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ARTICLE

First record of *Eumops glaucinus* (Wager, 1843) (Chiroptera, Molossidae) to the Brazilian state of Maranhão

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Abstract. The study provides the first record of *Eumops glaucinus* in the Maranhão state, located in the northern region of Brazil. The collected specimen was a non-lactating adult female, with grayish pelage, broad ears, smooth face, a well-developed and squarish tragus, and elongated snout. The combined analysis of the morphological and molecular data (COI, Cyt b, and rRNA 16S genes) confirmed the occurrence of *E. glaucinus* in the state of Maranhão. This record extends the known species range area by 660 km easternward from the closest locality, Belém, Pará.

Keywords. Wagner's bonneted bat; Morphology; Mitochondrial DNA; Maranhão.

INTRODUCTION

Eumops Miller (1906) is the most diversified bat genus of the family Molossidae, which currently includes 14 species, worldwide (Medina *et al.,* 2012; Gregorin *et al.,* 2016). These species considerably vary in body size (Eger, 2008) and several qualitative traits of external, cranio-dental and penial morphology (Gregorin, 2009). The 14 *Eumops* species recognized at present are *E. auripendulus* (Shaw, 1800), *E. bonariensis* (Peters, 1874), *E. glaucinus* (Wagner, 1843), *E. hansae* (Sanborn, 1932), *E. perotis* (Schinz, 1821), *E. patagonicus* (Thomas, 1924), *E. maurus* (Thomas, 1901), *E. delticus* (Thomas, 1923), *E. trumbulli* (Thomas, 1901), *E. chimaera* (Gregorin *et al.,* 2016), *E. dabbenei* (Thomas, 1914), *E. nanus* (Miller, 1900), *E. wilsoni* (Baker *et al.,* 2009), and *E. floridanus* (Allen, 1932).

The external morphology of *E. glaucinus* is defined by its short and shiny hair, a light chestnut to yellowish or occasionally grayish colored pelage. However, some individuals are darker and blackish. Its venter is lighter than the dorsum, the snout is elongated, the tragus is well developed and it has a squarish shape (Gregorin & Taddei, 2002). *E. glaucinus* is widely distributed in South America, between Venezuela and northern Argentina, occurring in all countries except French Guiana, Suriname, Chile and Uruguay (McDonough *et al.,* 2008; Eger, 2008). In Brazil, these species has been recorded in 14 states – Acre, Alagoas, Amazonas, Bahia, Espirito Santo, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pará, Paraíba, Pernambuco, Paraná, Rio de Janeiro and São Paulo (Guerra, 2007; Mendes *et al.,* 2009; Ramos *et al.,* 2013).

The mitochondrial Cytochrome Oxidase subunit I gene (COI), a fragment of 650 nucleotides

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from the 5′ extremity, was proposed by Hebert *et al.* (2003a, 2003b) as a molecular marker for the identification of species, which has been widely adopted as a identification tool for vertebrates species. The Cytochrome *b* gene (Cyt b) has provided excellent results in phylogenetic analyses (Meyer & Wilson, 1990; Esposti *et al.,* 1993; Gregorin *et al.,* 2016), while the 16S rRNA gene has been widely used for the identification of animal species and their relatedness, given its species-specific characteristics (Coleman, 2003; Naegele *et al.,* 2006).

The combined analysis of these molecular markers provides a reliable approach for the identification of species. In the present study, the analysis of morphological and molecular data has been used to identify a specimen of *E. glaucinus* collected in the eastern of the Maranhão state, which represents the first record for the state of Maranhão.

MATERIAL AND METHODS

The *E. glaucinus* specimen recorded here was collected in the municipality of Codó (04°27′41″S, 43°53′11″W) located in the eastern of the Maranhão state, this state is located in the northern region of Brazil (IBGE, 2019). The specimen was collected in a disused textile factory, located in the center of the city of Codó, in July 2018. This place is located in an area that has a high trees density, with several large houses, retail stores, and also street vendors. The specimen was collected with a mist net which dimensions are: 3 meters high for 12 meters length, with a 25 mm mesh, this was fixed to the ground by being tied to poles.

We took the specimen to the Genetics and Molecular Biology Laboratory (GENBIMOL) of the Center for Higher Studies at Maranhão State University (CESC/UEMA) based in the city of Caxias, Maranhão. The bat was weighed with a Pesola spring balance and its morphological length was measured using a caliper (300 mm). A series of external measurements were obtained (Table 2) using a caliper: The length of the right (RF) and the left forearm (LF), ear (E), tragus (TG), foot (F), and tail (TL). Ten craniometric measurements were also taken (Table 3): The greatest length of the skull (GLS), condylobasal length (CBL), height of the braincase (HBC), braincase breadth (BB), zygomatic arch (ZA), postorbital breadth (PB), width across upper canines (CC), height of the first molar (M1M1), length of the mandible (LMAN) and the palate length (PL). The specimen was treated with formalin at 10% of concentration and conserved in a 70% ethanol concentration fluid into a sealed glass jar. The specimen was identified taxonomically using the classification keys of Gardner (2008) and Reis *et al.* (2011, 2013, 2017). The collection of specimen was authorized by ICMBio through IBAMA/SISBIO license number: 42670-3.

For the molecular analyses, the total DNA was extracted from muscle tissue using Promega's Wizard Genomic DNA Purification kit. The mitochondrial COI, Cyt b, and rRNA 16S genes were amplified by Polymerase Chain Reaction (PCR). The COI gene was amplified using the primers LCO-1490 and HCO-2198 described by Folmer *et al.* (1994), while the Cyt b sequence was amplified with the primers L14121-H15318 described by Redondo *et al.* (2008), and the rRNA 16S gene was amplified using the primers 16SL-1987 and 16SH-2609 described by Palumbi *et al.* (1991).

The samples were sequenced using the dideoxy-terminal method (Sanger *et al.,* 1977) in an ABI Prism™ 3500 automatic DNA sequencer using the Big Dye kit. The phylogenetic analysis was run in MEGA X (Kumar *et al.,* 2018), using the Tamura Nei algorithm as the evolutionary model. To determine the degree of similarity among the different species, the COI sequence was plotted in the BOLD

Table 1. The GenBank accession numbers of the sequences included in the present analysis of the Cyt b and COI gene sequences.

GenBank accession numbers of the species used to analyze the:			
Cyt b gene		COI gene	
Species	Accession number	Species	Accession number
Eumops glaucinus	EU350038.1	*Eumops glaucinus floridanus*	KR337729.1
Eumops glaucinus	EU350033.1	*Eumops glaucinus floridanus*	KR337728.1
Eumops glaucinus	EU350041.1	*Eumops glaucinus floridanus*	KT000579.1
Eumops glaucinus	EU350035.1	*Eumops perotis*	KP734219.1
Eumops glaucinus	EU350030.1	*Eumops perotis*	KT000578.1
Eumops glaucinus	EU350029.1	*Eumops auripendulus*	JF454657.1
Eumops glaucinus	EU350031.1	*Eumops auripendulus*	MF362181.1
Eumops glaucinus	EU350041.1	*Eumops hansae*	JF448844.1
Eumops glaucinus	EU350036.1	*Eumops hansae*	JF435947.1
Eumops glaucinus	EU350034.1	*Eumops hansae*	JN312047.1
Eumops glaucinus	EU350020.1	*Lasiurus blossevilli**	JF448048.1
Eumops glaucinus	EU350017.1	** Outgroup for the COI gene	
Eumops glaucinus floridanus	EU350025.1		
Eumops glaucinus floridanus	EU350024.1		
Eumops glaucinus floridanus	EU350026.1		
Eumops glaucinus	EU350011.1		
Eumops glaucinus	EU350019.1		
Eumops glaucinus	EU350018.1		
Eumops glaucinus	EU350016.1		
Eumops glaucinus	EU350006.1		
Eumops glaucinus	EU350004.1		
Eumops glaucinus	EU350002.1		
Eumops glaucinus	EU350001.1		
Eumops glaucinus	EU350000.1		
Eumops glaucinus	EU350003.1		
Eumops wilsoni	JQ731827.1		
Eumops wilsoni	JQ731804.1		
Eumops perotis	EU349990.1		
Eumops perotis	EU349991.1		
Eumops maurus	JQ731821.1		
Eumops auripendulus	MH058046.1		
Eumops auripendulus	MH058045.1		
Eumops bonariensis	JQ731832.1		
Eumops bonariensis	JQ731829.1		
Eumops patagonicus	JQ731828.1		
Eumops patagonicus	JQ731831.1		
Eumops patagonicus	JQ731830.1		
Eumops patagonicus	JQ731833.1		
Eumops hansae	JQ731815.1		
Eumops hansae	JQ731813.1		
*Lasiurus blossevilli**	KC747683.1		

* Outgroup for the Cyt b gene

Systems (Barcode of Life Data) platform, v3 (Ratnasingham & Hebert, 2007), while the sequences of the Cyt b and rRNA 16S genes were plotted in the BLAST (Basic Local Alignment Search Tool) platform (Myers *et al.,* 2014).

The Cyt b and COI sequences obtained in the present study were compared with those obtained from GenBank for *E. glaucinus* and other *Eumops* species (Table 1). The rRNA 16S gene was not included in this analysis because no GenBank sequences are available for *E. glaucinus*. The sequence presented here is thus the first *E. glaucinus* rRNA 16S sequence deposited in the GenBank.

RESULTS

In the present study was collected an individual of *E. glaucinus* (field number: CUMA 72, voucher: 12156), identified as a non-lactating adult female, with a short, shiny, and grayish coat, wide ears, smooth face, well-developed square tragus, and elongated snout (Fig. 1). The bat weighed 35 g and had a dental formula of 1/2, 1/1, 2/2, and 3/3 = 30 teeth.

The species was identified based on the morphological measurements of the forearm (left and right), ear, tragus, foot, and tail (Table 2), and the set of craniometric parameters (Table 3). The skull is elongated with a rounded braincase, which is flattened dorsally, with a high and well-developed occipital protuberance. The rostrum is flat and is only slightly lower than the braincase. The presence position of the first upper premolar is a diagnostic characteristic of *E. glaucinus* (Fig. 2).

The *E. glaucinus* specimen recorded in current study confirms the occurrence of the species in the state of Maranhão and extends the known distribution of the species by 660 km from the nearest locality, at Belém (01°27′18″S, 48°30′09″W) in Pará, Brazil (Fig. 3).

The analysis of the COI, Cyt b and rRNA 16S molecular markers, confirmed the morphological diagnosis

Figure 1. The *E. glaucinus* specimen collected in the Brazilian state of Maranhão.

Table 2. Morphological measurements of the *E. glaucinus* specimen collected in eastern Maranhão, Brazil.

Morphological character	Length (mm)
Right forearm	59
Left forearm	58.5
Tragus	5
Ear	21
Foot	9.5
Tail	50

of the occurrence of *E. glaucinus* in the Brazilian state of Maranhão. The COI sequence had 617 base pairs (bps) and the specimen described here was 96.20% similar to *E. floridanus,* based on the analysis on the BOLD Systems platform. The Maximum Likelihood, Maximum Parsimony, and Neighbor-Joining phylogenetic trees all had a similar topology, in which the *E. glaucinus* specimen from Maranhão formed a clade (99% bootstrap value) with *E. floridanus* from the United States, which was, in turn, a well-supported sister group of *E. perotis* and *E. auripendulus* (Fig. 4). The mean interspecific nucleotide divergence of *E. glaucinus* from the other *Eumops* species varied from 4.3% concerning *E. floridanus,* to 18% in comparison with *E. hansae* (Table 4).

Table 3. Comparison of the craniometric measurements of the *E. glaucinus* specimen collected in the present study with those of the holotype presented by Medina *et al.* (2014).

Craniometric trait	Measurements (mm) of the specimen analyzed in the present study	Medina *et al.* (2014) holotype
Maximum length of the skull	24.0	24.31
Condylobasal length	21.9	—
Braincase height	10.2	—
Braincase breadth	13.1	11.8
Zygomatic arch	14.7	14.52
postorbital breadth	4.8	5.49
Width across upper canines	7.0	6.38
Height of the first molar	10.2	10.3
Mandible length	17.9	18.59
Palate length	9.71	9.71

The Cyt b sequence had 598 bps, and the specimen collected in the present study was 98.44% similar to the *E. glaucinus* sequences on the BLAST platform. The Maximum Likelihood, Maximum Parsimony, and Neighbor-Joining trees all had a similar topology, in which the specimen from Maranhão formed a well-supported clade with the *E. glaucinus* sequences from Venezuela and Paraguay, with 99% bootstrap support,

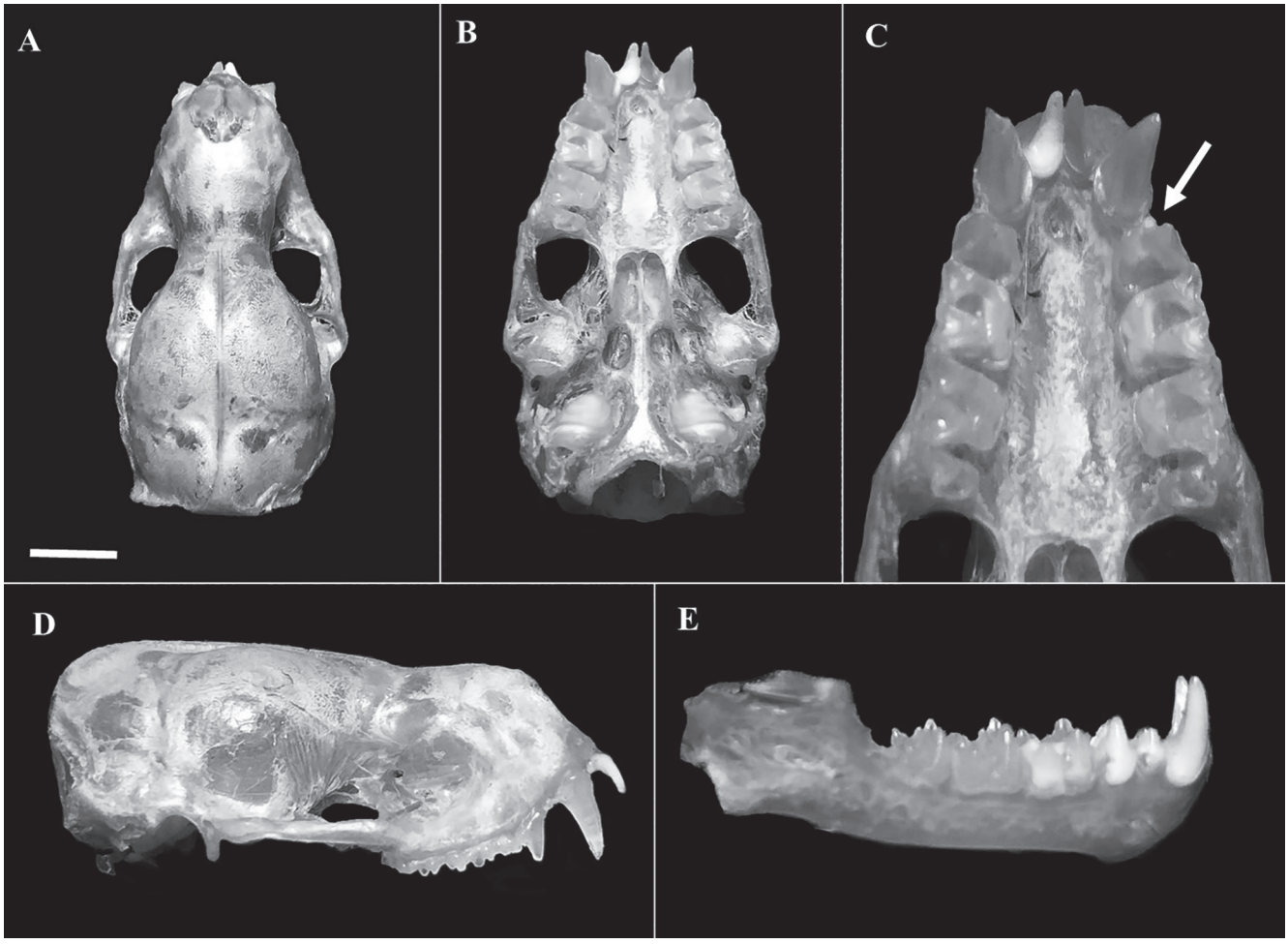

Figure 2. Skull of *E. glaucinus:* (A) dorsal view, showing the bifid upper incisors and diastema, (B) ventral view, showing the size of the canines relative to the incisors, (C) upper tooth row, showing the size and position of the first upper premolar, (D) lateral view, showing the elongation of the flattened, rounded braincase, and (E) lateral view showing the length of the mandible. Scale bar = 10 mm.

Table 4. Matrix of the mean genetic distances between the *Eumops* species (and the outgroup) based on the COI sequences, with the Tamura Nei algorithm. Interspecific divergence is shown below the diagonal, and intraspecific distances are shown along the diagonal, in **bold** type.

Species	Mean genetic distance (%)					
	1	2	3	4	5	6
1. *Eumops glaucinus floridanus* USA	**0.5**					
2. *Eumops glaucinus* MA	4.3	**n/c**				
3. *Eumops hansae* CAN	15.7	18.0	**2.7**			
4. *Eumops perotis* USA	10.8	11.3	21.6	**11.9**		
5. *Eumops auripendulus* FG	14.5	13.6	21.6	15.9	**0.0**	
6. *Lasiurus blossevilli* PAN	30.5	31.2	29.1	31.7	31.7	**n/c**

Legend: n/c = not calculated; USA = United States; MA = Maranhão; CAN = Canada; FG = French Guiana; PAN = Panama.

which is the sister group of the *E. glaucinus* specimens from Cuba and Mexico, with 99-100% bootstrap support (Fig. 5). The mean interspecific nucleotide divergence between *E. glaucinus* (including the specimen from Maranhão) and the other *Eumops* species ranged from 5.5% to 20.9%, while the intraspecific divergence between the specimen from Maranhão and *E. glaucinus* from Paraguay and Venezuela was 1.4%, increasing to 4.9% for the *E. glaucinus* sequences from Cuba and 5.2% for those from Mexico. The divergence from the American *E. floridanus* sequences (5.5%) was virtually the same as that for the Mexican *E. glaucinus* (Table 5).

DISCUSSION

The genus *Eumops* is made up of fast-flying insectivorous bats, which forage in open environments or above the forest canopy (Sodré *et al.,* 2008). These features of the behavior of *Eumops* are assumed to account for the infrequent capture of *E. glaucinus* specimens in Brazil. This species appears to inhabit mainly forests, but

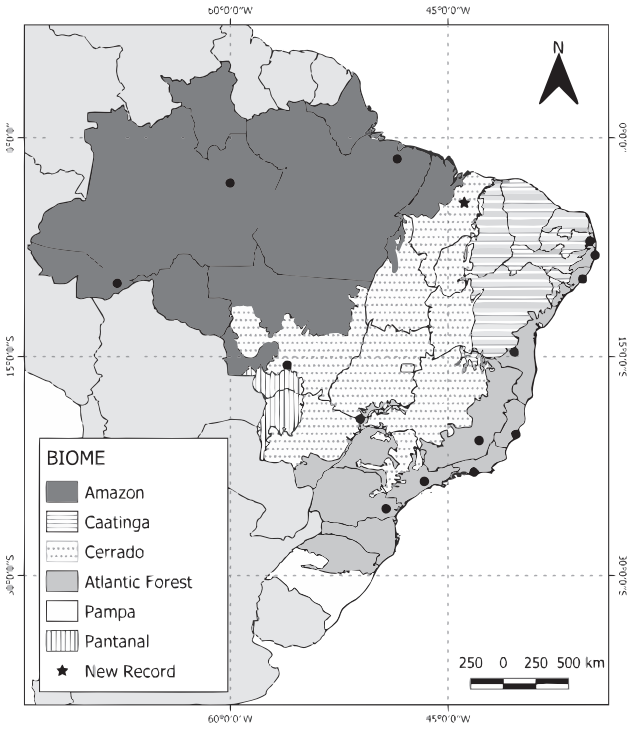

Figure 3. Geographic distribution of *E. glaucinus* in Brazil. The star indicates the collecting locality of the present study, which is the first record of the occurrence of the species in the state of Maranhão. The dots indicate all previous localities in Brazil, as recorded by Manhães (2017).

may roost in rock crevices, tree holes, and even buildings, where it forms small colonies. *Eumops glaucinus* hunts insects in flight, in particular species of the orders Coleoptera, Diptera, Orthoptera, and Hemiptera (Reis *et al.,* 2007). The abundance of insects found in the vicinity of old buildings, such as the disused textile factory in Codó, is favorable to the presence of insectivorous bats, and was presumably a factor contributing to the capture of the *E. glaucinus* individual for the present study.

Table 5. Matrix of the mean genetic distances between the *Eumops* species (and the outgroup) based on the Cyt b sequences, with the Tamura Nei algorithm. Interspecific divergence is shown below the diagonal, and intraspecific distances are shown along the diagonal, in **bold** type. Legend: n/c = not calculated; USA = United States; MA = Maranhão; VEN = Venezuela; MEX = Mexico; ECU = Ecuador; PAR = Paraguay; FG = French Guiana.

Species	Mean genetic distances (%)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Eumops glaucinus* MEX	**0.1**														
2. *Eumops glaucinus* CUB	1.2	**0.4**													
3. *Eumops glaucinus* PAR	4.9	4.6	**1.1**												
4. *Eumops glaucinus* MA	5.2	4.9	1.4	**n/c**											
5. *Eumops glaucinus* VEN	4.0	3.7	0.9	1.4	**n/c**										
6. *Eumops glaucinus floridanus* USA	1.8	1.0	5.2	5.5	4.3	**1.1**									
7. *Eumops bonariensis* MEX	17.9	17.7	18.0	17.3	17.2	18.6	**n/c**								
8. *Eumops hansae* ECU	22.4	22.3	19.7	20.9	19.9	23.0	22.6	**n/c**							
9. *Eumops hansae* VEN	22.0	21.9	19.3	20.2	19.6	22.6	21.7	0.7	**0.5**						
10. *Eumops auripendulus* FG	13.1	13.8	13.6	13.5	12.7	15.0	17.6	21.2	20.8	**0.2**					
11. *Eumops perotis* USA	13.0	13.3	14.5	14.0	13.6	13.5	18.9	20.1	19.3	13.7	**0.3**				
12. *Eumops patagonicus* PAR	16.3	16.0	18.9	18.2	18.1	16.8	6.5	20.2	19.4	16.6	17.9	**n/c**			
13. *Eumops wilsoni* ECU	16.0	15.7	18.2	17.6	17.4	16.5	6.3	19.5	18.7	16.2	17.3	0.4	**8.8**		
14. *Eumops maurus* ECU	9.6	10.0	10.9	11.0	10.0	10.6	18.1	19.1	18.8	9.3	11.7	17.2	16.7	**n/c**	
15. *Lasiurus blosevillii* BOL	32.5	32.9	31.9	32.9	32.0	33.6	39.0	35.3	36.0	35.6	35.9	37.2	36.2	35.5	—

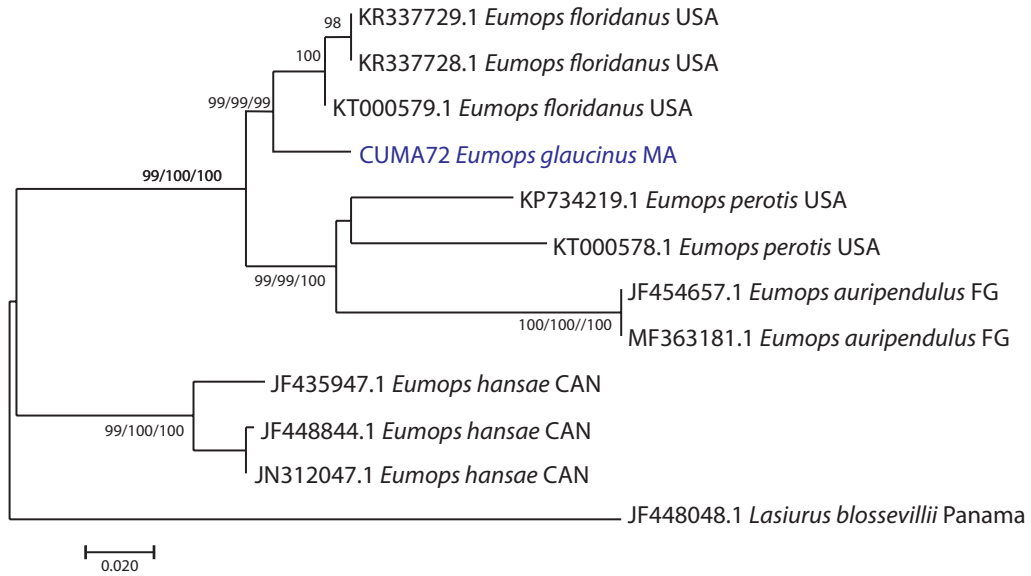

Figure 4. Neighbor-Joining phylogenetic tree of *Eumops* species based on the analysis of the COI sequences, using the Tamura-Nei algorithm. The *E. glaucinus* specimen from Maranhão (CUMA72) is highlighted. The values at each node refer to the Neighbor-Joining/Maximum Parsimony/Maximum Likelihood bootstrap scores. Legend: USA = United States; CAN = Canada; FG = French Guiana; MA = Maranhão, Brazil.

The external morphology of *E. glaucinus* is similar of the other species of the genus, although it can be differentiated from *E. floridanus* by its medium size, in comparison with the much larger *E. floridanus* (Timm & Genoways, 2004) and the far geographic distance. The cranial features of *E. floridanus* are also distinct from those of *E. glaucinus,* including the maximum cranial length, condylobasal length, and the width of the zygomatic arch, which are all relatively large, and also differentiate the species from the other members of the genus (Eger, 1977; Peracchi *et al.,* 2011). In general, the craniometric and morphological data collected from the Maranhão specimen support its identification as *E. glaciunus* (Fig. 2 and Tables 2 and 3).

The Cyt b sequence of the specimen from Maranhão diverges only marginally (1.4%) from those of the *E. glaucinus* specimens from Paraguay and Venezuela, which is consistent with an intraspecific level of differentiation, considering the 2% threshold proposed by Bradley & Baker (2001). The Maranhão sequence nevertheless diverges considerably from the *E. glaucinus* sequences from Cuba (4.9%) and Mexico (5.2%), and in fact, these distances are similar to that recorded for *E. floridanus* from the United States (5.5%). In the case of the COI gene, the smallest distance recorded between the Maranhão sequence and the other *Eumops* sequences was recorded in the case of the American *E. floridanus,* with a distance of 4.3%. This level of divergence is typical of that found between populations (Bradley & Baker, 2001).

McDonough *et al.* (2008) concluded that *E. glaucinus* are complex species, and Gregorin (2009) was unable to elucidate the relationship between this complexity and the other *Eumops* species. Bartlett *et al.* (2013) has failed to elucidate the relationship of the *E. glaucinus* complexity to other species of the genus *Eumops,* Gregorin *et al.* (2016) recognized four species groups, including an *E. glaucinus* species group, which contained *E. floridanus, E. glaucinus, E. wilsoni, E. ferox, E. dabbenei,* and *E. underwoodi*. Despite these advances, some of the relationships within the genus remain unresolved, including those of the *E. glaucinus* complexity, and a more definitive arrangement will require more systematic and integrative analyses that include a broader selection of taxa. In this context, it will be especially important to integrate different approaches to the understanding the diversity of the genus, given the often-inconclusive findings of the genetic analyses.

CONCLUSIONS

The combined analysis of the morphological and molecular data confirmed the occurrence of *E. glaucinus* in the Brazilian state of Maranhão, it's a location that extends its known distribution by 660 km east from the nearest locality, Belém, Pará. The study also provides the first sequence of the mitochondrial rRNA 16S gene of *E. glaucinus* deposited on the Genbank in addition to the sequences of the other genes (COI and Cyt b).

AUTHORS' CONTRIBUTIONS: FHSC, CLSC, APMO: Conceptualization; **FHSC, ACSL, CLSC, SBM, APMO:** Visualization; **FHSC, SBM, MCB:** Data curation; **FHSC, MCB:** Formal analysis; **FHSC, ECF, MCB:** Validation; **ACSL, SBM:** Methodology; **ACSL, CLSC, SBM, APMO, ECF, MCB:** Writing – review & editing; **ECF, MCB:** Supervision, Funding acquisition, Project administration; **FHSC:** Writing – original draft, Investigation. All authors actively participated in the discussion of the results; they reviewed and approved the final version of the paper.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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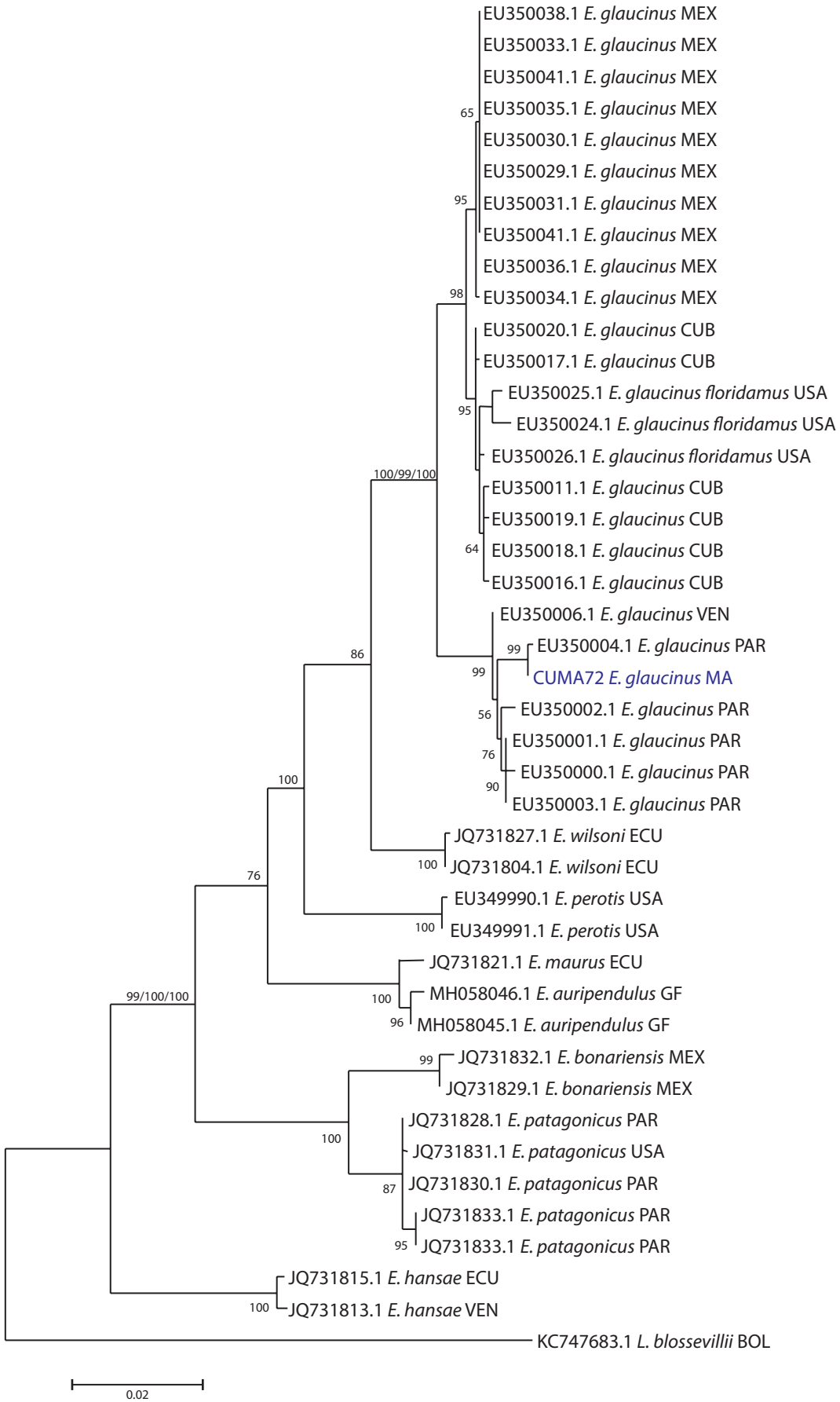

Figure 5. Neighbor-Joining phylogenetic tree of *Eumops* species based on the analysis of the COI sequences, using the Tamura-Nei algorithm. The *E. glaucinus* specimen from Maranhão (CUMA72) is highlighted. The values at each node refer to the Neighbor-Joining/Maximum Parsimony/Maximum Likelihood bootstrap scores. Legenda VEN = Venezuela, CUB = Cuba, MEX = Mexico, ECU = Ecuador, FG = French Guiana; PAR = Paraguai, USA = United States, BOL = Bolivia, MA = Maranhão, Brazil.

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