

First record of *Eptesicus furinalis* (Chiroptera, Vespertilionidae) from the state of Maranhão, Brazil

Primer registro de *Eptesicus furinalis* (Chiroptera, Vespertilionidae) en el estado de Maranhão, Brasil

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ABSTRACT

A specimen of *Eptesicus furinalis* was collected in the municipality of Cândido Mendes, in the state of Maranhão, Brazil. It was a non-lactating adult female, with dark chestnut dorsal coloration, yellow venter, hairless membranes, short and rounded ears, and a pointed tragus. The analysis of the DNA barcode of the COI mitochondrial gene revealed a 99.80 % similarity with the sequence of *E. furinalis* deposited in the BOLDSystems platform. The combined analysis of the morphological and molecular data confirmed the occurrence of *E. furinalis* in the state of Maranhão. This extends the known distribution of the species 676.1 km from the nearest recorded locality in the Ceará State.

Keywords: Bat, Cândido Mendes, DNA barcode, Mitochondrial gene.

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RESUMEN

Un espécimen de *Eptesicus furinalis* fue recolectado en la ciudad de Cândido Mendes, en el estado de Maranhão, Brasil. El ejemplar era una hembra adulta no lactante, con coloración dorsal castaña oscura, ventral amarilla, membranas sin pelos, el hocico inflado, orejas cortas y redondas, y trago puntiagudo. El análisis del código de barras de ADN del gen mitocondrial COI reveló una similitud del 99,80 % con la secuencia de *E. furinalis* depositada en la plataforma BOLDSystems. El análisis combinado de los datos morfológicos y moleculares confirma la presencia de *E. furinalis* en el estado de Maranhão. Este registro extiende la distribución conocida de la especie 676,1 km oeste desde la localidad registrada más cercana, en el estado de Ceará.

Palabras clave: Cândido Mendes, Código de barras de ADN, Gen mitocondrial, Murciélago.

INTRODUCTION

The genus *Eptesicus* belongs to the family Vespertilionidae (Chiroptera) and Garbino *et al.* (2020) confirmed the occurrence of five species in Brazil *Eptesicus brasiliensis* (Desmarest, 1819), *Eptesicus chiriquinus* Thomas, 1920, *Eptesicus diminutus* Osgood, 1915, *Eptesicus furinalis* (D'Orbigny and Gervais, 1847), and *Eptesicus taddeii* Miranda and Bernardi Passos, 2006. These species occur sympatrically (Reis *et al.* 2011, 2017).

Species of the genus *Eptesicus* exhibit close morphological similarity relationships with *Myotis* (Tirira 2017). These species are distinguished by the absence of hair at the base of the urothagio and by the smaller distance between the canine and first molar and reduced number of premolars (pm 1/2) (Reis *et al.* 2017). These bats are insectivorous, and present sexual dimorphism, with males being larger than the females (Laval and Fitch 1977).

Eptesicus furinalis is distributed between Mexico and northern Argentina, including Bolivia, Paraguay, the Guianas, and Brazil (Simmons 2005). In Brazil, this species has been recorded in the states of Amazonas, Amapá, Bahia, Ceará, Espírito Santo, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pará, Pernambuco, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo, and Tocantins, as well as the Federal District (Peracchi 2006, Tavares and Aguirre 2008a, Gregorin *et al.* 2008, Bernard *et al.* 2011).

The present study applies molecular tools sequencing mitochondrial Cytochrome Oxidase I (COI) gene, together with morphological and morphometric data, to confirm the

occurrence of *E. furinalis* in the state of Maranhão, which extends the known distribution of this species in Brazil.

MATERIALS AND METHODS

The *E. furinalis* specimen was collected in an area of dense forest in the municipality of Cândido Mendes (1°27'21" South, 45°43'32" West) on April 26th 2017 by Costa, CLS, Ventura, MCS and Mendes, SB. This municipality is within the Amazon domain, and its vegetation is dominated by dense rainforest (Brasil 2015). The specimen was collected with a mist-net, 3x 12 m, with a 25 mm mesh. The specimen was weighed using a Pesola balance and the basic external and cranial measurements were using a manual calliper Carbografite (0-150 mm, precision 0,02mm). The specimen was classified as a young adult, based on the ossification of the phalangeal epiphyses (Anthony 1988). The identification and morphological and craniometric measurements were obtained followed by Taddei *et al.* (1998), Barquez *et al.* (1999), Van Cakenberghe *et al.* (2002) Díaz *et al.* (2011) and Reis *et al.* (2011, 2013, 2017).

The skull was extracted through the buccal aperture. Once clean, the skull was clarified with 10 % peroxide and dried in a stove at 30 °C, after which, it was labeled and stored in a clean recipient. The study was authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) through licenses IBAMA/SISBIO 42670-3 and 54384-1. The specimen with field number (MRR 128) was fixed in 10 % formaldehyde and preserved in 70 % ethanol at the Laboratory of Genetics and Molecular Biology, Caxias/MA, and then transferred to the mammal collection of the Federal University of

Paraíba, in João Pessoa, Brazil where it is deposited and received the voucher n°12155.

Total DNA was extracted from muscle tissue using a Wizard Genomic DNA Purification kit from Promega, following the company instructions. The mitochondrial Cytochrome Oxidase subunit I (COI) gene was amplified by Polymerase Chain Reaction (PCR) using the primers LCO-1490 and HCO-2198 described by Folmer *et al.* (1994). The samples were sequenced by the dideoxyterminal method (Sanger *et al.* 1977) with a Big Dye kit in an automatic ABI Prism™ 3500 DNA sequencer. The COI gene sequences were edited and aligned using CLUSTAL W (Thompson *et al.* 1994) using default program parameters. The MEGA X program (Kumar *et al.* 2018) was used to obtain the Maximum Likelihood (MV) phylogenetic tree and the evolutionary model General Time Reversible (GTR+G+I). The estimated significance of the clusters was assessed by bootstrap analysis (Felsenstein 1985). The database consisted of twelve sequences with 581 base pairs, with species of *Eptesicus* occurring in Brazil available on online platforms. The species and Genbank access codes are: *E. furinalis* (JF454656, EF080339, JF448033, EU096738 and KU295475), *E. brasiliensis* (JF444299 and MG191855), *E. chiriquinus* (EU096715 and JF448026), *E.*

fuscus (MG423513 and JF498646). The species *Lasiurus ega* Gervais, 1856 (KP734216) and *L. blossevillii* Lesson & Garnot, 1826 (JF446799 and JF448048) were used as an outgroup. The similarity of the COI sequence with those of the *Eptesicus* was verified on the BOLD Systems V3 (Barcode of Life Data) platform (Ratnasingham and Hebert 2007). The sequence was deposited at Genbank with the access code OL504970.

RESULTS

The specimen was a non-lactating adult female (♀), with dark chestnut dorsal pelage, yellow venter, small brain box, the rostrum is flat, undeveloped sagittal crest, second upper incisor tooth half the size of the first, elongated snout, short, rounded ears with points, a pointed tragus, and hairless membranes. The morphometric measurements were: right forearm = 43.00 mm, left forearm = 43.5 mm, tragus = 7 mm, ear = 14 mm, and foot = 9 mm. The weighed 9 g and had 32 teeth, with a dental formula of 2/3 incisors, 1/1 canines, 1/2 premolars, and 3/3 molars (Figs. 1 and 2, Table 1).

A COI sequence of 687 base pairs was obtained from the *E. furinalis* specimen, which was 99.80% similar to the *E. fu-*



Figure 1. The *E. furinalis* specimen collected in Maranhão, northern Brazil.

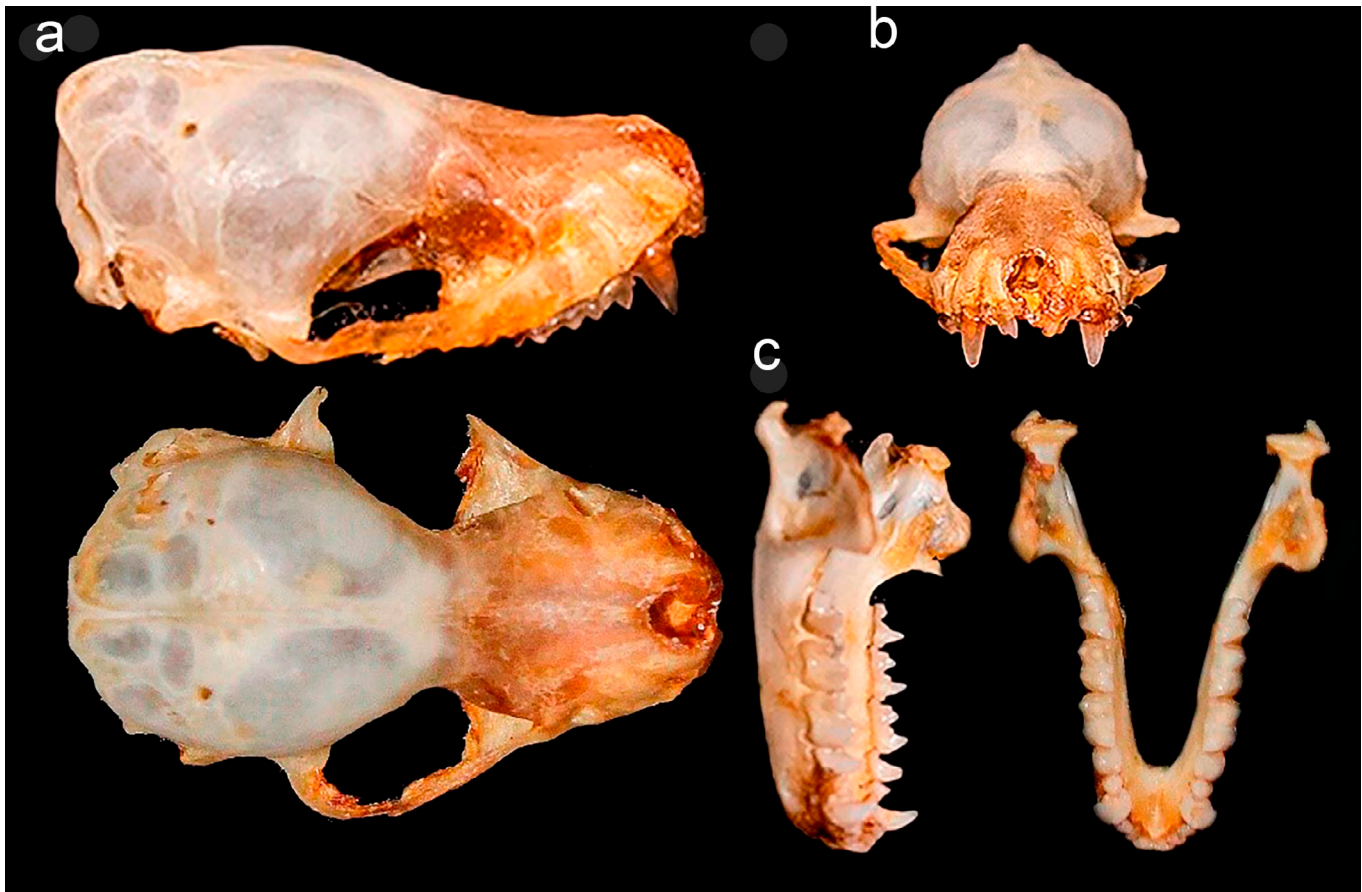


Figure 2. Skull of *Eptesicus furinalis*. **a.** Dorsal and side view showing the small braincase, **b.** Presence of poorly developed sagittal crest, **c.** side and front view of small molars with a series of maxillary teeth. Source: Olímpio, A.P.M.

furinalis sequences with which it was compared. This analysis of the COI marker thus confirmed the morphological identification of *E. furinalis*. The clusters observed in the Maximum Likelihood analysis showed a strongly supported clade with 99% bootstrap clustering *E. furinalis* specimens from Maranhão (present study), Guyana, Suriname and French Guiana; the other species grouped in separate and well-supported clades (Fig. 3).

This is the first record of *E. furinalis* specimen in the Brazilian state of Maranhão, in the municipality of Cândido Mendes (Fig. 4) extends the known distribution of the species by 676.1 km to the west and north from the nearest known locality at the RPPN Serra das Almas, in the Brazilian state of Ceará (05°05' South, 40°50' West) (Silva et al. 2015)

DISCUSSION

In the present study, *E. furinalis* was recorded in the Amazon biome of Maranhão, which may be linked to the

enormous diversity of insects and the abundance of other feeding resources, which would support the occurrence of the species in this biome (Bernard et al. 2011). The paucity of data on this species in most studies, is likely related to the difficulty of capturing this bat in mist-nets, given that it typically flies at much high levels, and may thus be under-represented in most scientific collections (Reis et al. 2013).

Although *E. furinalis* appears to have wide distribution (Reis et al. 2007), its occurrence in Brazil is still poorly understood, and many areas have yet to be inventoried adequately. When new areas are surveyed, as in the present study, the occurrence of *E. furinalis* in a new region is almost invariably confirmed.

The *E. furinalis* specimen collected in the present study in the Amazon forests of western Maranhão extend the known distribution of the species in Brazil 791 km to the north, from Colinas, in Tocantins state, 719.5 km in a

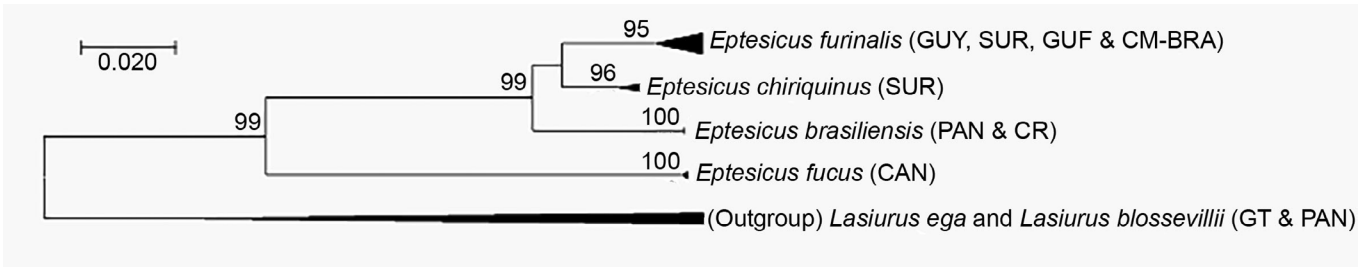


Figure 3. Maximum Likelihood phylogenetic tree generated using the General Time Reversible model based on COI gene data, for species of the *Eptesicus* genus. The numbers on the branches correspond to bootstrap values. CM-BRA = Cândido Mendes-Brazil, GUY= Guyana, GT= Guatemala, GUF= French Guyana, SUR= Suriname, PAN= Panama, CAN= Canada, and CR= Costa Rica.

straight line to the east, from the Amapá National Forest, in Amapá state, and 676.1 km to the west, from Serra das Almas, in Ceará. A further increase in the sampling effort within this region will probably provide further insights into the current distribution of this species.

The morphometric parameters recorded for the specimen (craniometrics, forearm, ear, tragus, foot and tail) were consistent with those reported for *E. furinalis* by Taddei *et al.* (1998), Barquez *et al.* (1999), Van Cakenberghe *et al.* (2002) Díaz *et al.* (2011) and Reis *et al.* (2013, 2017). In general, the external and cranial characteristics of *E. furinalis* are very similar to those of *E. brasiliensis*, although

Búrquez *et al.* (1999) note that the former has a smaller cranium and molars, which supports the identification of the specimen from Maranhão as *E. furinalis*.

The COI sequence obtained from the Maranhão specimen was closely similar to that of the same species from Suriname, with a genetic distance well below 1%. Hebert *et al.* (2003a) defined an infraspecific threshold of 3% for the COI barcode, and Clare *et al.* (2011), Olímpio *et al.* (2018), and Mendes *et al.* (2019) confirmed that this marker is effective for the identification of bat species. In the present study, then, the sum of the morphological and molecular data validated the occurrence of *E. furinalis* in Maranhão.

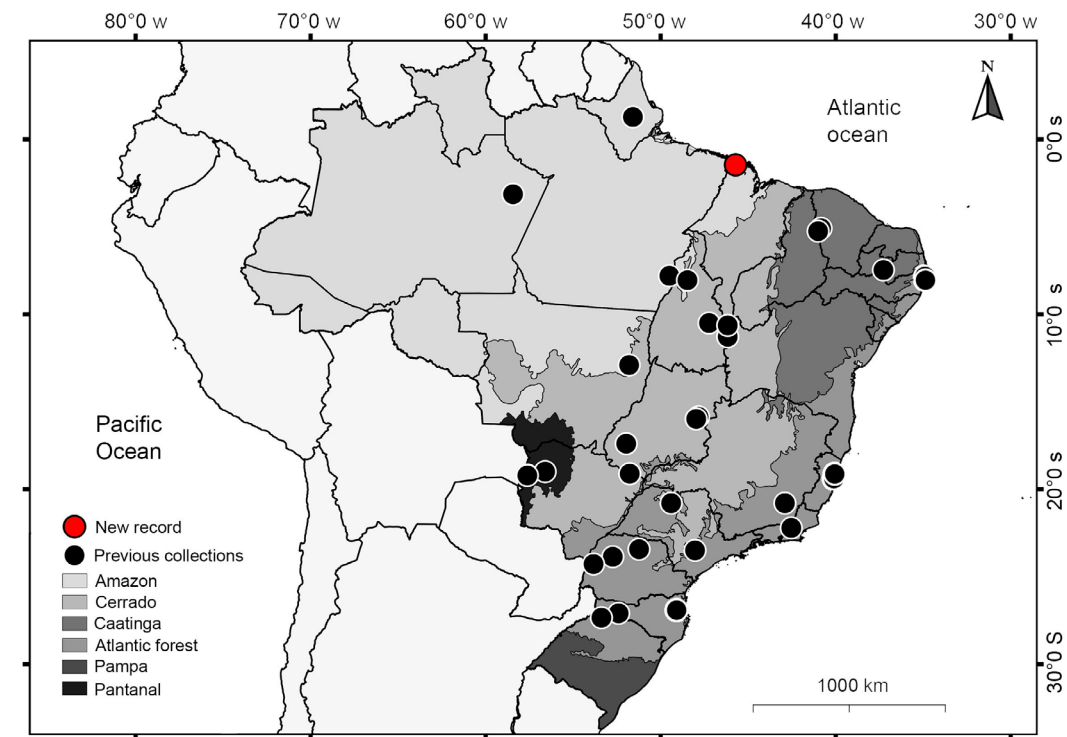


Figure 4. Geographic distribution of *E. furinalis* in Brazil. The black dots indicate the known occurrence localities of the species in Brazil, the red dot, the new record, in the state of Maranhão.

Table 1. Craniometric measurements (mm) of the *Eptesicus furinalis* specimen from Cândido Mendes, Maranhão, Brazil (present study), with values recorded in specimens of the same species from the Yucatán Peninsula (Mexico) and Chaco Borealis of Argentina, Bolivia and Paraguay.

Craniometric characters	Present study	Myers and Wetzel (1983)		Birney et al. (1974)
	Female (n=1)	Males (n=20)	Female (n=29)	Female (n=1)
Maximum skull length	14,8	14,7	15,0	15,5
Length of basal condyle	14,2	14,0	14,3	-
Maxillary tooth length	5,3	5,4	5,4	-
Mandibular length	11,7	11,4	11,6	-
Lower tooth length	6,8	6,9	6,9	-
Skull height	10,0	-	-	-
Zygomatic width	10,3	10,2	10,3	10,6
Mastoid width	8,2	8,1	8,3	-
Upper tooth width	6,4	6,3	6,4	5,6
Width between upper canines	4,5	4,6	4,6	-
Width of the interorbital constriction	3,9	3,7	3,9	-

PARTICIPATION OF AUTHORS

CLSC data collection, identification, analysis and manuscript review; APMO data analysis, image production and manuscript review; SBM data collection and identification of bat; ACSL collection, identification of bats and manuscript review; MCSV data collection; ECF article writing and correction; MCB writing, correction and translation of the article.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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