

## Artículo



# FIRST RECORD OF *Glyphonycteris sylvestris* THOMAS, 1896 (CHIROPTERA: PHYLLOSTOMIDAE: GLYPHONYCTERINAE) FOR ARGENTINA, WITH COMMENTS ON ITS KARYOTYPE

Mariano S. Sánchez<sup>1,2</sup>, Carolina A. Labaroni<sup>1,2</sup>, Francisco X. Castellanos Insuasti<sup>2</sup> and Diego Baldo<sup>1,2</sup>

<sup>1</sup>Instituto de Biología Subtropical (IBS)-nodo Posadas, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de Misiones (UNaM), Posadas, Misiones, Argentina.

[Correspondence: Mariano S. Sánchez <[mariannoseb.sanchez@gmail.com](mailto:mariannoseb.sanchez@gmail.com)>]

<sup>2</sup>Laboratorio de Genética Evolutiva, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones (UNaM), Posadas, Misiones, Argentina.

**ABSTRACT.** *Glyphonycteris* is a poorly known genus of bats that inhabit lowlands and forested mountains of Central and South America. This genus comprises three nominal species, with *G. sylvestris* being the most widely distributed species in South America. The karyological data previously available for this genus are restricted to those resulting from conventional staining techniques applied to a few specimens from Surinam. Here, we report the first record of *G. sylvestris* in Argentina extending its southern distribution. Moreover, our new locality represents the seventh record from the Atlantic Forests Biome and the first from Araucaria Moist Forest ecoregions. Our specimen overall exhibits the diagnostic characteristics described for the species except for some differences in the distribution of hairs on the wings and limbs. The fecal sample obtained from our specimen contained only seeds of *Piper aduncum*. The karyotype was  $2n = 22$  with a  $FN = 42$ ; the analysis of fluorochrome banding patterns in the chromosomal pericentromeric regions shows DAPI (4,6-diamidino-2-phenylindole) positive blocks in pairs 5 and 8, whereas pericentromeric regions stained with CMA<sub>3</sub> (Chromomicine A<sub>3</sub>) are positive in pairs 1, 2, 3, 4 and 11. Moreover, the interstitial region of chromosomes shows DAPI and CMA<sub>3</sub> positive blocks in pairs 1, 2, 3, 4 and 5. These karyological data are novel to *G. sylvestris*, contributing to the scarce knowledge of the genus. Thus, filling blanks of information and enabling further comparative chromosome analysis.

**RESUMEN.** Primer registro de *Glyphonycteris sylvestris* Thomas, 1896 (Chiroptera: Phyllostomidae: Glyphonycterinae) para Argentina, con comentarios sobre su cariotipo. *Glyphonycteris* es un género poco conocido de murciélagos que habita los bosques de tierras bajas y de montañas de Centroamérica y Sudamérica. Este género incluye tres especies nominales; donde *G. sylvestris* es la más ampliamente distribuida al sur de Sudamérica. Los datos de cariotipo para este género están restringidos a técnicas de tinción convencional realizadas sobre unos pocos especímenes de Surinam. En este trabajo se presenta el primer registro de *G. sylvestris* en Argentina extendiendo su distribución sur. Además, esta nueva localidad representa el séptimo registro para el Bioma del Bosque Atlántico y el primero para la ecorregión de los Bosques Húmedos de Araucaria. Nuestro espécimen exhibe los caracteres diagnósticos para la especie excepto por algunas diferencias en la distribución de los pelos en alas y miembros. La muestra fecal obtenida de nuestro espécimen solo contenía semillas de *Piper aduncum*. El cariotipo tuvo un  $2n = 22$  y  $FN = 42$ ; los patrones de bandeo con fluorocromos en la región pericentromérica muestra bloques de DAPI (4,6-diamino-2-fenilindol) positivo en los

pares 5 y 8, mientras que las regiones pericentroméricas teñidas con CMA<sub>3</sub> (Chromomicina A<sub>3</sub>) son positivas en los pares 1, 2, 3, 4 y 11. Además, en las regiones intersticiales de los cromosomas se observaron bloques DAPI y CMA<sub>3</sub> positivos en los pares 1, 2, 3, 4 y 5. Estos datos cariológicos son novedosos para *G. sylvestris* contribuyendo al escaso conocimiento del género. Por ende, llenan un vacío de información que permitiría futuros análisis cromosómicos comparativos.

**Key words:** Araucaria Moist Forest, Atlantic Forests, chromomicine, DAPI, diet, distribution.

**Palabras clave:** Bosque Húmedo de Araucaria, cromomicina, DAPI, dieta, distribución.

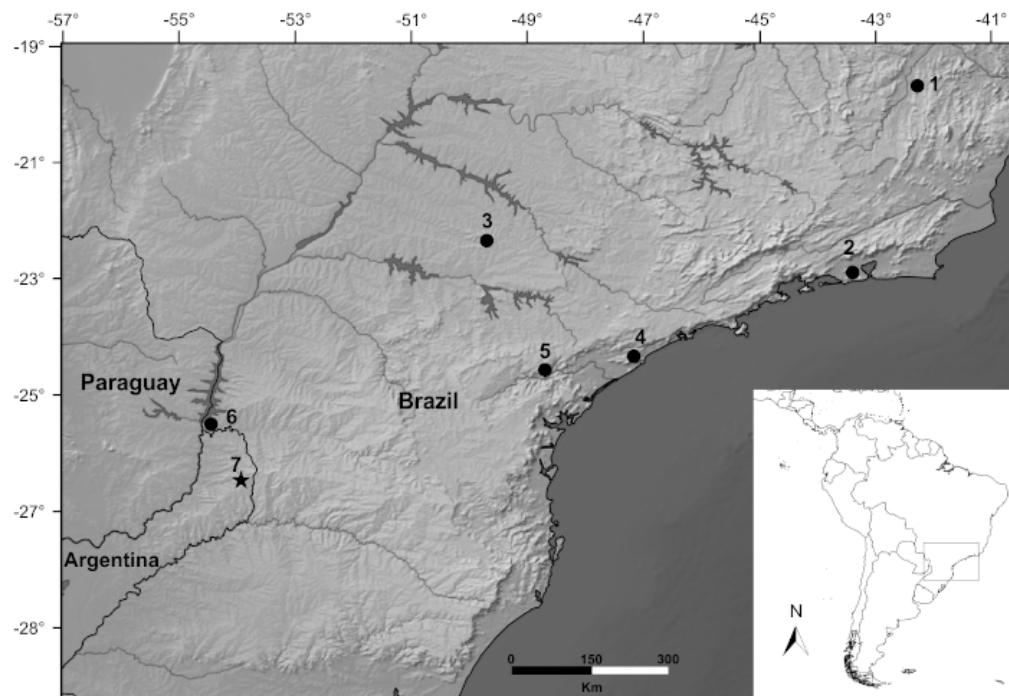
## INTRODUCTION

*Glyphonycteris* Thomas, 1896 is a poorly known genus of New World leaf-nosed bats that inhabit lowland and mountain forests of Central and South America (Williams & Genoways 2007; Zortea et al. 2008; Tirira et al. 2016; Solari 2018). This genus comprises gleaning foraging bats that are less frequently collected in studies using mist net or harp trap and are therefore poorly represented in museums (Simmons & Voss 1998; Pedro et al. 2001; Sampaio et al. 2003; Gregorin & Rossi 2005). *Glyphonycteris* and other related genera, such as *Lampronycteris*, *Neonycteris*, and *Trinycteris*, were traditionally treated as subgenera of *Micronycteris* after Sanborn's (1949) revision. However, this taxonomic arrangement was frequently questioned due to the paraphyly of *Micronycteris* (see Simmons 1996; Simmons & Voss 1998; Wetterer et al. 2000). In a taxonomic revision of the mammals of Paracou, Simmons & Voss (1998) included *Barticonycteris daviesi* (Hill 1965) in *Glyphonycteris*; the authors recognized it as a full genus and provided an emended diagnosis. More recently, several molecular phylogenetic analyses recovered *Glyphonycteris* + *Trinycteris* as a sister clade of *Carollia* (Baker et al. 2002; 2003; 2012; 2016; Amador et al. 2018). In this phylogenetic context, Baker et al. (2012; 2016) proposed and diagnosed the subfamily Glyphonycterinae in which the authors included *Glyphonycteris*, *Trinycteris*, and *Neonycteris*, and suggested that the observed morphological similarities with *Micronycteris* and *Macrotus* represent plesiomorphic features.

Currently, *Glyphonycteris* comprises three nominal species: *Glyphonycteris behnii* (Peters 1865), *G. daviesi* (Hill 1965), and *G. sylvestris* (Thomas 1896) (Simmons 2005). *Glyphonycteris behnii* is a rare species that has been suggested to be a senior synonym of *G. sylvestris* (Simmons & Voss 1998) and it is only known by two specimens from Cuzco in Peru, one from Mato Grosso and one from Minas Gerais in Brazil (Andersen 1906; Peracchi & Albuquerque 1985). *Glyphonycteris daviesi*, the

largest of the genus (forearm 53.8–58.1 mm and skull over 25.0 mm; Williams & Genoways 2007), is distributed in Central America (Honduras, Costa Rica and Panama), island of Trinidad and South America (Venezuela, Colombia, Guiana, Surinam, French Guiana, Brazil, Ecuador, Peru, and Bolivia; Williams & Genoways 2007; Morales-Martínez & Suárez-Castro 2014). Lastly, *G. sylvestris*, the type species of the genus (Thomas 1896, b:301), is also the smallest species (forearm 38.0–44.0 mm and the skull less than 22.0 mm; Williams & Genoways 2007), and inhabits Central America (Mexico, Panama and Costa Rica) and northern South America (Colombia, Venezuela, Trinidad and Tobago, Surinam, French Guiana, Ecuador, and Peru; Williams & Genoways 2007; Datzman et al. 2010; Morales-Martínez & Suárez-Castro 2014; Tirira et al. 2016). Additional populations were reported from Atlantic Forests Ecoregion Complex in southeastern Brazil, with a few records from Minas Gerais, Rio de Janeiro, São Paulo, and Paraná States (Trajano 1982; Sekiama et al. 2001; Pedro et al. 2001; Dias et al. 2003; Tavares et al. 2010), including a new record from the Cerrado in the Tocantins state (Felix et al. 2016).

Cytogenetic studies of bats from the family Glyphonycterinae are scarce, and the karyotype of only three species has been previously studied with the use of conventional chromosome staining techniques (Baker & Hsu 1970; Patton 1976; Honeycutt et al. 1980; Baker et al. 1982). Karyological data are restricted to just one specimen of *G. daviesi* from Surinam with  $2n = 28$  chromosomes and autosomal fundamental number FN = 52 described from one specimen (Honeycutt et al. 1980; Baker et al. 2016), whereas  $2n = 22$  chromosomes and FN = 36 was described from seven female specimens of *G. sylvestris* (Honeycutt et al. 1980). Other cytogenetic methods such as staining with different base-specific fluorochromes to determine DNA sequence composition (banding technique) have not been employed for species of *Glyphonycteris*, evidencing the lack of knowledge of this genus of bats.



**Fig. 1.** Location of records of *Glyphonycteris sylvestris* in the Atlantic forests from Brazil (1-6) and a new record for Argentina (7). All numbered localities are listed in **Table 2**.

Here, we report the first record of *G. sylvestris* in Argentina and in the Araucaria Moist Forest ecoregion extending its latitudinal distribution. In addition, the goals of this study were to examine dental features, external and skull measurements of our specimen, and compare them with information of bats of this species from other tropical sites. We described for the first time the karyotype for *G. sylvestris* from the Atlantic Forest populations and compared our results with the available information.

## MATERIALS AND METHODS

The study site was located in Parque Provincial El Piñalito, 19.5 km NE of Cruce Caballero along National Route 14, Department of San Pedro ( $26^{\circ} 25' 41.28''S$ ,  $53^{\circ} 50' 39.6''W$ , 762 m a.s.l.; Fig. 1), northeast of Misiones Province, Argentina, in the Araucaria Moist Forests. This forest is one of the 15 terrestrial ecoregions that compose The Atlantic Forests Global 200 Ecoregion (hereafter “Atlantic Forest”) (Dinerstein et al. 1995; Olson et al. 2001). In Argentina, the Araucaria Moist Forest forms a narrow belt along the Sierra of Misiones in the center and northeast of Misiones province (Giraudo et al. 2003). The forest canopy is characterized by the presence of conifer trees (*Araucaria angustifolia*; Araucariaceae). The subcanopy is characterized by *Nectandra megapotamica* (Lauraceae), *Balfourodendron riedelianum* (Rutaceae),

*Cordia americana* (Boraginaceae), *Diatenopteryx sorbifolia* (Sapindaceae), *Parapiptadenia rigida* and *Myrcarpus frondosus* (Fabaceae). Understory is characterized by *Alsophila setosa* (Cyatheaceae), *Piper* (Piperaceae), *Sorocea bonplandii* (Moraceae), *Trichilia* (Meliaceae), *Psichotria*, *Ixora* (Rubiaceae) and ferns were dominant in the herbaceous stratum. Vascular epiphytes such as ferns, cacti (*Lepismium* and *Rhipsalis*), and bromeliads are abundant (Cabrera 1976). The climate is hot and wet, with mean maximum temperature of  $17.7^{\circ}C$ , and only mildly seasonal, since the annual rainfall (1921 mm) is distributed rather uniformly throughout the year (data estimated from Worldclim using DIVA-GIS, version 4.2; Hijmans et al. 2005).

We deployed 10 mist-nets/night at the same sampling site from 6 to 9 March 2016. Nets were placed at the ground level up to the subcanopy level (6–8 m high) inside the forest, in flight pathways, riparian forest and forest edge. Mist nets were left opened for approximately 6 h from sunset and were checked every 30 min. Each captured bat was removed from the net and placed in cloth bags for data collection and identification; taxonomic treatment follows Gardner (2007). Bats were marked on the back by trimming the hair to avoid overestimation of the catches and were released near the capture site. Fecal samples were obtained from the bags and the bats handled in the net, and each sample was preserved as an individually labelled dry pellet. The plant dietary items were identified using a reference collection of seeds that was initiated by Giannini (1999) and continued by Sánchez et al. (2012) and Sánchez & Dos Santos (2015).

Voucher specimen was preserved as skin, skull and skeleton after recording the basic external measurements, and finally housed in the Mammal Collection of the Laboratorio de Genética Evolutiva (CM-LGE) of the Instituto de Biología Subtropical (IBS)-nodo Posadas, CONICET-UNaM. Specimen was collected under permit obtained from the Ministerio de Ecología y Recursos Naturales Renovables of Misiones Province (Collection Permit N° 002/2016). External and cranial measurements were taken using a digital caliper of 0.01 mm sensitivity DIGIMESS® (Buenos Aires, Argentina) and body mass to the nearest 0.5 g using a spring scale PESOLA™ (Baar, Switzerland). Measurements follow Simmons & Voss (1998), and Giménez & Giannini (2016) (Table 1) and include: weight (g); total length; tail length; hindfoot length; ear length; forearm length; tibia length; greatest length of skull excluding incisors; condyleincisive length; postorbital breadth; zygomatic breadth; braincase breadth; mastoid breadth; length of maxillary toothrow; length of palatal; breadth across upper molars; width across upper canines; length of mandible; length of mandibular toothrow.

Mitotic chromosomes were obtained from drip of cell suspension from bone marrow of one female specimen using the cell suspension technique, after *in vivo* injection of colchicine, following Ford & Hamerton (1956). Twenty metaphase spreads were selected in order to perform the karyotype characterization. This characterization was performed using different staining techniques: conventional staining with Giemsa 10% (Ford & Hamerton 1956), and differential staining with fluorochromes DAPI (4,6-diamidino-2-phenylindole), and CMA<sub>3</sub> (Chromomycin A<sub>3</sub>) to identify regions rich in AT and GC base pairs, respectively (Schweizer 1976; 1980). Diploid number (2n) and fundamental number (FN) were calculated on conventionally stained metaphases using photomicrographs obtained with an Olympus BX50 epifluorescent photomicroscope equipped with Sony Exwave-Had digital camera. Subsequently, ten metaphases were used for the construction of an idiogram using Drawid V0.26 software (Kirov et al. 2017).

## RESULTS AND DISCUSSION

We obtained the first record of *Glyphonycteris sylvestris* for Argentina, which is an adult female without evidence of reproductive activity, captured at 19:30 in a ground-level mist net placed in a forest edge. Simultaneously, we also captured individuals of *Artibeus lituratus*, *A. fimbriatus*, *Pygoderma bilabiatum*, and *Sturnira lilium*. Our specimen shares the diagnostic external characters for this species as described by Thomas (1896), Simmons & Voss (1998), and Williams & Genoways (2007): short and pointed ear, with the lower part of the outer edge ending in a low rounded lobule, and with a concavity on the posterior border near the tip, short fur (2.71 mm) at the external surface of the leading edge of the ear, ventral margin of narial horseshoe grading gradually into upper lip, interauricular band absent, chin with a pair of dermal pads arranged in a "V", with no central papilla, calcar shorter than hindfoot, soft and fine fur, smoky to dark gray and tricolored dorsally, and

gray fur on its ventral side. However, unlike Thomas' description, the dorsal fur of our specimen extends to wings and limbs, including pollical metacarpals and first finger. Forearm hairs extend to approximately up to one third of this bone, and a thin tuft of hairs cover the hind limb dorsally. Tail is short but it does not perforate the interfemoral membrane on its upper surface as in the type specimen (see Thomas 1896).

Like previous descriptions (e.g., Goodwin & Greenhall 1961; Simmons & Voss 1998) the skull of our specimen has two pairs of upper incisors, outer incisors reduced and hidden by cingulum of canine, low incisors trifid with crown height approximately equal to crown width, P3 and P4 subequal in crown height, P3 molariform with well-developed lingual cingulum and cusp, P4 with lingual cingulum of convex outline, edge not raised, and lingual cusp well developed (Fig. 2), upper canine much less than twice the height of the inner upper incisor, lower premolars aligned in row on mandible, coronoid process low with little slope along dorsal margin. The skull has a domed braincase, without sagittal crest, divided into single or two raised ridges, as in specimens from Trinidad and Ecuador (see Goodwin & Greenhall 1961; Tirira et al. 2016), rostrum and anterior orbital region of skull inflated, the dorsum of the rostrum is flat, the basisphenoid pits are deep, and mastoid breadth is shorter than zygomatic breadth. All measurements of our specimen fall within the range of size variation reported for specimens from Mexico, Trinidad and Panama (Goodwing & Greenhall 1964), French Guiana (Simmons & Voss 1998), Colombia (Morales-Martínez & Suárez-Castro 2014), Ecuador (Tirira et al. 2016) and Brazil (Dias et al. 2003) (Table 1).

This is the southernmost record for this species, and the first from the Araucaria Moist Forest ecoregions. Despite the scarce records throughout its distribution, *G. sylvestris* widely distributed and occurs in different ecoregions, from 45 to 1476 m a.s.l (e.g., Simmons & Voss 1998; Tirira et al. 2016). This species was recorded in 12 ecoregions from the northern populations of South America, four ecoregions from the southern populations of the Atlantic forest (Table 2), and in a moist forest relict in the Cerrado, between the northern and southern populations (Felix et al. 2016). This broad distribution suggests that *G. sylvestris* would be flexible in habitat use and highly tolerant to different environmental conditions. Populations from Cerrado might indicate a historical connection between both

**Table 1**

External and cranial measurements (in mm) of the specimen of *Glyphonycteris sylovestris* (CM-LGE 200) reported here, compared to specimens reported by Dias et al. (2003) for Brazil; Tirira et al. (2016) for Ecuador; Simmons & Voss (1998) for French Guiana; Morales-Martínez & Suárez-Castro (2014) for Colombia; Jones & Carter (1976) for Panama; Thomas (1896); Andersen (1906); Goodwing & Greenhall (1961); Goodwing & Greenhall (1964) for Costa Rica; Goodwing & Greenhall (1964) for Trinidad and Mexico. Institutional abbreviations are: CM-LGE, Mammal Collection of the Laboratorio de Genética Evolutiva of the Instituto de Biología Subtropical, Universidad Nacional de Misiones; ALP, Collections of Adriano Lúcio Peracchi from the Instituto de Biología da Universidade Federal do Rio do Janeiro; QCAZ, Mammal Division of Museo de Zoología de la Pontificia Universidad Católica del Ecuador; AMNH, American Museum of Natural History; ICN, Instituto de Ciencias Naturales Universidad Nacional de Colombia; USNM, National Museum of Natural History; BM, British Museum (Natural History). Measurements are expressed as the mean  $\pm$  standard deviation for specimens from Trinidad and Mexico. LMnT: Length of mandibular toothrow. LMxT: Length of maxillary toothrow. WAUC: Width across upper canines.

Measurements	Argentina	Brazil	Ecuador	French	Colombia	Panama	Costa Rica	Trinidad	Mexico
	CM-LGE 200	ALP 5856	QCAZ 15197	Guiana	ICN 13313	USNM 396399	BM 96.10.12	(N = 5)	(N = 2)
Sex									
Weight (g)	Female	Female	Female	Male	Male	Female	Male	Male	Females
Total length	10.50	9.00	7.70	8.00					10.70 ( $\pm$ 0.4)
Tail length	67.00	68.40	57.00	65.00					
Hindfoot length	8.00	12.00	9.00	9.00					
Ear length	11.00	10.60	12.00	12.00					
Forearm length	20.50	20.00	22.00	17.00					
Tibia length	41.00	41.10	42.20	38.70	39.00	42.00	40.00 <sup>a</sup> -40.30 <sup>b</sup>	40.60 ( $\pm$ 0.7)	42.40 ( $\pm$ 0.2)
Greatest length of skull	15.08	15.70	14.60				15.00 <sup>a</sup>		
Condyloincisor length	19.86	20.20	19.50				19.60 <sup>a</sup> -19.80 <sup>b</sup>	20.40 ( $\pm$ 0.4)	20.80 ( $\pm$ 0.4)
Postorbital breadth	19.05					17.30		20.80 ( $\pm$ 0.4)	19.00 ( $\pm$ 0.1)
Zygomatic breadth	4.88	4.70	4.80					4.90 ( $\pm$ 0.3)	4.90 ( $\pm$ 0.2)
Braincase breadth	10.40	10.00	10.10				10.70	10.20 <sup>b</sup>	10.20 ( $\pm$ 0.3)
Mastoid breadth	8.81	8.40	8.20				8.20	8.70	8.80 ( $\pm$ 0.2)
LMnT	9.42	9.00	8.30					9.50 <sup>b</sup>	8.50 ( $\pm$ 0.4)
Length of palatal	8.19	8.10	7.80						
Breadth across molars	8.97	9.40	8.70						
WAUC	6.94	6.50	6.80						
Length of mandible	3.44	3.60							
LMxT	13.64	13.10							
	8.67								

<sup>a</sup>Thomas (1896)

<sup>b</sup>Andersen (1906)



**Table 2** Occurrences of *Glyphonycterus sylvesteris* in the Atlantic Forest ecoregion. Each locality was assigned to an ecoregion according to the World Wildlife Fund (WWF). Locality number matches geographical location on map in Fig. 1.

N°	Locations	Country	Latitude (N)	Longitude (W)	Elevation (m a.s.l.)	Ecoregion	Source
1	Piracicaba river basin, between the municipalities of Antônio Dias and Nova Era, Minas Gerais Estação Ecológica dos Caetetus, São Paulo	Brazil	19°40'	42°5'	791	Bahia inferior forest	Tavares et al. (2010)
3	Parque Estadual da Pedra Branca, Rio de Janeiro	Brazil	22°15'– 22°30'	49°30'– 49°45'	516	Atlantic forests	Pedro et al. (2001)
2	Juréia-Itatins, São Paulo	Brazil	23°52'– 23°04'	43°23'– 43°32'		Serra do Mar coastal forests	Dias et al. (2003)
4	Iporanga, São Paulo	Brazil	24°24'	47°01'	235	Alto Paraná Atlantic forests	Gimenez & Ferrareze (2004)
5	Parque Nacional do Iguaçu, Paraná	Brazil	24°34'	48°42'	580	Alto Paraná Atlantic forests	Trajano (1982)
6	Parque Provincial El Piñalito, Misiones	Argentina	25°05'– 26°25'	53°40'– 54°38'		Alto Paraná Atlantic forests	Sekkama et al. (2001)
7			41°28"	53°50'– 39°6"	762	Araucaria moist forests	This study

**Fig. 2.** Dorsal, ventral, lateral and frontal views of the skull of the female specimen of *Glyphonycteris sylvestris* (CM-LGE 200) captured in Parque Provincial El Piñalito. Note the skull with domed braincase without sagittal crest, and the pair of incisors reduced and hidden by canine cingulum. See Table 1 for measurements. Scale = 5 mm.

tropical populations, which should be tested in the future by biogeographical analyzes.

The fecal sample obtained from the Argentine specimen only contained plant material, includ-

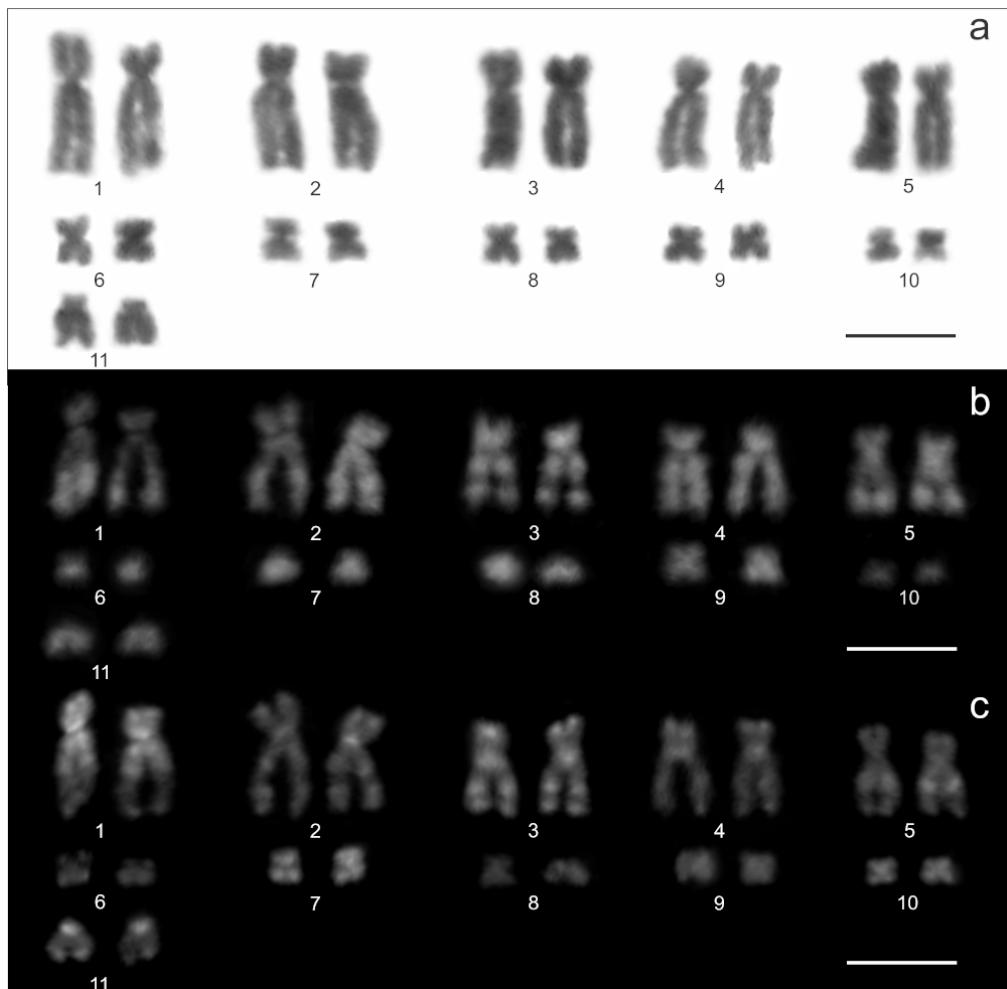
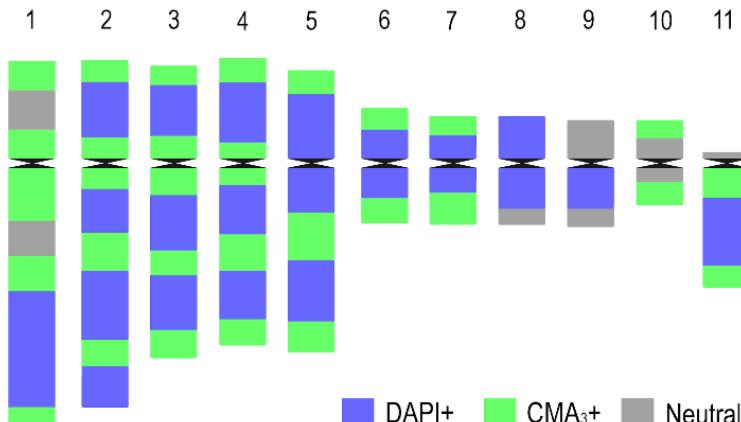


Fig. 3. Female karyotype of *Glyphonycteris sylvestris* (CM-LGE 200) with  $2n = 22$ ; FN = 42. a) Giemsa-stained mitotic chromosomes; b) fluorochrome DAPI staining; c) fluorochrome Chromomycin A<sub>3</sub> staining. Scale = 10  $\mu\text{m}$ .

ing seeds of *Piper aduncum*. *Glyphonycteris* + *Trinycteris* has always been recovered as the sister clade of *Carollia* (see Baker et al. 2012; Rojas et al. 2016; Amador et al. 2018). Both *Glyphonycteris* and *Trinycteris* are insectivores that include plant material in their diets, whereas *Carollia* and successive branches in the phyllostomid tree (e.g., Rhinophyllinae and Stenodermatinae) are obligate frugivores. This suggests that frugivory strategies evolved from the feeding strategy of a basal insectivore (Baker et al. 2012). *Piper* is the main resource of *Carollia* throughout the Neotropics (e.g., Lobova et al. 2009) and their mutualistic interaction was associated with the phylogeny and evolution of this basal lineage of frugivorous bats

(Sánchez & Giannini 2018). Thus, the use of *Piper* by *G. sylvestris* could indicate that its diet may be related to a phylogenetic constraint due to its affinity to the Carollinae clade (see Sánchez & Giannini 2018).

The diploid chromosome number of our *Glyphonycteris sylvestris* specimen is  $2n = 22$ , and the fundamental number (including the chromosomal sex pair) FN = 42. Almost all chromosomes of the complement are biarmed, except for one pair of small acrocentric chromosomes (Fig. 3). Although this description agrees, in general, with that found in the literature for *G. sylvestris*, we observed a single pair of acrocentric chromosomes instead of two, as described by Honeycutt et al. (1980).



**Fig. 4.** Haploid idiogram of *Glyphonycteris sylvestris*. Fluorochrome DAPI and Chromomycin A<sub>3</sub> positive bands are shown in blue and green respectively. Grey corresponds to neutral regions.

This implies a change in the fundamental number described previously for *G. sylvestris*, from FN = 40 to FN = 42. These discrepancies seem to be due to different criteria in the assignment of chromosomes, since we also detected one acrocentric chromosome in the figure of Honeycutt et al. (1980). It was also considered by Honeycutt et al. (1980) that because the closely related species *Glyphonycteris daviesi* and *Trinycteris nicefori* have biarmed sexual chromosomes (Patton & Baker 1978; Honeycutt et al. 1980), the sexual pair XX would be a biarmed element. Nevertheless, we cannot discuss that as we have not analyzed male specimens. The analysis of fluorochrome banding patterns in the chromosomal pericentromeric regions shows DAPI positive blocks in pairs 5 and 8, whereas pericentromeric regions stained with CMA<sub>3</sub> are positive in pairs 1, 2, 3, 4 and 11. Moreover, the interstitial region of chromosomes shows DAPI and CMA<sub>3</sub> positive blocks in pairs 1, 2, 3, 4 and 5 (Fig. 3 and 4).

Among the phyllostomids, diploid chromosome numbers are highly variable, with karyotypes ranging from 2n = 14 to 2n = 46 chromosomes in 106 studied species (see Sotero-Caio et al. 2017 and references therein). In Glyphonycterinae only the conventional karyotypes of *Trinycteris nicefori*, *Glyphonycteris daviesi* (both with 2n = 28, NF = 52), and *G. sylvestris* (2n = 22) are known (Patton & Baker 1978; Honeycutt et al. 1980, this work). To date, a 2n = 22 karyotype seems to be unique within Glyphonycterinae, and although two species in the sister clade Carolliinae (*Carollia benkeithi* and *C. castanea*; Solari & Baker 2006) share this diploid number, both karyotypes have clear differences in chromosomal morphology, and different rearrangements would be involved. The fluorochrome bands of *G. sylvestris* presented here are the first for Glyphonycterinae and the lack

of data for related species limits comparisons at this time. Thus, it would be of high interest to develop further molecular cytogenetic studies that could help understand chromosomal evolution from a phylogenetic perspective in Glyphonycterinae.

## ACKNOWLEDGMENTS

We thank the staff of Ministerio de Ecología y Recursos Naturales Renovables of Misiones province, Argentina, for fieldwork permits and logistical support. We thank Roman M. Sánchez for his help with figure preparation and editing. The study was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) through the research grants PICT2013-2759 and PICT2016-0608 assigned to MSS and DJB. This manuscript was greatly improved with comments from Valeria Tavares, the Associate Editor, and one anonymous reviewer, to whom we thank especially.

## LITERATURE CITED

- AMADOR, L. I., R. L. MOYES ARÉVALO, F. C. ALMEIDA, S. A. CATALANO, & N. P. GIANNINI. 2018. Bat Systematics in the Light of Unconstrained Analyses of a Comprehensive Molecular Supermatrix. *Journal of Mammalian Evolution* 25:37–70. <https://doi.org/10.1007/s10914-016-9363-8>
- ANDERSEN, K. 1906. On the bats of the genera *Micronycteris* and *Glyphonycteris*. *Annals and Magazine of Natural History* 18:50–65. <https://doi.org/10.1080/00222930608562579>
- BAKER, R. J., & T. HSU. 1970. Further studies on the sex-chromosome systems of the American leaf-nosed bats (Chiroptera, Phyllostomatidae). *Cytogenetic and Genome Research* 9:131–138. <https://doi.org/10.1159/000130083>
- BAKER, R. J., M. HAIDUK, L. W. ROBBINS, A. CADENA, & B. KOOP. 1982. Chromosomal studies of South American bats and their systematic implications. *Mammalian Biology in South America* (M. A. Mares & H. H. Genoways, eds.). The Pymatuning Symposia in Ecology, Special Publications Series, Pittsburgh. <https://doi.org/10.2307/1380542>
- BAKER, R. J., O. R. P. BININDA-EMONDS, H. MANTILLA-MELUK, C. A. PORTER, & R. A. VAN DEN BUSSCHE. 2012. Molecular timescale

- of diversification of feeding strategy and morphology in New World leaf-nosed bats (Phyllostomidae): a phylogenetic perspective. Evolutionary history of bats fossils, molecules and morphology (G. F. Gunnell & N. B. Simmons, eds.). Cambridge University Press, New York. <https://doi.org/10.1017/cbo9781139045599.012>
- BAKER, R. J., S. R. HOOFER, C. A. PORTER, & R. A. VAN DEN BUSCHE. 2003. Diversification among New World leaf-nosed bats: an evolutionary hypothesis and classification inferred from genomic congruence of DNA sequence. Occasional papers, Museum of Texas Tech University 230:1-32. <https://doi.org/10.5962/bhl.title.156931>
- BAKER, R. J., S. SOLARI, & F. G. HOFFMANN. 2002. A new Central American species from the *Carollia brevicauda* complex. Occasional papers, Museum of Texas Tech University 217:1-12. <https://doi.org/10.5962/bhl.title.156831>
- BAKER, R. J., S. SOLARI, A. CIRRANELLO, & N. B. SIMMONS. 2016. Higher level classification of phyllostomid bats with a summary of DNA Synapomorphies. Acta Chiropterologica 18:1-38. <https://doi.org/10.3161/15081109acc2016.18.1.001>
- CABRERA, A. L. 1976. Regiones fitogeográficas Argentinas. Encyclopedie Argentina de Agricultura y Jardinería. Segunda Edición. Editorial ACME, Buenos Aires.
- DATZMAN, T., O. VONHELVERSEN, & F. MAYER. 2010. Evolution of nectarivory in Phyllostomid bats (Phyllostomidae Gray, 1825, Chiroptera: Mammalia). BMC Evolutionary Biology 10:1-14. <https://doi.org/10.1186/1471-2148-10-165>
- DIAS, D., S. S. PEREIRA DA SILVA, & A. L. PEREACCHI. 2003. Ocorrência de *Glyphonycteris sylvestris* Thomas (Chiroptera, Phyllostomidae) no Estado do Rio de Janeiro, sudeste do Brasil. Revista Brasileira de Zoologia 20:365-366. <https://doi.org/10.1590/s0101-81752003000200030>
- DINERSTEIN, E. ET AL. 1995. A Conservation Assessment of the Terrestrial Ecoregions of Latin America and the Caribbean. World Bank, Washington, DC.
- FELIX, S., R. L. M. NOVAES, R. F. SOUZA, & L. S. AVILLA. 2016. Bat assemblage in karstic area from northern Brazil: seven new occurrences for Tocantins state, including the first record of *Glyphonycteris sylvestris* Thomas, 1896 for the Cerrado. Check List 12:1-13. <https://doi.org/10.15560/12.6.1999>
- FORD, C. E., & J. L. HAMERTON. 1956. A colchicine, hypertonine citrate, squash sequence for mammalian chromosomes. Stain Technology 31:247-251. <https://doi.org/10.3109/10520295609113814>
- GARDNER, A. L. 2007. Order Chiroptera Blumenbach, 1779. Mammals of South America, Volume 1 (A. L. Gardner, ed.). The University of Chicago Press, Chicago.
- GIANNINI, N. P. 1999. Selection of diet and elevation by sympatric species of *Sturnira* in an Andean rainforest. Journal of Mammalogy 80:1186-1195. <https://doi.org/10.2307/1383169>
- GIMÉNEZ, A. L., & N. P. GIANNINI. 2016. Morphofunctional segregation in molossid bat species (Chiroptera: Molossidae) from the South American Southern Cone. Hystrix, the Italian Journal of Mammalogy 27:1-11.
- GIMÉNEZ, E. A., & H. FERRAREZZI. 2004. Diversidade de morcegos no sudeste da Mata Atlântica. Estação Ecológica Juréia-Itatins: ambiente físico, flora e fauna (O. A. V. Marques & W. Duleba, eds.). Editora Holos, Ribeirão Preto.
- GIRAUDET, A. ET AL. 2003. Biodiversity Status of the Interior Atlantic Forest of Argentina. The Atlantic Forest of South America. Biodiversity Status, Threats, and Outlook (C. Galindo-Leal & I. de GusmaoCamara, eds.). Island Press, Washington, DC. <https://doi.org/10.1007/s10980-005-1788-z>
- GOODWING, G., & A. M. GREENHALL. 1964. New records of bats from Trinidad and comments on the status of *Molossus trinitatis* Goodwin. American Museum Novitates 2195:1-23.
- GOODWIN, G. G., & A. M. GREENHALL. 1961. A review of the bats of Trinidad and Tobago: descriptions, rabies infection, and ecology. Bulletin of the American Museum of Natural History 122:187-302. <https://doi.org/10.2307/1377397>
- GREGORIN, R., & R. ROSSI. 2005. *Glyphonycteris daviesi* (Hill, 164), a rare Central American and Amazonian bat recorded for Eastern Brazilian Atlantic Forest (Chiroptera, Phyllostomidae). Mammalia 69:427-430. <https://doi.org/10.1515/mamm.2005.035>
- HIJMANS, R., L. GUARINO, P. MATHUR, & A. JARVIS. 2005. DIVA-GIS Version 4.2. Available at: <http://www.diva-gis.org/download>
- HONEYCUTT, R. L., R. J. BAKER, & H. H. GENOWAYS. 1980. Results of the Alcoa Foundation-Suriname Expeditions III. Chromosomal data for bats (Mammalia: Chiroptera) from Suriname. Annals of Carnegie Museum 49:237-250. [https://doi.org/10.2992/0097-4463\(2005\)74\[225:ROTAFE\]2.0.CO;2](https://doi.org/10.2992/0097-4463(2005)74[225:ROTAFE]2.0.CO;2)
- JONES, J. K., & D. D. CARTER. 1976. Annotated checklist, with keys to subfamilies and genera. Part I. Biology of bats of the New World family Phyllostomatidae (R. J. Baker, J. K. Jones & D. C. Carter Jr., eds.). Special Publications of the Museum, Texas Tech University 10:1-218. <https://doi.org/10.5962/bhl.title.142603>
- KIROV, I. ET AL. 2017. DRAWID: user-friendly java software for chromosome measurements and idiogram drawing. Comparative Cytogenetics 11: 747-757. [doi:10.3897/compcytogen.v11i4.20830](https://doi.org/10.3897/compcytogen.v11i4.20830)
- LOBOVA, T. A., C. K. GEISELMAN, & S. A. MORI. 2009. Seed dispersal by bats in the Neotropics. The New York Botanical Garden, New York.
- MORALES-MARTÍNEZ, D. M., & A. F. SUÁREZ-CASTRO. 2014. New records for *Glyphonycteris* Thomas, 1896 (Chiroptera: Phyllostomidae) from Colombia. Check List 10:639-644. <https://doi.org/10.15560/10.3.639>
- OLSON, D. M. ET AL. 2001. Terrestrial Ecoregions of the World: a new map of life on earth. BioScience 51:933-938.
- PATTON, J. C. 1976. Evolutionary implications of the G-Banded and C-Banded Karyotypes of Phyllostomatoid bats. Master of Science Thesis. Graduate Faculty of Texas Tech University, Lubbock, United States of America.
- PATTON, J. C., & R. J. BAKER. 1978. Chromosomal homology and evolution of phyllostomatoid bats. Systematic Zoology 27:449-462. <https://doi.org/10.2307/2412927>
- PEDRO, W. A., F. C. PASSOS, & B. K. LIM. 2001. Morcegos (Chiroptera, Mammalia) da Estação Ecológica dos Caetetus, estado de São Paulo. Chiroptera Neotropical 7:136-140
- PERACCHI, A. L., & S. T. ALBUQUERQUE. 1985. Considerações sobre a distribuição geográfica de algumas espécies do gênero *Micronycteris* Gray, 1866 (Mammalia, Chiroptera, Phyllostomidae). Arquivos da Universidade Federal Rural do Rio de Janeiro 8:23-26.
- ROJAS, D., O. M. WARSI, & L. M. DAVALOS. 2016. Bats (Chiroptera: Noctilionoidea) challenge a recent origin of extant Neotropical diversity. Systematic Biology 65:432-448. <https://doi.org/10.1093/sysbio/syw011>
- SAMPAIO, E., E. K. V. KALKO, E. BERNARD, B. RODRÍQUEZ-HERRERA, & C. O. HANDLEY JR. 2003. A biodiversity assessment of bats (Chiroptera) in a tropical lowland rainforest of central Amazonia, including methodological and conservation considerations. Studies on Neotropical Fauna and Environment 38:17-31. <https://doi.org/10.1076/snfe.38.1.17.14035>
- SANBORN, C. C. 1949. Bats of the genus *Micronycteris* and its subgenera. Fieldiana Zoology 31:215-233.
- SÁNCHEZ, M. S., & D. A. DOS SANTOS. 2015. Understanding the spatial variations in the diets of two *Sturnira* bats (Chiroptera: Phyllostomidae) in Argentina. Journal of Mammalogy 96:1352-1360. <https://doi.org/10.1093/jmammal/gvy144>
- SÁNCHEZ, M. S., & N. P. GIANNINI. 2018. Trophic structure of frugivorous bats in the Neotropics: emergent patterns in evolutionary history. Mammal Review 48:90-107. <https://doi.org/10.1111/mam.12116>
- SÁNCHEZ, M. S., L. V. CARRIZO, N. P. GIANNINI, & R. M. BARQUEZ. 2012. Seasonal patterns in the diet of frugivorous bats in the subtropical rainforests of Argentina. Mammalia 76:269-275. <https://doi.org/10.1515/mammalia-2011-0059>

- SCHWEIZER, D. 1976. Reverse Fluorescent Chromosome Banding with Chromomycin and DAPI. *Chromosoma* 58:307-324. <https://doi.org/10.1007/bf00292840>
- SCHWEIZER, D. 1980. Simultaneous fluorescent staining of R-bands and specific heterochromatic regions (DA/DAPI bands) in human chromosomes. *Cytogenetics and Cell Genetics* 27:190-193. <https://doi.org/10.1159/000131482>
- SEKIAMA, M. L., N. R. REIS, A. L. PERACCHI, & V. J. ROCHA. 2001. Morcegos do Parque Nacional do Iguaçu, Paraná (Chiroptera, Mammalia). *Revista Brasileira de Zoologia* 18:749-754. <https://doi.org/10.1590/s0101-81752001000300011>
- SIMMONS, N. B. 2005. Order Chiroptera. Mammal species of the World: a taxonomic and geographic reference (D. E. Wilson & D. M. Reeder, eds.). The Johns Hopkins University Press, Baltimore.
- SIMMONS, N. B. 1996. A new species of *Micronycteris* (Chiroptera: Phyllostomidae) from Northeastern Brazil, with comments on phylogenetic relationships. *American Museum Novitates* 3158:1-34.
- SIMMONS, N. B., & R. S. VOSS. 1998. The mammals of Paracou, French Guiana: a Neotropical lowland rainforest fauna. *Bulletin of the American Museum of Natural History* 237:1-219. [https://doi.org/10.1206/0003-0090\(2001\)263<0003:tmopfg>2.0.co;2](https://doi.org/10.1206/0003-0090(2001)263<0003:tmopfg>2.0.co;2)
- SOLARI, S. 2018. *Glyphonycteris daviesi*. The IUCN Red List of Threatened Species 2018:e.T13377A22124873. <http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T13377A22124873.en>
- SOLARI, S., & R. J. BAKER. 2006. Mitochondrial DNA sequence, Karyotypic, and Morphological variation in the *Carollia castanea* species complex (Chiroptera, Phyllostomidae) with description of a new species. *Occasional papers, Museum of Texas Tech University* 254:1-16. <https://doi.org/10.5962/bhl.title.156889>
- SOTERO-CAIO, C. G., R. J. BAKER, & M. VOLLETH. 2017. Chromosomal evolution in Chiroptera. *Genes* 8:272. <https://doi.org/10.3390/genes8100272>
- TAVARES, V. C., L. M. S. AGUIAR, F. A. PERINI, F. C. FALCÃO, & R. GREGORIN. 2010. Bats of the state of Minas Gerais, southeastern Brazil. *Chiroptera Neotropical* 16:675-705.
- THOMAS, O. 1896. XLVI.—On new small mammals from the Neotropical region. *Annals and Magazine of Natural History: Series 6*, 18:301-314. <https://doi.org/10.1080/00222939608680459>
- TIRIRA, D. G., M. A. CAMACHO, N. TINOCO, M. F. SOLÓRZANO, & S. F. BURNEO. 2016. Genus *Glyphonycteris* Thomas, 1896 (Mammalia: Chiroptera) in Ecuador: first confirmed record of *G. sylvestris* Thomas, 1896 and a geographical review to *G. daviesi* (Hill, 1965). *Check List* 12:1-10. <https://doi.org/10.15560/12.5.1965>
- TRAJANO, E. 1982. New records of bats from southeastern Brazil. *Journal of Mammalogy* 63:529-531. <https://doi.org/10.2307/1380462>
- WETTERER, A. L., M. V. ROCKMAN, & N. B. SIMMONS. 2000. Phylogeny of phyllostomid bats (Mammalia: Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. *Bulletin of the American Museum of Natural History* 248:1-248. [https://doi.org/10.1206/0003-0090\(2000\)248<0001:POPBMC>2.0.CO;2](https://doi.org/10.1206/0003-0090(2000)248<0001:POPBMC>2.0.CO;2)
- WILLIAMS, S. L., & H. H. GENOWAYS. 2007. Subfamily Phyllostominae Gray, 1825. *Mammals of South America, Volume 1* (A. L. Gardner, ed.). The University of Chicago Press, Chicago.
- ZORTEA, M., E. SAMPAIO, B. LIM, S. PETERS, & J. ARROYO-CABRALES. 2008. *Glyphonycteris sylvestris*. The IUCN Red List of Threatened Species 2008.