012	Mai=y 2012	Jun 2012
Espermatogonia	x	x	x	x	x	x	x	x	x
Espermatocyte	x	x	x				x	x	x
Spermatid	x	x	x				x	x	x

TABLE 1 - Cell types found in the testes of Lithobates palmipes.

The degree of development of germ cells within the loculi varied throughout the period analyzed. Spermatogonia cysts were found throughout the entire period analyzed (Table 1). Spermatocyte and elongated spermatid cysts were found in six of the nine months analyzed and in thirteen of the twenty slides analyzed. No spermatozoa were observed in the slides analyzed, possibly due to the absence of material from the tail of the epididymis, the region where sperm maturation occurs. Lithobates palmipes (Spix, 1824) presented bundles of elongated spermatids between September and December 2011 and (dry season), interspersed by three periods where only spermatogonia cysts were found in January, February, and March 2012 (Table 1) and again presenting elongated spermatids in April, May, and June 2012 (rainy season) (Table 1; Graph 1).



GRAPH 1 – Spermatogenic stages found in Lithobates palmipesand their association with rainfall and evaporation rates in the study area (abiotic data obtained by INMet).

The testicular parenchyma consists of seminiferous cords, formed by Sertoli cells and gonocytes, surrounded by a layer of smooth muscle, the myoid cells. The production of male gametes involves the processes of cell division and differentiation, by which sperm are produced in the testes, a process called spermatogenesis (10). Sperm are formed within the seminiferous tubules of the testes. These tubules contain a complex series of developing germ cells that later form highly specialized cells, the male gametes[10].

Spermatogonia, derived from primordial germ cells, undergo cellular modifications to form primary spermatocytes, which undergo meiotic reduction division to form secondary spermatocytes. In the next stage, they undergo equational meiosis, giving rise to spermatids, which undergo the process of spermiogenesis, in which they undergo several modifications and form spermatozoa[10]. Primary spermatocytes originating from the last mitotic division are smaller cells than their predecessor and have a spherical, basophilic nucleus[11]. The germ cell organization is cystic, as described for anurans, and each cyst contains germ cells at the same stage[12]. Primary spermatocyte cysts are the most frequent, as are spermatogonia, which can be found isolated along the tubules or forming spermatogonial clusters at the periphery of the testes[11]. In some species, only the apical portion of Sertoli cells is involved in the process of spermatogenesis, while the basal portion, containing the nucleus, is retained for the next generation of cysts[13].

Elongated spermatids and early spermatozoa are arranged in parallel bundles with their nuclei embedded in the vacuolated apical parts of Sertoli cells and their flagella directed toward the lumen of a seminiferous tubule. At this stage of development, cysts have opened due to a rupture of the Sertoli cells and finally form spermatozoa with a flagellum [14].

Environmental and behavioral factors affecting anurans may also influence the spermatogenic process, both in terms of the variation of certain cell types throughout the year and in terms of the variation in germ organization in the testes observed between species[12]. Based on this description and the fact that elongated spermatid cysts in the final stage of maturation were found inside the seminiferous tubules of adults in the Lithobates palmipes (Spix, 1824) population studied, both in the dry season (January, February, and March) and in the months considered rainy (April, May, and June), we consider that Lithobates palmipes(Spix, 1824) in this region has a continuous gametogenic cycle, reproducing throughout the year, as also found for other species of anurans in tropical regions. In these species with a continuous gametogenic cycle, sperm are generally produced throughout the year, and the testes always contain nests of spermatogenic cells[15].

In addition to natural environmental factors such as temperature and humidity, anthropogenic activities in reproductive environments or their surroundings can have diverse effects on the development of these animals [16]. However, reproduction in the studied population appears to occur independently of the area's rainfall regime. This is possible in part due to the lower heterogeneity of habitats and the constant availability of permanent water bodies and temporary aquatic environments throughout the year in the area studied. Although this rainfall regime establishes a short dry season for the area studied, these factors may favor the maintenance of the species' reproductive activity even during the driest period. In addition, the period established as dry was preceded by above-average rainfall, which promoted vegetation growth and consequently favored the development of reproductive sites in the area that were maintained during the dry period. However, there is limited information on the urban environment and the effects of urbanization on the local anuran population. Therefore, further studies are needed to assess these effects and draw conclusions[12].

FINAL CONSIDERATIONS

The study of the histological aspects of the testes of Lithobates palmipes(Spix, 1824) reveals the complexity of amphibian reproduction in response to environmental variables. This study reinforces the importance of histological analysis of the reproductive biology of tropical amphibians, especially in areas of fragmented urban forests, where environmental factors can significantly influence reproductive patterns. Understanding how the species Lithobates palmipes (Spix, 1824) adapts to such conditions is essential for the development of conservation strategies, ensuring the maintenance of populations in environments increasingly impacted by human activities.

BIBLIOGRAPHIC REFERENCES

1. Braga RR, Castro DP, Lima DC, Oliveira CR, Ávila RW. Achados patológicos e incidentais em anuros do Ceará, nordeste brasileiro. Rev. Cienc. anim. bras. 2024;25(1) p. 2-18.
https://www.scielo.br/j/cab/a/FCn6kfQd3HWxjsmjMXwH7gq/?format=pdf&lang=pt

2. Ferreira-Silva SK, Alexandre RJR, Penac SA, Lucenab MDL, Vieirade TB, Gomesum FBR. Associations between morphological attributes and food resources in anurans from the Middle Xingu region, Brazil. Rev. Braz. J. Biol. 2025;85(1) p. 1-13
https://www.scielo.br/j/bjb/a/P3TzjcCTbf97FKM6b3PjPjz/?format=pdf&lang=en

3. Ryan MJ. Amphibians: biology of amphibians. Rev. Scien. 1986;232(4747):271

4. Santos LRS, Oliveira C. Morfometria testicular durante o ciclo reprodutivo de Dendropsophus minutus (Peters) (Anura, Hylidae). Rev. Bras Zool. 2007;24(1) p.70-64. https://www.scielo.br/j/rbzool/a/j8PzkhxfbmJPKsph7HmFhzR/?format=pdf&lang=pt.

5. Bovo RP, Simon MN, Provete DB, Lyra M, Navas CA, Andrade DV. Beyond Janzen's Hypothesis: How Amphibians That Climb Tropical Mountains Respond to Climate Variation. Rev. Integr Org Biol. 2023;5(1) p. 2-19
https://pmc.ncbi.nlm.nih.gov/articles/PMC10155226/pdf/obad009.pdf

6. Chaves MF. Caracterização histologia e biologia reprodutiva de Leptodactylus macrosternum (Anura, Leptodactylidae), nordeste do Brasil. Pernambuco: Universidade Federal Rural de Pernambuco. 2016 Dez; p. 74-111. http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/7307

7. Mechkarska M, Barran G, Kolodziejek J, Coquet L, Leprince J, Jouenne T, et al. Peptidomic analysis of the host-defense peptides in skin secretions of the Amazon River frog Lithobates palmipes (Ranidae). Rev. Comp Biochem Physiol Part D Genomics Proteomics. 2023;46(1) p. 1-7.
https://www.sciencedirect.com/science/article/pii/S1744117X2300014X?via%3Dihub

8. Barbosa GG, de Santana JC, Silva TL, Santana BCG, Paiva PMG, de Freitas GG, et al. A new temporin with antibacterial activity and cytotoxicity from the skin secretion of Lithobates palmipes (Spix, 1824) (Amphibia: Ranidae) from Brazilian Atlantic Forest. Rev. Comp Biochem Physiol B Biochem Mol Bio. 2024;275(1) p. 1-6.
https://www.sciencedirect.com/science/article/pii/S1096495924001088?via%3Dihub

9. Lima PJ, Heckendorff WD. Atlas geográfico do Estado da Paraíba. João Pessoa: Grafset; 1985.

10. Alcântara LM, Toniolli R. A Espermatogênese. Rev. Cienc. Anim. 2023;33(4) p. 101-121.

11. Chagas JMA, Ninhaus-silveira A, Veríssimo-silveira R. Ciclo Testicular de Devario aequipinnatus (Teleostei, Cyprinidae): Um Ptencial Modelo Biológico Em Experimentação Anaimal. Rev. Bol. Inst. Pesca. 2016;42(4) p.766-780.
http://hdl.handle.net/11449/173975

12. Bordin RO, Fernandes CES, Franco-Belussi L, Leão TRF, Sanabria M. Sperm morphology and testicular histology of the polyandric species Leptodactylus podicipinus (Anura: Leptodactylidae) from an urban environment. Rev. Anat Rec. 2021;305(12) p. 1-11.
https://anatomypubs.onlinelibrary.wiley.com/doi/10.1002/ar.24928

13. Pudney J. Spermatogenesis in nonmammalian vertebrates. Rev. Microsc Res Tech. 1995;32(6) p. 459-97.
https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/epdf/10.1002/jemt.1070320602

14. Haczkiewicz K, Rozenblut-Kościsty B, Ogielska M. Prespermatogenesis and early spermatogenesis in frogs. Rev. Zoology. 2017;122 p. 1-17
https://www.sciencedirect.com/science/article/abs/pii/S0944200616301258?via%3Dihub

15. Duellman WE, Trueb L. Biolology of Amphibia. New York: McGraw-Hill Book Company; 1994.

16. Borges RE, Santos LRS, Assis RA, Benvindo-Souza M, Franco-Belussi L, & Oliveira C. Monitoramento da integridade morfológica de anuros neotropicais. Ciências Ambiental e Pesquisa em Poluição, 2019;26 p. 23–34.