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Morphological Variation of Primary Reproductive Structures in Males of Five Families of Neotropical Bats

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ABSTRACT

Bats present unique features among mammals with respect to reproduction, and although neotropical bats do not have a hibernation period, many of their reproductive characteristics vary seasonally and interspecifically. Thus, this work aimed to examine the reproductive structures of 18 species belonging to five families of Brazilian bats. The gross anatomy of the testes varied little; however, the epididymis of Emballonuridae and Vespertilionidae showed exceptional structures with a large elongation of the caudal region. We observed a wide variation in the positioning of the testes: Phyllostomidae and Noctilionidae presented external testes; Emballonuridae and Molossidae presented migratory testes that may be in intra-abdominal or external positions; and Vespertilionidae displayed scrotal testes. In the histological evaluation, we observed a different pattern in vespertilionid species, with testicular regression and sperm retention/storage in the cauda epididymis in the five species analyzed. Similar testicular regression was observed in *Molossops temminckii*; however, sperm retention/storage was not observed in this species. These data suggest that although the species analyzed are tropical species that do not present a prolonged period of torpor (hibernation), they still maintain a period of seminiferous tubule regression and sperm storage very similar to that observed in hibernating bats. Anat Rec, 296:156–167, 2013. ©2012 Wiley Periodicals, Inc.

Key words: Chiroptera; epididymis; quiescence; testes; Vespertilionidae

The order Chiroptera is the second largest order of mammals, with approximately 202 genera and 1116 species widely distributed among all tropical and temperate regions, except for some remote oceanic islands and Antarctica (Reis et al., 2007). It is classically divided into two suborders: Megachiroptera and Microchiroptera. The first is found exclusively in the Old World and is composed of only one family (Pteropodidae), with approximately 42 genera and 186 species; while the second is cosmopolitan, with 17 families and 930 species (Simmons, 2005).

In Brazil, there are approximately 165 species of Microchiroptera, distributed into nine families: Emballonuridae, Furipteridae, Molossidae, Mormoopidae, Natalidae, Noctilionidae, Phyllostomidae, Thyropteridae, and Vespertilionidae (Reis et al., 2006). Of these families, Furipteridae, Mormoopidae, Natalidae, Noctilionidae, Phyllostomidae, and Thyropteridae are found exclusively in the Americas, while Emballonuridae, Molossidae, and Vespertilionidae are also found occurred in the Old World.

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TABLE 1. List of specimens analyzed in the present study

Family	Species	São Paulo[a]	Goiás[b]	Collection[c]
Emballonuridae	*Peropteryx macrotis*	–	–	5
	Rhynchonycteris naso	–	2	3
Molossidae	*Eumops glaucinus*	5	–	–
	Molossops temminckii	4	4	–
	Molossus molossus	25	–	–
	Molossus rufus	5	–	3
Phyllostomidae	*Artibeus lituratus*	18	–	–
	Artibeus planirostris	59	–	–
	Carollia perspicillata	8	–	–
	Glossophaga soricina	14	–	–
	Phyllostomus discolor	5	–	–
	Platyrrhinus lineatus	20	–	–
Noctilionidae	*Noctilio albiventris*	5	–	5
Vespertilionidae	*Eptesicus furinalis*	5	–	–
	Histiotus velatus	5	–	1
	Lasiurus blossevillii	1	–	4
	Myotis albescens	–	3	2
	Myotis nigricans	30	1	–

[a] Specimens collected at São Paulo state, Brazil (49W 22′ 45″ 20S 49′ 11″).
[b] Specimens collected at Goiás state, Brazil (49W 15′ 14″ 16S 40′ 43″).
[c] Specimens obtained from the Chiroptera collection at the Sao Paulo State University, UNESP-IBILCE.

It has been a great effort by researchers aiming to study the characteristics of species of temperate and tropical regions. Although most emphasis has been to the temperate species, which are subject to abrupt changes in temperature, the results observed for tropical species, has been very interesting. The most studies in tropical species generally deal with aspects of female reproduction and pregnancy (Rasweiler, 1972, 1974, 1978, 1982, 1987, 1993; Quintero and Rasweiler, 1974; Badwaik et al., 1997; Rasweiler and Badwaik, 1997; Badwaik and Rasweiler, 2001; Komar et al., 2007; Nolte et al., 2009), and only a few examinations are focused on male patterns, and these are primarily based in ecological–behavioral perspectives (Wilson and Findley, 1971; Fabián and Marques, 1989; Heideman et al., 1992; Heideman and Bronson, 1994; Zortéa, 2003; Léon-Galván et al., 2005; Chaverri and Kunz, 2006; Costa et al., 2007; Ortêncio-Filho et al., 2007).

The characteristics exhibited by females are directly related to the specializations of males and anatomical, physiological, embryological, and behavioral specializations observed in females of some species with wide distribution and representation in the tropics constitute significant evidence that they have diverse reproductive patterns (Rasweiler, 1987; Rasweiler and Bonilla, 1992; Rasweiler and Badwaik, 1997; Rasweiler et al., 2000; Badwaik and Rasweiler, 2001; Rasweiler et al., 2011).

Despite all these observations, almost no evaluations of these species from a morphological/histological viewpoint have been made (Krutzsch et al., 2002; Krutzsch and Nellis, 2006; Beguelini et al., 2009; Duarte and Talamoni, 2010). Thus, this study aimed to analyze the male primary reproductive structures (testes and epididymis) of 18 species belonging to five of the great families of neotropical bats, to investigate the possible morphological differences.

MATERIALS AND METHODS

Species, Aging, and Licenses

The primary reproductive structures (testes and epididymis) of 18 species of exclusively neotropical bats, which belong to five different families, Emballonuridae (two spp.), Molossidae (four spp.), Phyllostomidae (six spp.), Noctilionidae (one spp.), and Vespertilionidae (five spp.), were morphologically analyzed in this study. Most specimens were collected in northwest São Paulo state, Brazil (São José do Rio Preto: 49W 22′ 45″ 20S 49′ 11″); however, some species were obtained from captures in the central region of Goiás state, Brazil (Goiânia: 49W 15′ 14″ 16S 40′ 43″), and the more difficult-to-collect species were obtained from the Chiroptera collection at the São Paulo State University, UNESP-IBILCE (Table 1). The captures occurred from June 2008 to June 2010.

Only adult males were used in this study. The bats were classified as adults based on their body weight, complete ossification of the metacarpal epiphyses, wear of the teeth (De Knegt et al., 2005), and the presence of spermatozoa inside the testes and/or the cauda epididymis. All of the studied animals were sexually mature adults; however, we were unable to determine whether the animals were in basal reproductive or peak breeding conditions or their social status, so our studies indicate a mixture of these cases, with animals collected during or outside of their peak breeding periods.

The capture and captivity of all specimens were authorized by the Brazilian institution responsible for wild animal care (Instituto Brasileiro do Meio Ambiente, IBAMA, Process: 21707-1), and a proposal of this study was assessed and approved by the Ethics Committee for Animal Experimentation (Document: Protocol. 013/2009 CEEA/IBILCE-UNESP).

The animals were treated according to the recommendations of the Committee on Care and Use of Laboratory Animals from the Institute of Laboratory Animal Resources, National Research Council, Guide for the Care and Use of Laboratory Animals" (Committee on Care and Use of Laboratory Animals, 1980), and after being sacrificed they were deposited in the Chiroptera collection at the São Paulo State University (UNESP-IBILCE).

Animal Processing and Documentation

The animals were euthanized by cervical dislocation and placed supinely on the dissection board. An incision was made in the abdominal region in the caudal direction, and the skin was moved to the sides. The locations of the testes and epididymis were documented, and these organs were then removed for future histological analysis. In animals with testes located inside the abdominal cavity, the subcutaneous tissue was cut, the area was exposed with forceps, the region was photographed, and the testes and epididymis were removed. The gross documentation was accomplished with a 7.0-mp HP Photosmart M627 camera (6.0–18.0 mm) with a 3x optical zoom and a 5x digital zoom.

Corporal and Testicular Weights, Gonad-Somatic Index, and Statistical Analysis

The animals and the testes were weighed on a balance with an accuracy of three decimal places. The specimens from the UNESP-IBILCE collection were weighed and their weights were increased by 33% to compensate for the weight lost during the fixation process, as described by Baker (1958).

The gonad-somatic index was calculated by dividing the weight of the testes by the corporal weight of the animal and recording the result to the fourth decimal place. The values were expressed using the mean value ± standard deviation.

All data were analyzed by a nonparametric multiple independent test (Kruskal–Wallis: $P \leq 0.05$) using the Statistica 7.0 software (Copyright© Statsoft, Inc. 1984–2004).

Histology

The reproductive status of each specimen was evaluated histologically to confirm its sexual maturity. The presence of spermatozoa inside the testes and in the cauda epididymis was used as an indication of sexual maturity and activity. The testicular regression was characterized by the observation of only the spermatogonia and Sertoli cells in the seminiferous epithelium and by a relative decrease in epithelium height and increase of interstitial tissue.

After surgical excision, the testes and epididymis were immersed in Bouin fixative solution for at least 24 hr, dehydrated in a graded ethanol series, embedded in glycol methacrylate (Historesin, Leica Instruments), and sectioned (1 μm thickness) using a Leica RM 2155 microtome. Tissue sections were stained with hematoxylin-eosin (Ribeiro and Lima, 2000) and analyzed using an Olympus BX60 microscope with Image-Pro-Plus for Windows computer image analysis software.

RESULTS

Position of the Testes–Epididymis Complex

The position and the framework support complex of the testes and epididymis widely varied in the five families analyzed.

The testes–epididymis complex (TEC) of all specimens of the six species of phyllostomid bats analyzed were found outside the abdominal cavity, located laterally to the abdomen (Fig. 1A, arrows), in the proximity of the inguinal canal (Fig. 1D, arrow). Its dislocation to a more caudal position, laterally to the base of the penis (parapenial), was observed in all species (Fig. 1B), with this movement apparently subject to the control of the animal and able to be performed within a few minutes. The TEC was located between the skin and muscles, with only a thin and transparent tunica vaginalis covering it (Fig. 1C). The presence of a true scrotum was not observed in these animals, but only a relaxation in the skin-muscle connection that allows the movement of the TEC (Fig. 1A,B).

Similar to phyllostomid bats, the TEC of *Noctilio albiventris* (Noctilionidae) was also observed outside the abdominal cavity, laterally to the abdomen; however, it is enclosed in an unusual pocket-like pouch that is directly associated with a true scrotum (Fig. 1E–H). The scrotum presents no hair on its surface and can be clearly distinguished by having a thin layer of skin with numerous scattered short barbs and long barbs in its periphery (Fig. 1F). The pocket-like pouch was constituted by a thick layer of tunica vaginalis surrounded by large amounts of adipose tissue (Fig. 1G,H). The dislocation of the TEC of the fold into the scrotum is easily observed (Fig. 1E,F and 1G,H).

The position of the TEC in Molossidae specimens varied as follows: (*i*) both TECs located outside the abdominal cavity, between the skin and muscles, inside a small scrotum (Fig. 1I) or dislocated to the vicinity of the inguinal canal (Fig. 1J, arrow); (*ii*) a TEC located outside and another inside the abdominal cavity (Fig. 1K); or (*iii*) both TECs located within the abdominal cavity, dorsally arranged below the kidneys (Fig. 1L–M).

The TEC position in the emballonurid bats was unique, with each cauda epididymis always externally located in the lateral of the base of the penis (parapenial), within a subcutaneous pouch (Fig. 1O), whereas the testes varied in position from the parapenial (Fig. 1O) to an intra-abdominal position (Figs. 1P–Q). When the testes were externally located, the epididymis made a loop around them (U-shaped), with the caput and cauda epididymis close to each other (Fig. 1O). When within the abdominal cavity, the testes were located behind the prostate complex, arranged caudally to the kidneys (Fig. 1P–Q), and the caput epididymis was joined with the testes. Additionally, in these cases, the corpus epididymis elongates a great deal (Fig. 1Q), and the cauda epididymis remained enclosed in the pouch. The maintenance of the cauda epididymis position is due to the presence of suspensor ligaments (connective tissues) that attach it directly to the subcutaneous pouch. Despite the presence of an external pouch, we do not observe a true scrotum in these species (Fig. 1N).

We observed that the testes of all five vespertilionid species studied were external and permanently scrotal (Fig. 1R–U). The maintenance of their position is achieved by suspensor ligaments (connective tissues) that connect the prolonged cauda epididymis to the caudal skeleton and the juxtaposition of the cauda epididymis between the two interfemural membranes (uropatagium). The length of the cauda epididymis seems to vary seasonally and, at the apex of its elongation, it may be observed in the living animal (Fig. 1R, arrow).

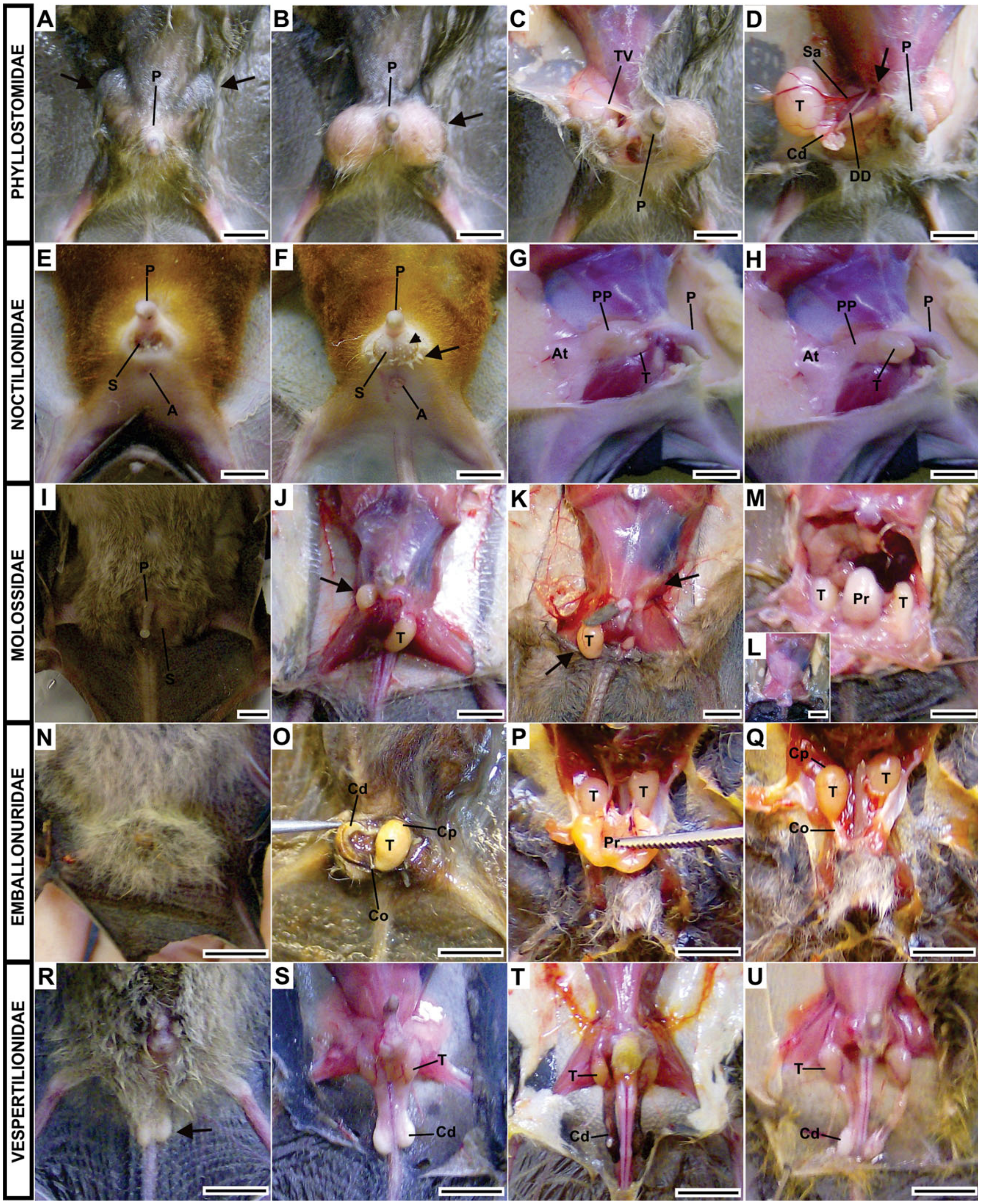

Figure 1 (Legend, overleaf.)

Morphology of the Testes and Epididymis

The gross anatomy of the testes varied little, being more rounded in the Phyllostomidae species (except *P. discolor*, which had bean-shaped testes) (Fig. 2A–F), oval in the Emballonuridae (Fig. 2M–O), and more elongated in the Molossidae (Fig. 2I–L), Noctilionidae (Fig. 2G–H), and Vespertilionidae species (Fig. 2P–T).

The epididymis in all species analyzed was formed by a long and highly convoluted tubule that can be divided into three main regions: (*i*) caput, the initial portion that is juxtaposed with the confluence of the rete testes network; (*ii*) corpus, the medial part of the epididymis; and (*iii*) cauda, the end portion of the epididymis that communicates directly with the deferens ducts (Fig. 2). In contrast, the size and positioning, especially of the cauda epididymis, varied considerably.

The epididymides of Phyllostomidae, Molossidae, and Noctilionidae were connected laterally to the testes for their entire extension. Phyllostomidae had a low degree of adhesion to the testis and a highly developed caudal region, which remained fully attached to the testis in all species (Fig. 2A–F), except in *G. soricina*, where the caudal region appeared to be free (Fig. 2D), and in *P. discolor*, where it formed a lateral elevation (Fig. 2E). Molossidae presented the highest degree of adhesion to the testis and the smallest size of the caudal region (Fig. 2I–L). Unlike the other families, Noctilionidae presented a further development of the caput than the cauda epididymis (Fig. 2G–H).

In Emballonuridae and in the four genera of Vespertilionidae analyzed, the epididymis showed a large elongation of the caudal region that was not attached to the testes. The size of this elongation varied individually and in some specimens was larger than the testis itself. In all of the emballonurid specimens analyzed, the cauda epididymis remained enclosed inside the scrotum, forming a loop around the testes (Fig. 2M–N), when it was external or had an elongated shape (Fig. 2O) when it was internal. However, in Vespertilionidae, it extended parallel to the caudal skeleton, with its position being maintained by filaments that connected it to the caudal skeleton and by juxtapositions between the anterior and posterior interfemural membranes (uropatagium). It was not directly attached to the testes, but, in most specimens, remained near them because both were covered with a highly pigmented fascia (black) (Fig. 2P–T).

Corporal and Testicular Weights, Gonad-Somatic Index, and Statistical Analysis

The corporal and testicular weights and the gonad-somatic index of all analyzed species and the statistical analysis are shown in Figure 3. Because of an inability to acquire data from *M. albescens* and *R. naso*, they are absent from the figure. The Phyllostomidae family presented the highest values and the largest variation in the corporal weights, presenting significant differences among all species (Fig. 3A). The Molossidae family also presented a large variation; however, their corporal weights were intermediate between those of the Phyllostomidae and Vespertilionidae families. In contrast, the Vespertilionidae species presented the lowest corporal weights values and the smallest variation between species. The corporal weight of *N. albiventris* closely approximates that of the medium-sized Phyllostomidae species and that of the large Molossidae species, whereas the corporal weight of *P. macrotis* approximates that of the Vespertilionidae species (Fig. 3A).

The Phyllostomidae family also presented the highest testicular weight values (Fig. 3B) and high gonad-somatic index values (Fig. 3C). *Phyllostomus discolor*, although not the largest species, presented the largest gonads and also the highest index. The smallest species of Phyllostomidae, *C. perspicillata* and *G. soricina*, also had the smallest gonads (Fig. 3B); however, their indexes were greater than those of larger species, such as *A. lituratus* (Fig. 3C).

The Molossidae family presented intermediate gonad weights, with the exception of *M. temminckii* (Fig. 3B). Again, we observed that smaller species have higher indexes (*M. temminckii* and *M. molossus*; Fig. 3C). Vespertilionidae presented the lowest gonad weights (Fig. 3B). *Lasiurus blossevillii* presented the lowest index, whereas *E. furinalis* and *M. nigricans* presented high indexes. *Noctilio albiventris* presented a testicular weight and gonad-somatic index near those of the molossids, and *P. macrotis* (Emballonuridae) presented values near those of the vespertilionids (Fig. 3A–B).

Histological Evaluation

The histological evaluation of all the analyzed species of Phyllostomidae (Fig. 4A–L), Noctilionidae (Fig. 4S–T),

Fig. 1. General arrangement of the testes–epididymis complex (TEC) of the analyzed bats. **A–D.** Phyllostomidae pattern, *Artibeus planirostris*. Note the dislocation of the TEC from the lateral of the abdomen (A, arrows) to the base of the penis (B, arrow), the thin and transparent tunica vaginalis that covers it (C) and the passage of the duct deferens (DD) and spermatic artery (Sa) through the inguinal canal (D, arrow). **E–H.** Noctilionidae pattern, *Noctilio albiventris*. Note the presence of a true scrotum (E, TEC outside the scrotum; F, TEC inside the scrotum) that presented numerous scattered short barbs (F, arrow-head) and long barbs at its periphery (F, arrow); also, note that the TEC may dislocated from the scrotum to an unusual pocket-like pouch (E-F and G-H) and that this pocket-like consisted of a thick layer of tunica vaginalis surrounded by large amounts of adipose tissue (G-H). **I–M.** Molossidae pattern, *Eumops glaucinus* (I), *Molossops temminckii* (J and L-M) and *Molossus molossus* (K). Note the presence of a true scrotum (I) and the migratory pattern of the TEC: (*i*) both external TECs (J); (*ii*) a TEC outside and another inside the abdominal cavity (K, arrows); and (*iii*) both TECs located within the abdominal cavity (L-M). When both are outside, they may stay in the scrotum (I) or be dislocated to the vicinity of the inguinal canal (J, arrow). **N–Q.** Emballonuridae pattern, *Rhynchonycteris naso* (N and P-Q) and *Peropteryx macrotis* (O). Note the absence of a scrotum (N); the loop of the epididymis around the testis when it was located externally, inside the subcutaneous pouch (O); and the elongation of the corpus epididymis (Q) when the testes were located within the abdominal cavity, behind the prostate complex, arranged caudally to the kidneys (P). **R–U.** Vespertilionidae pattern, *Myotis nigricans* (R-S), *Histiotus velatus* (T), and *M. albescens* (U). Note the considerable elongation of the cauda epididymis and the external, permanently scrotal testes of all species, which may be observed in the living animal (R, arrow). (A, anus; At, adipose tissue; Cd, cauda epididymis; Co, corpus epididymis; Cp, caput epididymis; DD, ductus deferens; P, penis; PP, pocket-like pouch; Pr, prostate complex; S, scrotum; Sa; Spermatic artery; T, testis; TV, tunica vaginalis). Scale bars = 0.5 cm.

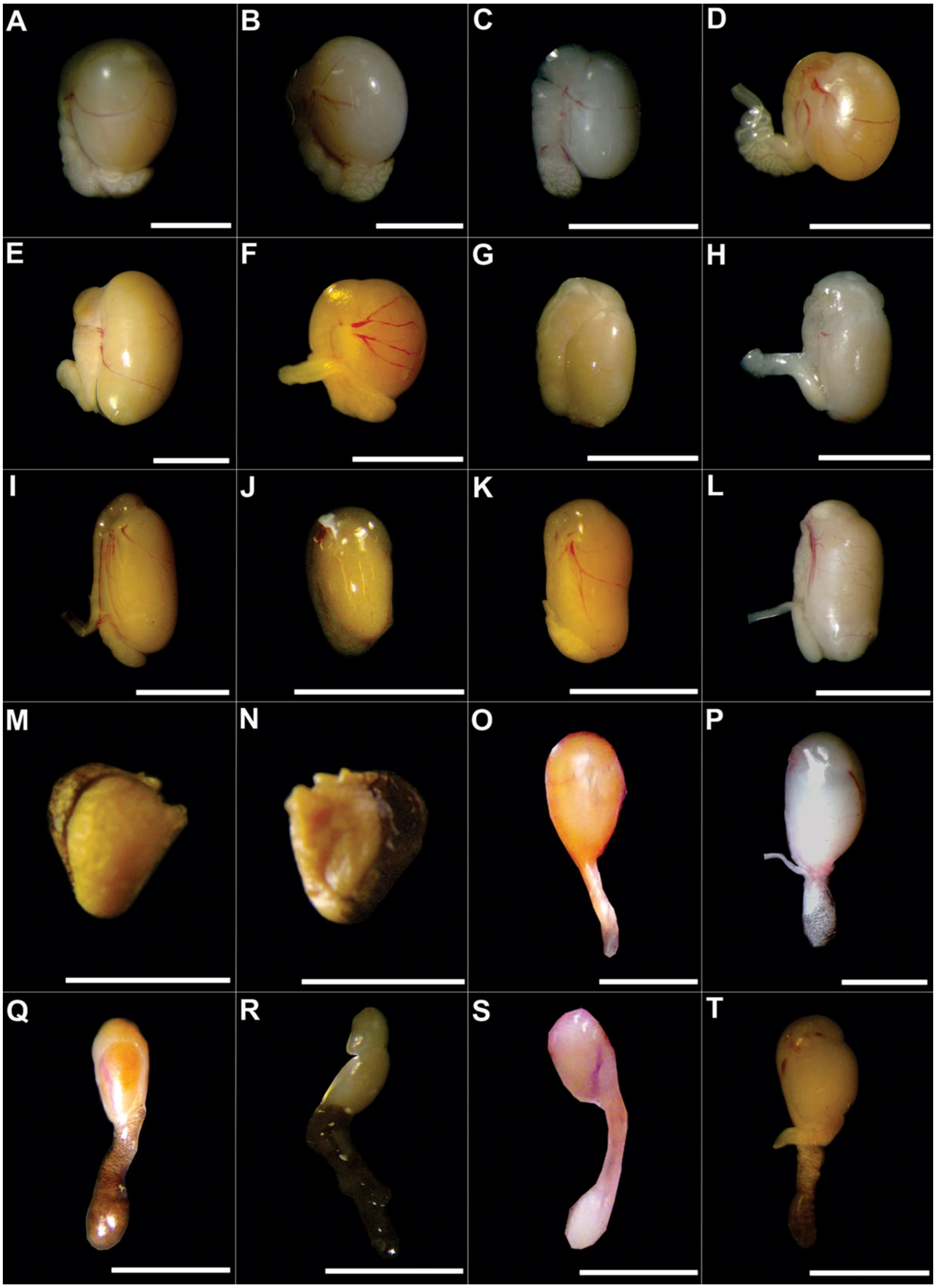

Fig. 2. Basic testicular and epididymal morphology of the 18 species analyzed. **Phyllostomidae: A.** *Artibeus lituratus*. **B.** *Artibeus planirostris*. **C.** *Carollia perspicillata*. **D.** *Glossophaga soricina*. **E.** *Phyllostomus discolor*. **F.** *Platyrrhinus lineatus*. **Noctilionidae: G-H.** *Noctilio albiventris*. **Molossidae: I.** *Eumops glaucinus*. **J.** *Molossops temminckii*. **K.** *Molossus molossus*. **L.** *Molossus rufus*. **Emballonuridae: M.** Ventral view of the gonad in *Peropteryx macrotis*. **N.** Dorsal view of the gonad in *Peropteryx macrotis*. **O.** *Rhynchonycteris naso*. **Vespertilionidae: P.** *Eptesicus furinalis*. **Q.** *Histiotus velatus*. **R.** *Lasiurus blossevillii*. **S.** *Myotis albescens*. **T.** *Myotis nigricans*. Scale bars = 0.5 cm.

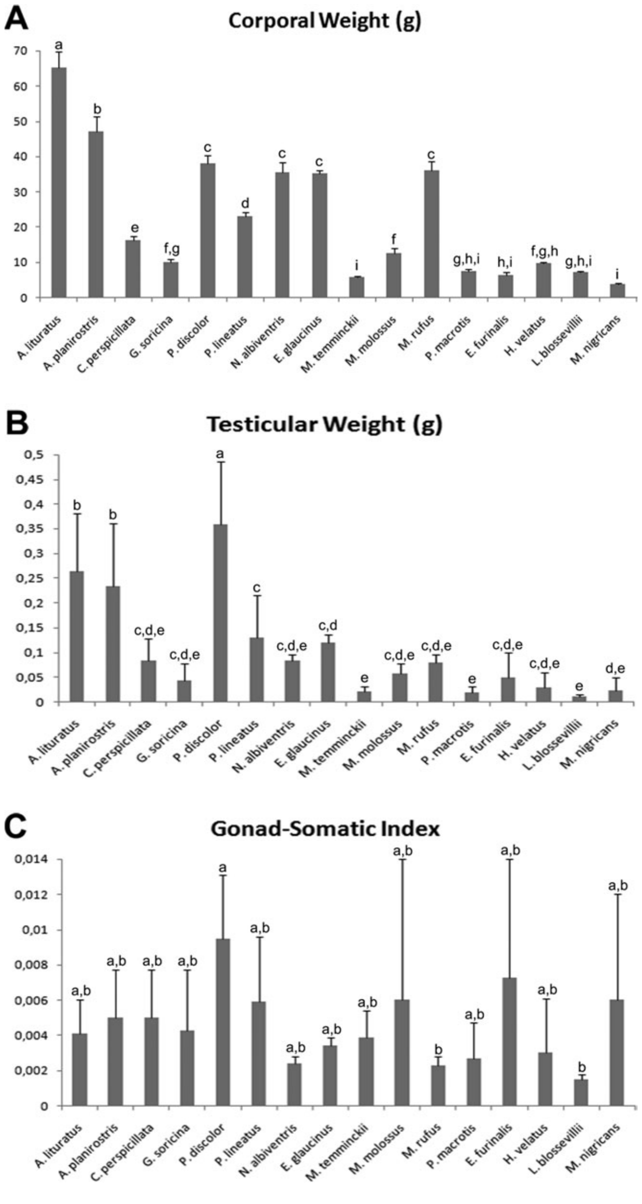

Fig. 3. Corporal (**A**) and testicular (**B**) weights and the gonad-somatic index (**C**) of all analyzed species. The different letters indicate significant differences ($P \leq 0.05$).

and Emballonuridae (Fig. 4U–Z) and of the molossid species *E. glaucinus* (Fig. 4M–N), *M. molossus* (Fig. 4O–P), and *M. rufus* (Fig. 4Q–R) corroborated the sexual maturity of their specimens and indicated that no cases of reproductive latency was presented in these species.

The analysis of Vespertilionidae showed a different pattern, with testicular regression and sperm retention in the cauda epididymis in the five species analyzed. In *E. furinalis*, we observed normal spermatogenesis in three specimens, regressed testes in two specimens (Fig. 5A) and sperm retention in the cauda epididymis in all specimens (Fig. 5B). In *H. velatus*, we observed normal spermatogenesis in two specimens, regressed testes in four specimens (Fig. 5C) and sperm retention in the cauda epididymis in all specimens (Fig. 5D). The testicular epithelium was regressed in all specimens of *L. blossevillii* (Fig. 5E) and *M. albescens* (Fig. 5G), with sperm retention in their cauda epididymis (Fig. 5F,H, respectively). In *M. nigricans*, we observed normal spermatogenesis in the majority of specimens, regressed testes in five specimens (Fig. 5I) and sperm retention in the cauda epididymis in all specimens (Fig. 5J).

Molossops temminckii also showed signs of testicular regression; we observed normal spermatogenesis in four specimens (Fig. 5K), testes in regression in two specimens (Fig. 5M) and regressed testes in two specimens (Fig. 5O). This pattern was similar to that observed in the vespertilionid species; however, it was different in that the sperm storage in the cauda epididymis was observed only in the specimens that were active (Fig. 5L) or in regression (Fig. 5N), with regressed individuals having only a few spermatozoa in their epididymides (Fig. 5P).

The period in which the specimens with regressed testes were captured varied from species to species: for *E. furinalis*, the captures were in December; for *H. velatus*, the captures occurred in July–August; for *L. blossevillii*, they occurred in November; for *M. albescens*, they occurred in June; for *M. nigricans*, they occurred in June and November; and for *M. temminckii*, they occurred in November–December.

DISCUSSION

Many species of Chiroptera share with other mammalian species, some reproductive characteristics that are observed commonly in the temperate species, such as enter into hibernation and present a regression in the seminiferous in which only spermatogonia and Sertoli cells can be observed (Racey, 1974; Fuentes et al., 1991; Lee et al., 2001; Kurohmaru et al., 2002; Lee, 2003; Lee and Mori, 2004). Possibly to adapt to the hibernation period, the mammals species have developed unique characteristics, such as prolonged sperm storage in the cauda epididymis in males and in the oviducts and/or uterine cornua in females, asynchrony between spermatogenesis and the mating period and late ovulation, fertilization and implantation in the female reproductive tract (Anand-Kumar, 1965; Racey, 1979; Rasweiler, 1993; Crichton and Krutzsch, 2000; Lee et al., 2001; Encarnação et al., 2004; Sharifi et al., 2004, Beguelini et al., 2009, 2011).

Although neotropical bats do not have a hibernation period, many of their reproductive characteristics vary interspecifically. According to Krutzsch (2000), the location of the TEC varies interfamiliarly and interspecifically and may also vary seasonally or daily. According to the authors, the position can be classified into four classes: (*i*) permanently abdominal; (*ii*) permanently inguinal or scrotal; (*iii*) migratory, in which they migrate (seasonally or daily) from the abdomen into the scrotum via the inguinal canal; and (*iv*) external, where they remain outside the abdominal cavity.

In the present study, we observed variation in the positioning of the TEC among families. We observed that Phyllostomidae and Noctilionidae have external TECs, with their position varying from the base of the penis to the vicinity of the inguinal canal in phyllostomids and from the vicinity of the inguinal canal to the scrotum in noctilionids. Emballonuridae and Molossidae presented

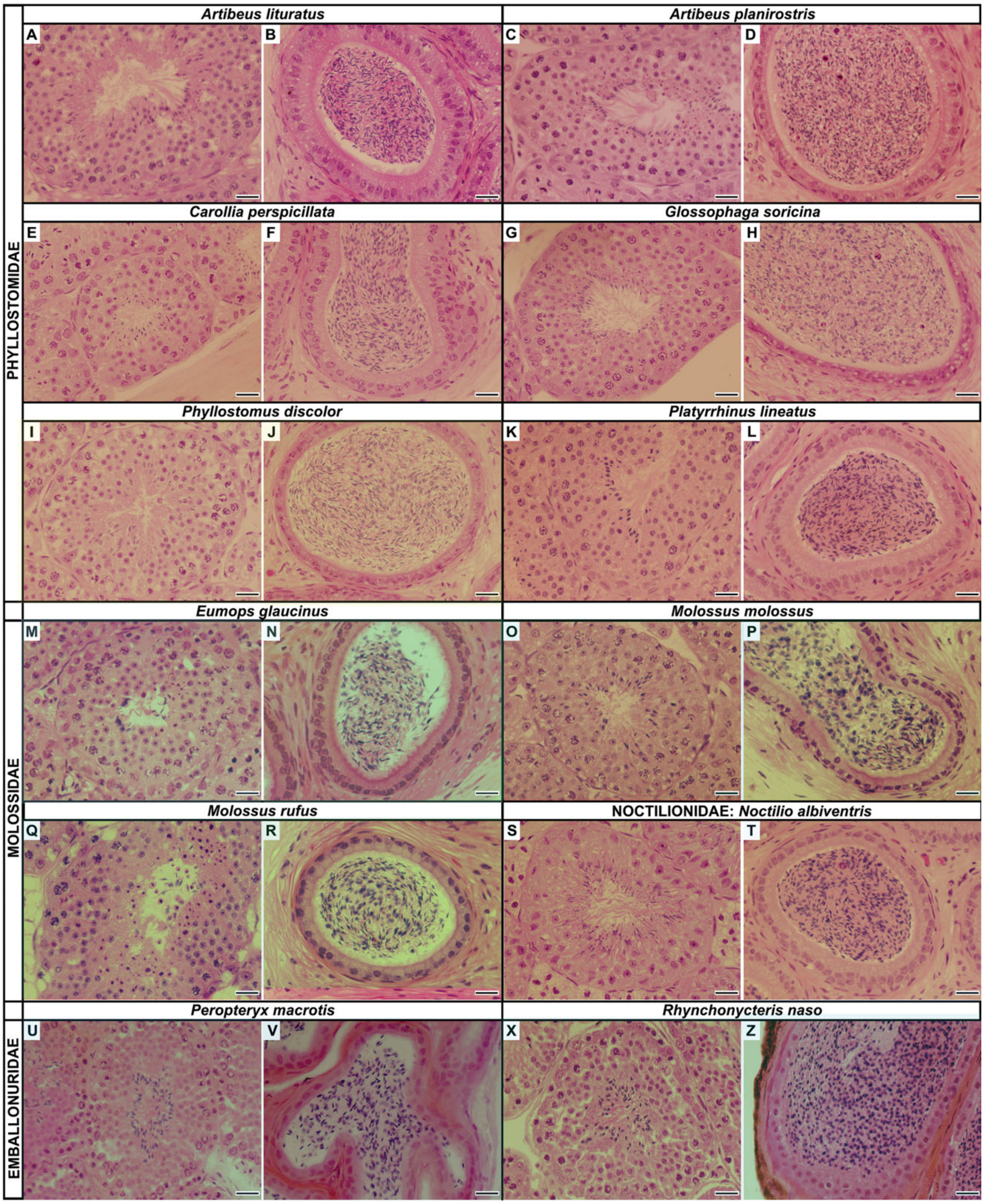

Fig. 4. General arrangement of the testicular and cauda epididymis tissues stained with hematoxylin eosin. **Phyllostomidae**: *Artibeus lituratus* (**A-B**); *Artibeus planirostris* (**C-D**); *Carollia perspicillata* (**E-F**); *Glossophaga soricina* (**G-H**); *Phyllostomus discolor* (**I-J**); *Platyrrhinus lineatus* (**K-L**). **Molossidae:** *Eumops glaucinus* (**M-N**); *Molossus molossus* (**O-P**); *Molossus rufus* (**Q-R**). **Noctilionidae**: *Noctilio albiventris* (**S-T**). **Emballonuridae**: *Peropteryx macrotis* (**U-V**); *Rhynchonycteris naso* (**X-Z**). Note the active pattern in the testes and the sperm storage in the cauda epididymis of all species. Scale bars = 20 μm.

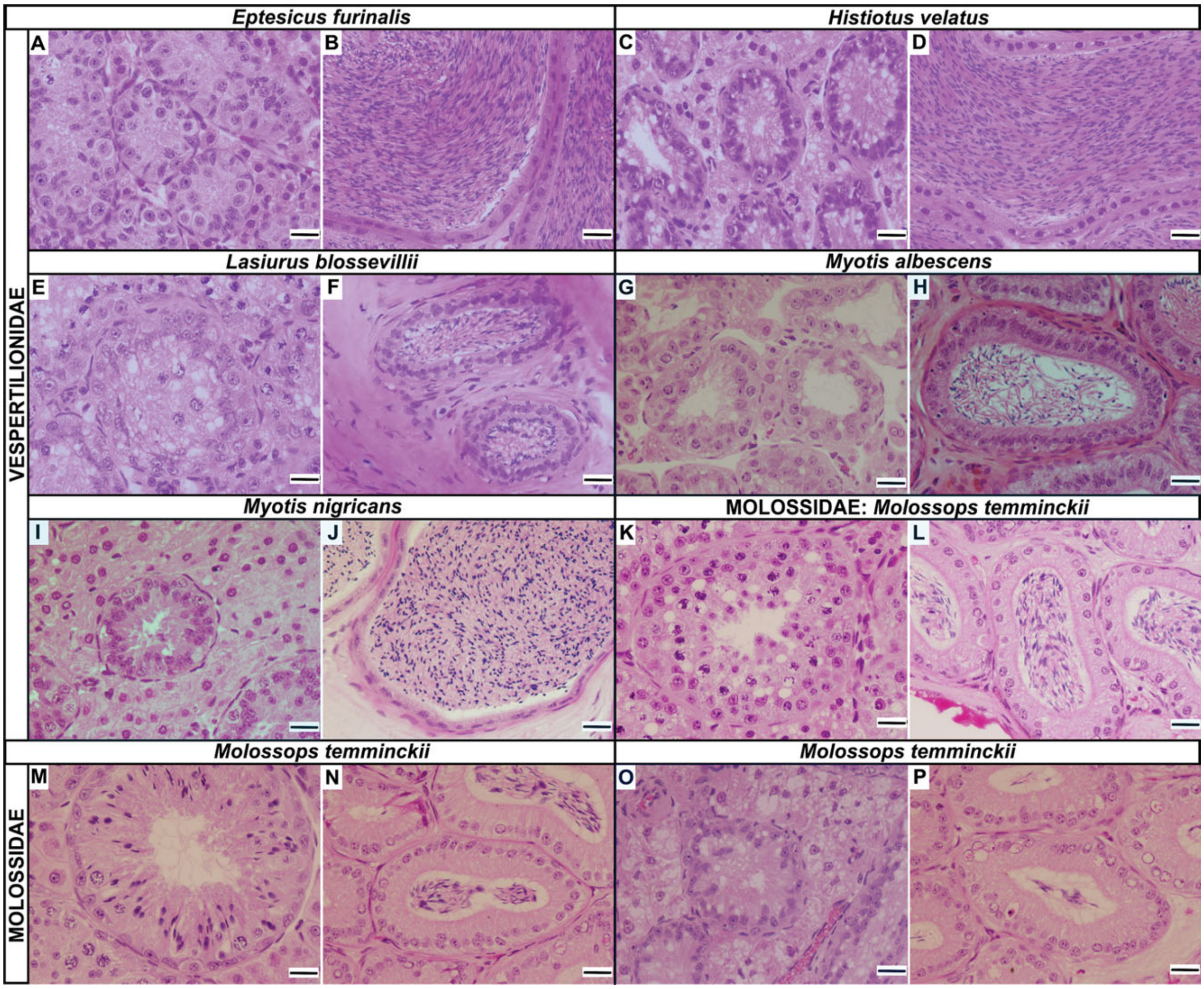

Fig. 5. General arrangement of the testicular and cauda epididymis tissues stained with hematoxylin eosin. **Vespertilionidae**: *Eptesicus furinalis* (**A-B**); *Histiotus velatus* (**C-D**); *Lasiurus blossevillii* (**E-F**); *Myotis albescens* (**G-H**); *Myotis nigricans* (**I-J**). **Molossidae:** *Molossops temminckii* (**K-P**). Note the testicular epithelium regressed in *E. furinalis* (**A**), *H. velatus* (**C**), *L. blossevillii* (**E**), *M. albescens* (**G**) and *M. nigricans* (**I**) with sperm storage in the cauda epididymis (**B**, **D**, **F**, **H** and **J**, respectively), and the active (**K**), in regression (**M**) and regressed (**O**) testicular epithelium of *M. temminckii*. Scale bars = 20 μm.

migratory TEC, and Vespertilionidae presented permanently scrotal TECs, with the scrotum enclosing only the testes and the caput and corpus epididymis.

These data from Phyllostomidae are similar to those described by Crichton and Krutzsch (2000) in *Macrotus californicus* and by Krutzsch and Nellis (2006) in *Brachyphylla cavernarum*, but differ somewhat from the findings of Orsi et al. (1990) in *Desmodus rotundus*, in which the TEC can migrate within the abdomen at will. Thus, despite the small number of species analyzed, we propose that the preferential localization of the Phyllostomidae TEC is external, outside the abdomen, near the crest of the pubis, lateral to the base of the penis (parapenial), or near the vicinity of the inguinal canal, with this dislocation subject to the control of the animal. Although the intra-abdominal condition was not observed in this study, it cannot be neglected in relation to other species of this family. Similarly, the observation of the dislocation of the TEC of *N. albiventris* from the pocket-like pouch to the scrotum agrees with the observations of Dunn (1934).

Migratory TECs in Molossidae were also observed in *Mormopterus planiceps* (Krutzsch and Crichton, 1987), *Tadarida brasiliensis mexicana* (Krutzsch et al., 2002), *T. condylurus*, *T. pumila* (Happold and Happold, 1989), and *T. hindei* (Crichton and Krutzsch, 2000). In all these species and in all species studied herein, we observed that the TEC might migrate seasonally from an intra-abdominal to an external position. We also note that, as observed in *T. condylurus* (Mutere, 1973; Happold and Happold, 1989) and in the species studied herein, the testicular position (intra-abdominal or external) did not provoke a significant variation in sperm production, differing from *T. hindei* (Marshall and Corbet, 1959), which intra-abdominal testes tend to be regressed.

Elongation of the cauda epididymis of vespertilionid bats was also observed in *Pipistrellus kuhlii* (Sharifi et al., 2004), *P. pipistrellus* (Racey and Tam, 1974), *P. subflavus* (Krutzsch and Crichton, 1986), *Myotis daubentonii* (Encarnação et al., 2004), *Corynorhinus mexicanus* (León-Galván et al., 2005), and *Neoromicia nanus* (Van der Merwe and Stirnemann, 2007), all of which are hibernating vespertilionids. Thus, we conclude that most vespertilionid bat genera already studied, including the four nonhibernating genera analyzed herein, present this elongation. A similar elongation of the cauda epididymis of *P. macrotis* was also observed in other species of emballonurid bats such as *Taphozous georgianus* (Jolly and Blackshaw, 1988).

The variation in the positioning of the testes and epididymis of eutherian mammals seems to be linked to a balance between the production, capacitation, and storage of spermatozoa (Bedford, 1978, 2004, 2008). The exposure of the testes to deep body temperatures (intra-abdominal condition) seems to directly influence the sperm production; however, it is not incompatible with normal spermatogenesis. Although body temperature does not suppress sperm maturation in the epididymis, it dramatically influences the storage capacity and the support of sperm viability and maturation in the cauda epididymis (Bedford, 2008).

It is known that the sperm production is directly correlated with the size of the testis (Short, 1997; Bedford, 2008) and to the number of germ cells nursed by each Sertoli cell in the seminiferous epithelium (Russell and Griswold, 1993; Hermo et al., 2010a,b). According to several authors, the lower epididymal temperatures facilitate sperm maturation and storage (Foldesy and Bedford, 1982; Djakiew and Cardullo, 1986; Jolly and Blackshaw, 1988), thereby clarifying that the positioning of the testes and epididymis directly influences the reproductive success of each species, with their positioning representing a balance between sperm production and storage. Optimizing sperm storage may enable lower sperm production by the testis without compromising male fecundity (Bedford, 2008), while saving energy in small animals that show great metabolic stress. In the same way, it seems that the sperm storage serves to ensure that adequate amounts of sperm are available at the peaks of reproductive activity, mainly in species that form harems.

The elongation of the cauda epididymis in Emballonuridae and Vespertilionidae was associated with the small size of their testes and was contrasted by the absence of elongation in others species (Molossidae, Phyllostomidae and Noctilionidae families). The fact that these species have larger testes seems to corroborate the trade-off between sperm production and storage.

Our data show similarities between the phyllostomid and noctilionid bats. This fact is not surprising because they belong to the same superfamily and present many other very similar features, such as characteristics of the female reproductive system and function and of early developmental biology (Bleier, 1975; Rasweiler, 1972, 1978; Rasweiler et al., 2011).

Male Reproductive Activity

The absence of cases of reproductive latency (regressed testes) in the six species of Phyllostomidae corroborated the observations of others authors: Hollis (2005) described the presence of scrotal males and pregnant females of *A. planirostris* in many months and predicted that this species may be able to produce young at any time throughout the year; Costa et al. (2007) described a similar pattern in *P. lineatus*; and Cloutier and Thomas (1992), Alvarez et al. (1991), and Kwiecinski (2006) observed a bimodal polyestry with great reproductive activity during the year for the species *C. perspicillata*, *G. soricina*, and *P. discolor*, respectively. However, in contrast to the postulation by Oliveira et al. (2009), the presence of a period of gonadal regression in *A. lituratus* was not observed.

Similarly, other studies also indicated the absence of regressed testes in *E. glaucinus* (Best et al., 1997) and *M. molossus* (Fabián and Marques, 1989), but no clear literature is available for *M. rufus* and *N. albiventris*.

Studies of the reproductive cycle of emballonurid bats are scarce for neotropical species. Plumpton and Jones Jr. (1992) postulated that the number of pregnancies per *R. naso* female of ranges from 0 to 2 per year, and Yee (2000) described that *P. macrotis* breeds during almost the entire year. These data along with our own observations suggest that both species may have active males during almost the entire year.

The Case of Vespertilionidae

Our data indicate the presence of a reproductive period of quiescence (regression of the seminiferous tubules) and sperm retention in the five species of neotropical Vespertilionidae analyzed that is very similar to that observed in hibernating bats. Other studies performed at latitudes, such as those by Van der Merwe and Rautenbach (1987) in *Nycticeius schlieffenii* at a latitude of 22°S, Van der Merwe and Rautenbach (1990) in *Pipistrellus rusticus* at a latitude of 24°S, and Van der Merwe and Stirnemann (2007) in *Neorimicina nanus* at a latitude of 25°S, also demonstrated a period of testicular regression in these vespertilionid bats.

These data suggest that, although the species analyzed are tropical species that do not present a prolonged period of torpor (hibernation period), they still maintain a period of seminiferous tubule regression and sperm retention/storage, a trait probably retained from the common temperate vespertilionid ancestor. The maintenance of these traits in a tropical environment is interesting and is worthy of further study.

The Case of *M. temminckii*

Although no clear data were observed for the reproductive patterns of *M. temminckii*, Krutzsch and Crichton (1987) and Krutzsch et al. (2002) observed a period of testicular regression in the molossid bats *Mormopterus planiceps* and *Tadarida brasiliensis mexicana*. The former study occurred in Southeast Australia (36°S) and the latter was in Texas (USA ~ 30°N). Thus, these data indicated that the occurrence of testicular regression in molossid bats might occur in both hemispheres. However, the fact that only one species of the four Brazilian molossid bats analyzed presented this pattern was intriguing and deserves future investigation.

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