



# Proteinaceous Plug and Urethral Ectasia in *Artibeus lituratus* (Chiroptera: Phyllostomidae)

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**ABSTRACT** – *Artibeus lituratus* (great fruit-eating bat; Phyllostomidae, Stenodermatinae) is a bat predominantly frugivorous species, with an important role in seed dispersal. The scarce literature on the morphology and pathology of bats is a challenge for professionals who study the species. This report presents the case of a free-living adult male of *A. lituratus*, captured during ecology and health studies in an Atlantic Forest remnant in the city of Rio de Janeiro, Brazil. The macroscopic examination of the structures showed a prostate, with about 0.5 cm in diameter, attached to the base of the urinary bladder, embracing part of the initial portion of the urethra and being composed of two well-defined regions: dorsal region and ventral region (involving the ventral part of the urethra) with the presence of homogeneous and whitish parenchyma. The urinary bladder was quite rigid and very thick with a lot of a whitish, friable and caseous material inside. On microscopic examination, this material was strongly eosinophilic and densely marked by PAS staining with the presence of sperm. The histology of prostatic region was preserved; however, the marked ectasia of the prostatic urethra was observed, with a large amount of refluxed material in its interior and flattening of the lining epithelium in part of the sample. This is the first report of this pathological process in *A. lituratus* and important to draw attention of veterinarians and other professionals about the presence of this pathology in bats.

**Keywords:** Proteinaceous plug; *Artibeus lituratus*; urethral ectasia.

## Plug Proteináceo e Ectasia Uretral em *Artibeus lituratus* (Chiroptera: Phyllostomidae)

**RESUMO** – *Artibeus lituratus* (Phyllostomidae, Stenodermatinae) é uma espécie de morcego predominantemente frugívora, com importante papel na dispersão das sementes, e a escassa literatura a respeito da morfologia e patologia de morcegos é um desafio para os profissionais que estudam a espécie. Neste relato é apresentado o caso de *plug* proteináceo e ectasia uretra em um macho adulto de *A. lituratus* de vida livre, capturado para análise de ecologia e patologia em um fragmento de Mata Atlântica na cidade do Rio de Janeiro, Brasil. O exame macroscópico evidenciou próstata com aspecto e tamanho aparentemente normal. A vesícula urinária apresentava-se bastante rígida, espessa e com presença, em seu interior, de material friável esbranquiçado e caseoso em grande quantidade. Ao exame microscópico o material era fortemente eosinofílico e densamente marcado pela coloração de com Ácido Periódico de Schiff, inclusive com presença de espermatozoides. Na região prostática, a histologia do parênquima estava preservada, porém, uma marcante ectasia da uretra prostática foi observada, com grande quantidade de material de refluxo em seu interior e achatamento do epitélio de revestimento em parte da amostra. Este é o primeiro relato desse processo patológico na espécie, e alerta os médicos veterinários e outros profissionais que trabalham com a espécie em cativeiro ou em vida livre sobre a presença dessa patologia em quirópteros.

**Palavras-chave:** Plug proteináceo; *Artibeus lituratus*; ectasia uretral.



## Tapón Proteináceo y Ectasia Uretral en *Artibeus lituratus* (Chiroptera: Phyllostomidae)

**RESUMEN** – *Artibeus lituratus* (Phyllostomidae, Stenodermatinae) es una especie de murciélago predominantemente frugívoro, con un papel importante en la dispersión de semillas. La escasa literatura sobre la morfología y patología de los murciélagos es un desafío para los profesionales que estudian la especie. Este informe presenta el caso de un macho adulto de vida libre de *A. lituratus*, capturado para análisis de ecología y patología en un fragmento de Mata Atlántica en la ciudad de Río de Janeiro, Brasil. El examen macroscópico de las estructuras mostró una próstata, de unos 0,5 cm de diámetro, unido a la base de la vejiga urinaria, abarcando parte de la porción inicial de la uretra y estando compuesto por dos regiones bien definidas: región dorsal y región ventral (involucra la parte ventral de la uretra) con presencia de parénquima homogéneo y blanquecino. La vesícula urinaria era bastante rígida, gruesa y con gran cantidad de material friable blanquecino y cursi en su interior. En el examen microscópico, el material era fuertemente eosinofílico y densamente marcado por la tinción PAS, incluida la presencia de espermatozoides. En la región prostática se conservaba la histología del parénquima, sin embargo, se observaba una marcada ectasia de la uretra prostática, con gran cantidad de material reflujo en su interior y aplanamiento del epitelio de revestimiento en parte de la muestra. Este es el primer reporte de este proceso patológico en la especie y alerta a veterinarios y otros profesionales que trabajan con la especie en cautiverio o en libertad sobre la presencia de esta patología en murciélagos.

**Palabras clave:** Tapón proteináceo; *Artibeus lituratus*; ectasia uretral.

### Introduction

*Artibeus lituratus* (Olfers 1818) (Phyllostomidae, Stenodermatinae), occurs from Mexico southward to the northern Argentina, and is widespread distributed in Brazil (Gardner, 2008). This Neotropical species is mainly frugivorous, but also eats insects, flowers and leaves (Reis et al., 2007).

The scarce literature the pathology of bats makes the histopathological analysis of these animals a great challenge. Kirejczyk et al. (2021) described the most common pathological findings in bats and in the results the non-infectious processes (especially musculoskeletal diseases and reproductive losses) were more common than infectious processes, and no case of urethral plug was cited. Some studies about the urinary and reproductive systems of *A. lituratus* are found in the scientific literature (Arandas, 2013; Miotti et al., 2018; Santos et al., 2017; Campolina-Silva et al., 2019; Albernaz et al., 2021); however, there are no descriptions in the most accessed databases about urinary bladder and urinary tract morphology. Regarding accessory glands, Santos et al. (2017) published a detailed study on their morphology in the species *A. lituratus*, including the prostate. According to these authors, this is formed by ventral and dorsal regions (Santos et al., 2017, confirmed by Albernaz et al., 2021) separated only by soft connective tissue. The ventral part is

formed by organized basal (elongated nuclei) and secretory (rounded and more abundant nucleus) cells, forming acini with epithelium ranging from cubic to pseudostratified or totally absent and in some cases presenting cells in apoptosis and luminal debris. In the dorsal region, the epithelial cells form a cubic to columnar pseudostratified epithelium (Santos et al., 2017).

### Case Description

Reports about pathological findings of the urinary and reproductive system in *A. lituratus* are not common; however, the proteinaceous plug is a commonly reported in other species of mammals (like rodents), and for professionals working in the area of toxicological and experimental pathology, it is a well-known nosological entity, especially in rodents (Frazier et al., 2012). This finding is reported associated with euthanasia and/or death, being common when ether inhalation has been used as euthanasia method (Rapp, 1962). Moreover, it can more commonly be associated with obstructive syndromes secondary to genitourinary tract infections (Hard et al., 1999; Frazier et al., 2012; NIH, 2021). Microscopically, the plug material can be observed in the urinary bladder or urethra of males and is extremely eosinophilic, containing sperm, leukocytes and desquamative cells (Hard et al., 1999; Frazier et al., 2012; NIH, 2021). In this study, we report

a proteinaceous plug with urethral ectasia in a free-living, unknown history, male of *A. lituratus* with an inactive reproductive condition, possibly a young adult, forearm measuring 71 cm, stored in biological collection at with voucher number RM1633, captured during ecology and health studies in a regenerated forest area in an Atlantic Forest remnant in the city of Rio de Janeiro (Ethics Committee in the Use of Animals of the: LICENCA CEUA LM-6/18 and Sisbio 19037-1). The individual was handled following capture, handling, and collection protocols defined by the American Society of Mammalogists, using acepromazine and ketamine (0.15 mL/100g of weight) for euthanasia. The prostate and urinary bladder set was collected and fixed in 10% formaldehyde in phosphate buffer (PBS) and sent for pathological analysis. On macroscopic examination, the prostate was apparently normal for the species, but the urinary bladder was quite rigid and with an apparent thickening of the wall. The cut surface of the prostate had a homogeneous, whitish parenchyma, while the urinary bladder had a very thick wall compared to other mammalian species and was filled by a large amount of whitish friable material (Figure 1 A and B). The material has been processed by the usual techniques for embedding in paraffin and stained by hematoxylin and eosin and by Periodic Acid Schiff (PAS) to assess the overall activity of the gland secretion and the presence of glycoproteins. Images were captured with the Zen 2 program (Carl Zeiss®) using the Zeiss Axio Lab® microscope associated with the AxioCam ERc5® camera.

Upon microscopic examination, it was observed that a large part of the urinary bladder wall was formed by a muscular layer with a plexiform aspect, with an outermost portion extending through a soft connective tissue and delimited by the mesothelium (Figure 1 C and D). Internally, its mucosa was composed of a sparse lamina propria, covered by a thin transitional epithelium and whose surface sometimes had papillary projections (Figure 1 D). No pathological conditions were observed in the bladder wall. Inside, the material was strongly eosinophilic and densely marked by PAS stain (indicating the presence of glycoproteins) and even with a small amount of sperm, suggesting that it was semen reflux and not just accessory gland products. The meatus could be visualized, also without pathological conditions in its wall, but full of the previously described content (Figure 1 C). The

prostatic parenchyma showed a clear distinction between its dorsal and ventral portion, this last one with a strongly positive PAS material, indicating and the presence of glycoproteins (Figure 1 E). A marked ectasia of the prostatic urethra was observed, with a large amount of material reflux in its interior and flattening of the lining epithelium in part of the sample (Figure 1 F). Testis were normal. The findings, therefore, evidenced a case of proteinaceous plug with associated urethral ectasia.

## Discussion

The histopathological evaluations of tissues from free-living animals are always challenging given the lack of reference material on their morphological and mainly pathological aspects. The information in the literature generally refers to exotic animals related to pet living or animals kept as biomodels, but may reflect little on the reality of free-living animals, as they are not kept in controlled or known survival conditions (Frazier et al., 2012). It is also important to highlight that the proteinaceous plug commonly observed in some animal species, including rodents, may have been related to the process of euthanasia and/or death, being a product of the agonal secretion of accessory sex glands, or associated with obstructive syndromes that may be related to genitourinary tract infections (NIH, 2021). Considering the large prostatic urethral ectasia observed in the studied case, where the urethra has approximately twice the size than the urethra of normal prostates identified in previous morphological studies, the second hypothesis is the most likely in this report.

In this study, the macroscopic structure of prostate was similar to those found by other authors, including for other bat species like *Sturnira* (*S. erythromos*, *S. lilium*, and *S. oporaphilum*) (Santos et al, 2011; Miotti et al, 2017) and microscopically it was possible to differentiate the dorsal portion from the ventral by the high positivity by P.A.S. as in other studies and other bat species (Puga et al, 2016; Miotti et al, 2017).

About the proteinaceous plug, microscopically, the plug material can be observed in the urinary bladder or urethra of males and is extremely eosinophilic, containing sperm, leukocytes and desquamative cells (Hard et al., 1999; Frazier et al., 2012; NIH, 2021) as in this study. Regarding the causes of the process, the



hypothesis of urethral obstruction is the most plausible considering the large prostatic urethral ectasia, where the urethra has approximately twice the diameter than the urethra of normal prostates identified in previous morphological studies (Hard et al., 1999; Frazier et al., 2012; NIH, 2021).

In conclusion, this report is very important to draw attention to the presence of proteinaceous

plug with associated urethral ectasia in bats through the description of a spontaneous case in free-living *Artibeus lituratus*. Given the lack of descriptions on the subject the need for further studies on the morphology and pathology of the species is clear, including about the urinary system.

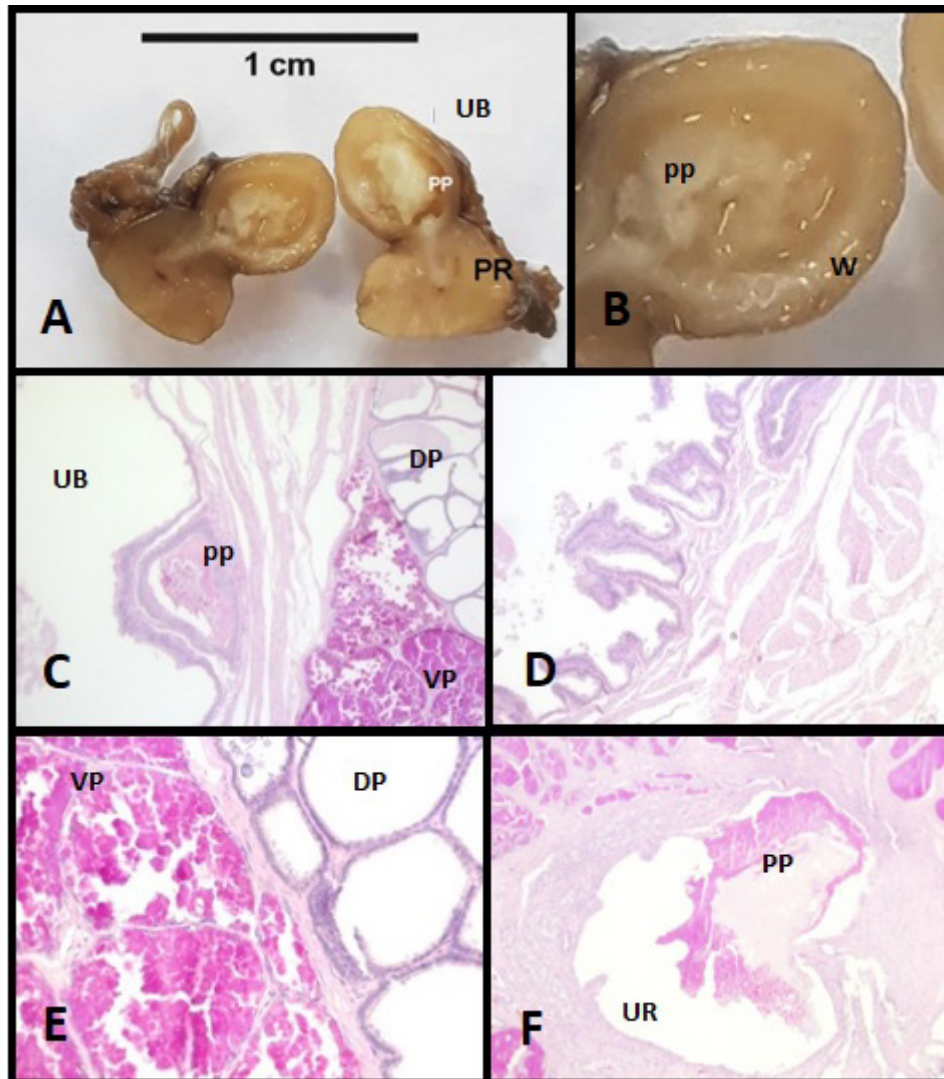


Figure 1 – A – Macroscopy of the prostate (PR) and urinary bladder (UB) where the cut shows the presence of the proteinaceous plug (PP) inside the UB; B – Detail of the previous image showing the thickness of the urinary bladder wall (W) and the proteinaceous plug (PP) inside the UB; C – Microscopic image of the urinary bladder (UB), initial portion of the urethra with proteinaceous plug (PP) in the luminal region and dorsal (DP) and ventral regions of the prostate (VP); D – Detail of the wall of the urinary bladder demonstrating its different layers and the papillary projections on the mucosa; E – Transition region between the ventral and dorsal region of the prostate, with detail of the PAS positive in the ventral portion (PAS obj.40x); F – Urethra (UR) in the prostatic region showing ectasia and a proteinaceous plug inside. All microscopic images were generated using PAS staining with 10x objective (C, D and F) or 40x objective (E). Slides of the species *Artibeus lituratus* (white-faced bat). [Credit: Kelly Demarque]



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