Shelters, Morphometry and Reproduction of *Histiotus velatus* (Chiroptera: Vespertilionidae)

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ABSTRACT – *Histiotus velatus* (Vespertilionidae) is an important synanthropic species, whose shelters and conditions are essential for its reproductive success. This study aimed was to diagnose the adaptive capacity of *H. velatus* in shelter occupation and reproductive patterns. A total of 47 individuals (F = 32/M = 15) of *H. velatus* deposited in a scientific collection were analyzed. Catalog sheets were examined for data referring to the origin of the animals, shelters and supposed foraging sites. The analysis of reproductive biology was based on morphological and morphometric aspects of the reproductive system. The results indicated that colonies of *H. velatus* generally do not exceed 15 animals per shelter. These refugia ranged from 2.5 to over 6 m in height from ground level. For the reproductive aspect, it was observed that 64% (n = 7) of the pregnant females presented the development of twins. For males, gamete storage and reproductive activity are continuous with a short period of interruption during the year. Such information is of great relevance for the establishment of policies for the conservation and management of *H. velatus*.

Keywords: Bats; synanthropic species; adaptation; conservation.

Abrigos, Morfometria e Reprodução de *Histiotus velatus* (Chiroptera: Vespertilionidae)

RESUMO – *Histiotus velatus* constitui uma importante espécie sinantrópica, cujos abrigos e condicionantes são imprescindíveis ao seu sucesso reprodutivo. O objetivo deste estudo foi diagnosticar a capacidade adaptativa de *H. velatus* na ocupação de abrigos e padrões reprodutivos. Um total de 47 indivíduos (F = 32/M = 15) de *H. velatus* depositados em coleção científica foram analisados. Fichas catalográficas foram examinadas quanto aos dados referentes à origem dos animais, aos abrigos e aos supostos locais de forrageamento. A análise da biologia reprodutiva foi baseada nos aspectos morfológicos e morfométricos do aparelho reprodutor. Os resultados indicaram que as colônias de *H. velatus* geralmente não ultrapassavam 15 animais por abrigo. Esses refúgios variaram de 2,5 até mais de 6 m de altura a partir do nível do solo. Para o aspecto reprodutivo, foi observado que 64% (n = 7) das fêmeas grávidas apresentaram o desenvolvimento de gêmeos. Para machos, a estocagem de gametas e a atividade reprodutiva são contínuas, com um curto período de interrupção durante o ano. Tais informações são de grande relevância para o estabelecimento de políticas de conservação e manejo de *H. velatus*.

Palavras-chave: Morcegos; espécie sinantrópica; adaptação; conservação.

Dormideros, morfometría y reproducción de *Histiotus velatus* (Chiroptera: Vespertilionidae)

RESUMEN – *Histiotus velatus* (Vespertilionidae) es una importante especie sinantrópica, cuyos refugios y condiciones son esenciales para su éxito reproductivo. El objetivo de este estudio fue diagnosticar la capacidad adaptativa de *H. velatus* en la ocupación de refugios y patrones reproductivos. Se analizaron un total de 47 individuos (F = 32/M = 15) de *H. velatus* depositados en una colección científica. Se examinaron hojas de catálogo en busca de datos referentes al origen de los animales, refugios y supuestos sitios de alimentación. El análisis de la biología reproductiva se basó en aspectos morfológicos y morfométricos del sistema reproductivo. Los resultados indicaron que las colonias de *H. velatus* generalmente no superan los 15 animales por albergue. Estos refugios oscilaron entre 2,5 y más de 6 m de altura desde el nivel del suelo. Para el aspecto reproductivo se observó que el 64% (n = 7) de las hembras preñadas presentaron el desarrollo de mellizos. Para los machos, el almacenamiento de gametos y la actividad reproductiva son continuos con un breve período de interrupción durante el año. Dicha información es de gran relevancia para el establecimiento de políticas de conservación y manejo de *H. velatus*.

Palabras clave: Murciélagos; especies sinantrópicas; adaptación; conservación.

Introduction

Bats comprise the world's second largest order of mammals with 1447 species, second only to rodents according to the Mammal Diversity Database (MDD, 2023). In Brazil, 181 species representing nine families are recognized (Garbino et al., 2020). Of the 21 families of bats distributed worldwide, the Vespertilionidae constitute the most diverse in species with 490 taxa, with about 25 registered in Brazil (Abreu et al., 2020). The genus *Histiotus* consists of eight species (Aramayo et al., 2019), five of which occur in Brazilian territory (Garbino et al., 2020). Being endemic to South America, these animals are easily recognized by their long ears (Feijó et al., 2015). This study focused on *H. velatus*, whose distribution in the limits of Brazil includes the Center-East and South regions (Vicente, 2005; Welter, 2009).

The species, in addition to being found in primary forests, is also common in shelters of an anthropic nature, mainly in human buildings (Vicente, 2005; Reis et al., 2013). The conditions of the diurnal shelters play an important role regarding the ecological and evolutionary aspects of the species. Such places provide for mating, hibernation, feeding of young, social interactions, protection against inclement weather and predators (Kunz, 1982; Vicente, 2005; Bernardi et al., 2009; Guedes and Vincent, 2012).

H. velatus is a species with an average body size of 62 mm, forearm between 42 and 50 mm, and body weight of 11 grams (Reis et al., 2013). They are insectivorous animals (Emiliano et al., 2017), and reinforces the importance of ecosystem services, given that bats from this trophic guild can especially suppress and control insect biomass (Moiseienko and Vlaschenko, 2021). However, there are few works available on ecology, behavior and interspecific associations, and exploration of refuges that refer to *H. velatus*. Available data on this species were reported by Vicente (2005) and Reis et al. (2011). For the conservation status, data is deficiently listed in the International Union for Conservation of Nature (González et al., 2021), highlighting the importance of studies of ecological character and aspects of the reproductive biology of the species.

Even though neotropical species do not have a hibernation period, many of their reproductive characteristics slip into Nearctic representatives and, according to Beguelini et al. (2013), vary seasonally and interspecifically. Among the bat families, Vespertilionidae is best known in terms of reproductive biology, with regard to species from North America and Europe (Mottin et al., 2018), and reiterates the need to understand Neotropical representatives. In view of the above, the main objective of this work is to reveal morphological and morphometric aspects of *H. velatus*, adding information relevant to reproductive biology and patterns of exploitation of diurnal shelters for the species in question.

Material and Methods

Obtaining samples

We examined 47 specimens of *H. velatus*, 15 males and 32 females. These animals were divided into 32 adults, 13 juveniles, and two fetuses. 14 of the females were pregnant, and seven were non-pregnant. The material consists of specimens deposited in the collection of the Laboratory of Chiropterology, Universidade Estadual Paulista (UNESP), São José do Rio Preto, São Paulo, Brazil. The samples were collected in different locations in the Southeast and South of Brazil (Figure 1), in different seasonal periods and in different chronology, since the 1960s. The individuals were collected using mist nets set up next to daytime shelters, in flight routes according to the catalog sheets of the aforementioned Collection.

The animals were anesthetized and sacrificed and during fixation, the mouth of which was kept open, allowing the examination of the dentition. The animals were mounted in the supine position. Fixation was performed with 10% formaldehyde, injected into the general cavity and cervical region, after which they were preserved and maintained in 70° G.L. alcohol. Each copy is registered under the collection protocol number (General Registry – GR) and the respective data in noted in the general catalog and in the official complementary sheets of the collection.

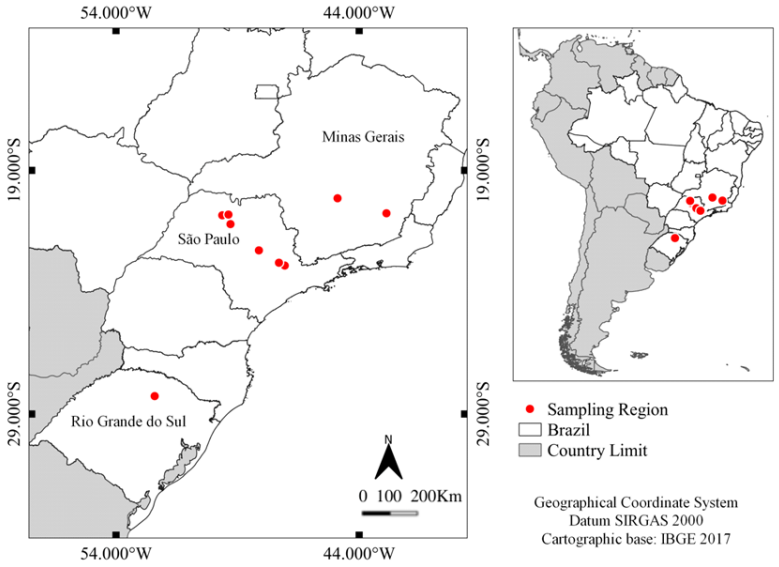

Figure 1 – Geographical distribution of the studied sample of *H. velatus*.

Examined specimens (47: 32 females, 15 males) DZSJRP 2325, 2403, 2416, 2424, 2537, 2533, 2554, 2566, 2623, 2958 a 2961, 3039, 3086, 3131, 3230, 3231, 3322, 3340, 3365, 3504, 3580, 3686, 4506, 4781, 4783, 4811, 10153, 10400 a 10403, 12484 a 12491, 12586, 14034, 14902 a 14904, 14924.

Reproductive biology

The analysis of reproductive biology was based on the morphological and morphometric aspects of the reproductive system. The level of development, activity and disposition of the mammary glands were verified, along the mammary crest or milky line, as a means of diagnosing the development and activity status of Young, nulliparous adult females or already in reproductive activity. Morphometric and morphological data of the other structures of the reproductive system (uterus and testes) of individuals at different stages of development were also gathered. The shape of the uterine structure was also considered for categorizing the evolutionary status of the organ. The format and characteristics observed allowed the attribution of a category, following the recommended reference for mammals and based on the constant in Weichert (1979).

To verify the preferential periods of reproduction, data related to each specimen corresponding to estrus, early pregnancy, presence of infants or developed breasts (lactation) and number of pups in the uterus were checked for the females in the sample. The metric values of the uterine horns refer to the diameter (largest width). In specimens with already developed embryos, measurements were taken related to the total length of the embryo and, when possible, the length of the forearm. The total length of the embryo was taken from the top of the head to the posterior end of the trunk, keeping the individual in the intrauterine position he occupied. Forearm measurements were performed according to the usual way for studies in adult specimens: from the proximal to the distal radioulnar end.

In males, measurements were taken regarding the length and width of the testis (usually the left) and of the epididymis (tail of the epididymis of the respective testis). All measurements were performed with a caliper and expressed in mm, with approximations to 0.1 mm. At the same time, photographic documentation of the male and female reproductive system was carried out, in closer approximation with the aid of a Leika MZ16 stereoscopic equipment.

Data analysis

The values obtained for the forearm were treated statistically and demonstrated as mean±standard deviation. Normality was tested by the Shapiro-Wilk test and then the difference in forearm size between males and females was verified using Students t test. A value of $p < 0.05$ was established as statistical significance. The test was run on Bio Stat 3.0. The morphometric variables of the reproductive system were expressed in averages or percentages.

Result

Characteristics of shelters and population sizes

Catalog data from 13 shelters were observed. The number of individuals registered in the respective resting places did not exceed 15. The data recorded in the catalog reveal that most specimens explore refuges in buildings, ranging from 2.5 to more than 6 m in height from the ground level. The structure of the explored shelters can also be variable, including wooden ceilings or the spaces between slabs, columns and tiles. When the distance between the base and the tiles is very small, the specimens can adopt a horizontal posture and remain in the shelters even in places where the movement of people is constant, as long as they are not directly disturbed. This fact is consistent with the synanthropic nature of *H. velatus*.

An unusual shelter recorded was a wicker lamp, from which eight females were collected. Among the females collected, there were five post-lactating, two nulliparous and one non-pregnant adult. The presence of post-lactating females (end of the reproductive period) grouped with nulliparous juveniles demonstrates the typical formation of a maternity colony. The record of use the natural shelter was recorded in a hollow tree, where a male of *H. velatus* was collected.

Morphometry of forearm relationships

The adult individuals in the sample had forearm measurements that ranged from 44.9 to 50.8 mm, with a general mean of 47.21 mm (SD = 1.36). The values obtained and treated for the forearm showed that the mean for females was 47.43 mm and for males 46.78 mm, with standard deviations of 1.45 and 1.10 respectively (Table 1). Individuals considered juveniles (non-ossified metaphyses) have forearm measurements on average of 44.66 mm (SD = 2.93), ranging from 38.2 to 48.1 mm. The highest mean values for the forearm were recorded for the female sample, however the lowest extreme value (44.9) also occurs in this set. For this collection of adult animals, males and females did not differ statistically in terms of forearm size (t = -1.48; $p = 0.1697$).

Table 1 – Data referring to the measured values of the forearm, expressed in mm.

Forearm analysis	Mean±SD
Fetus (n = 2)	12.15±0.07
Juveniles (n = 13)	44.66±2.93
Adult males (n = 11)	46.78±1.10
Adult females (n = 21)	47.43±1.45

Reproductive biology

In this species there is a pair of breasts, located in the cranial-thoracic region of the milky line (Figure 2A). The females of this species present uterine morphology compatible with an intermediate condition, between the evolutionary forms called bicornuate and bipartite, in which both horns and ovaries are functional. The occurrence of twin pregnancy (Figure 2B and C) totaled 36% of the females analyzed. Among the other representatives studied, 14% were pregnant, 18% were pregnant with only 1 embryo and 32% were nulliparous. Embryonic development occurs differently in each of the uterine horns, from the early stages to the term gestational condition.

When considering only pregnant females in the sample, the percentage of twin pregnancy rises to 64% compared to 36% of single pregnancy. In one case, both fetuses had identical measurements of body and forearm length, indicating full morphological development.

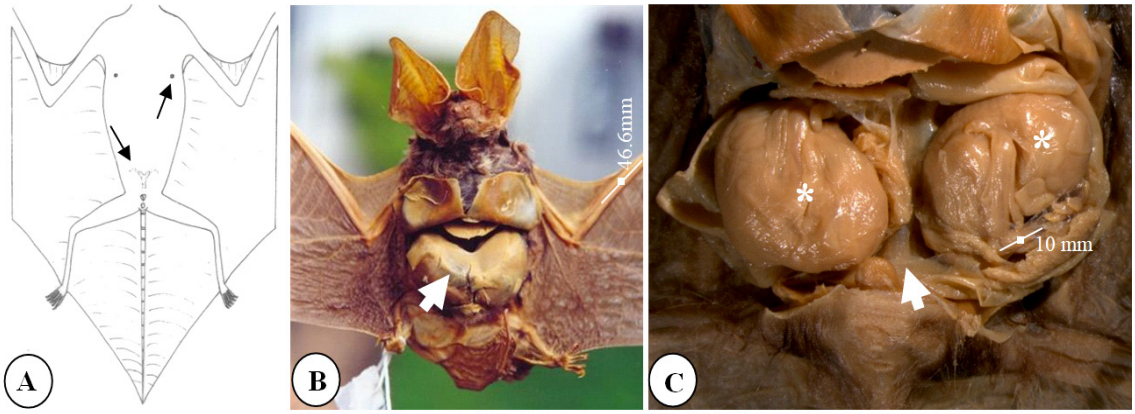

Figure 2 – Representation of the female reproductive system and pregnancy of twins of *H. velatus*. A) the arrows indicate the breasts in the cranial thoracic region and the position and shape of the uterus; B) General view of a pregnant female that, indicated by the arrow, the expanded uterine horns can be observed; C) Fetuses (*) in intrauterine position (arrow).

The morphological data of males indicate that the tail of the epididymis, in most individuals, is quite elongated, voluminous, with conspicuous pigmentation that can be seen through the translucent uropatagium (Figure 3).

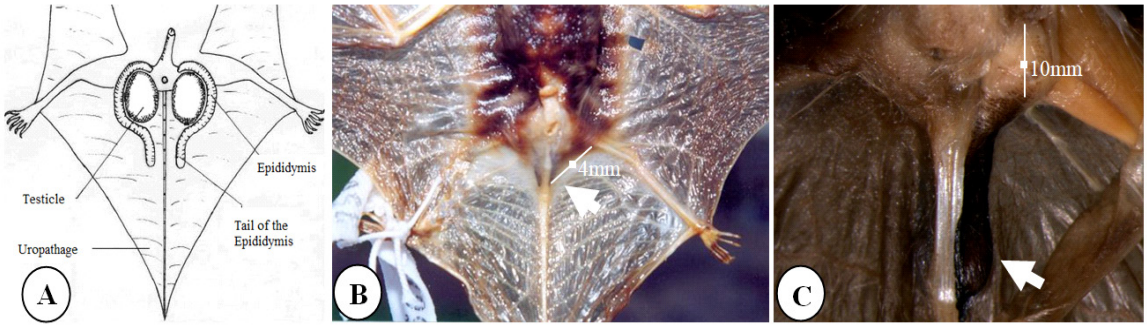

Figure 3 – Middle-posterior region of the trunk, pelvis and posterior appendages: A) standard schematic model of the location and constitution of the male genitalia; B and C) pigmentation and developmental levels of the testis and epididymis of *H. velatus*. Note in B that the tail of the epididymis can be seen through transparency, as indicated by the arrow.

The measurement of the length and width of the testes is seasonally variable. Measures between 2.4 and 4.3 mm were recorded for length and between 1.5 and 2.6 mm for width. The average of the values reported for the length of the testes of adult males was 3.61 mm and the width was 2.06 mm.

Discussion

In this study, it was observed that *Histiotus velatus* is a gregarious species that preferentially explores refuges located between 2.5 to about 6 m in height from the ground level, in human buildings. The grouping of post-lactating females and juveniles observed in this study indicated maternity colonies, and their occurrence in urban areas and usual proximity to men gives the species a synanthropic character (Vicente, 2005; Bernardi et al., 2009). According to Pacheco et al. (2010) colonies of this species range from one to forty individuals and with shelter fidelity that exceeds three years in urban areas.

The data revealed in this work point to the cost/benefit of the reproduction strategies of *H. velatus*. The literature on the reproductive aspects of this species is quite scarce and the most systematic work so far is that of Peracchi (1968 in Reis et al., 2011). In 1968, Peracchi describes observations made in three different shelters, located in the municipality of Itaguaí, Rio de Janeiro, Brazil. It has been reported that the reproductive period of *H. velatus* begins in mid-September and females with only one infant (Peracchi, 1968 in Reis et al., 2011). Here, in the present study, the reproductive onset is confirmed for this period, in addition, there were twins in pregnancy.

In *Lasiurus* species (Vespertilionidae) it is quite common to have two to five offspring per pregnancy and females have two pairs of breasts located in the thoracic region (Vicente, 2005; Vicente et al., 2012). In *H. velatus*, the presence of two pairs of breasts was not observed. According to Pough et al. (2008), the pairs of breasts are classified according to their location, in the different regions of the milky line. Generally, in bats, this variation is quite expressive and is consistent with the number of offspring produced per pregnancy.

The generation of two offspring followed by the lactation of just one, suggests energy investment that increases the success of an offspring and the nutritional return of the mother in postpartum condition, in view of the high energy demand of lactation. This suggestion was supported, in part, by Bradbury and Vehrencamp (1976) and corroborated by Garbino et al. (2021), who considered the synchronism of the peaks of feeding activities with embryonic development, a guarantee of survival of pregnant females, of increasing the viability of lactation and offspring development processes. Nevertheless, lactation requires much greater energy demand than pregnancy (Migula, 1969). The considerations presented here for *H. velatus* do not disregard the possibility of recording two infants, hitherto not observed (Vicente, 2005).

Added to the fact that, so far, females with two infants have not been recorded, suggests the viability of only one calf after delivery, however, the functionality of the ovaries and uterine horns (right and left) is definitely not an event isolated. Females from different locations and periods of the year were observed with this condition. The average values obtained for the uterine horns and testes, at different stages of development, serve as diagnostic parameters for the onset of sexual maturity in individuals, since no evidence of reproductive activity was recorded in juveniles. Other species of the Vespertilionidae family have already been documented for twin pregnancy. Examples include *Nyctalus noctula* (Knörnschild et al., 2007), the flat-headed bats *Tylonycteris pachypus* and *T. robustula* (Zhang et al., 2005) and the great brown bat, *Eptesicus fuscus* (Monroy et al., 2011), being one of the few species in which twin births are common.

Although the sample of this study consisted of specimens collected in different periods of the year, it is not uniformly represented in terms of monthly series, either by quantitative differences (number of individuals) from one series to another (in different months), or by sampling failures in some months of the year. However, considering the heterogeneity of age, the monthly samples allowed a very reliable estimate of birth rates and developmental time to be made. Many juveniles in a given month, obviously there were previous births at similar levels, and so on.

For the morphological aspects of males, elongation of the epididymis has also been observed in previous studies (Encarnação et al., 2004; León-Galván et al., 2005; Beguelini et al.,

2013). These characteristics possibly indicate the process of continuous gametogenesis, typical of the species. Beguelini et al. (2013) observed that elongation of the epididymal tail in Vespertilionidae was associated with the small size of their testes. In *Plecotus auritus*, for example, the size and shape of the tail epididymis appeared to be a better criterion for defining sexual maturity in this species (Entwistle et al., 1998). The main function of the epididymis is sperm maturation (Beguelini et al., 2015), in which the immobile cells (sperm) that leave the testes and fail to fertilize the oocytes transform into fully mature cells that have the ability to swim, recognize and fertilize eggs (Robaire et al., 2006).

Furthermore, the size of the testes seems to corroborate the relationship between sperm production and storage (Beguelini et al., 2013). The adjustment of reproductive patterns is variable according to latitude in many mammalian species, and the beginning of the annual reproductive cycle can be influenced by changes in photoperiod (Sadlier, 1969; Millar and Glover, 1973). The events of diapause or embryonic retardation ensure the birth of offspring at more favorable times of the year in terms of several factors, including food availability (Pough et al., 2008).

Considering morphological aspects of the gonads, in this work we identified an anatomical pattern in the male reproductive system of *H. velatus*, epididymal hyperpigmentation, which gives the organ a blackened color, visible through the thin skin of the uropatagium. The numerous types of pigments in animal tissues and membranes are generally not dissociated from light and heat intensity levels. Both melanin and carotenoid pigments are formed from the synthesis of proteins related to tyrosine production. Melanin is considered the main protective pigment against ultraviolet radiation and essential for animals in tropical regions (Silva, 2000).

The important phenotypic attribute in the epididymis in *H. velatus* seems to have an adaptive function, corresponding to the cytoprotection of gametes that are stored there, going through final maturation. However, future studies would be interesting to establish whether the other properties of melanization would still have a phylogenetic and evolutionary character along with the other taxonomic levels of bats. Future work efforts are still encouraged for the reproductive biology of *H. velatus*, especially to shed light on the twin pregnancy reported in the present work.

Conclusion

In this study, they presented characteristics of shelters, morphology and reproductive biology of *H. velatus*. This species has a great ability to adjust to environmental changes and explores a wide variety of shelters, especially those of an anthropic nature. More studies are needed, especially those focused on reproductive biology, extending other regions of occurrence of *H. velatus*, for a better understanding of the ecology and conservation of the species.

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