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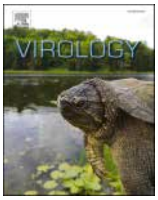

Bat coronavirus surveillance across different habitats in Yucatán, México

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ABSTRACT

Bats, which play a vital role in maintaining ecosystems, are also known as natural reservoirs of coronaviruses (CoVs), thus have raised concerns about their potential transmission to humans, particularly in light of the emergence of MERS-CoV, SARS-CoV, and SARS-CoV-2. The increasing impact of human activities and ecosystem modifications is reshaping bat community structure and ecology, heightening the risk of the emergence of potential epidemics. Therefore, continuous monitoring of these viruses in bats is necessary. Despite the rich diversity of bats species in México, few studies have been conducted to search for CoVs in these group of mammals. In the present study we conducted CoV surveillance across previously unexplored sites in the state of Yucatán, a state located within the ecologically diverse Yucatán Peninsula, a tropical region undergoing pronounced anthropogenic changes, including deforestation, agricultural expansion and urbanization. We captured 191 bats between 2021 and 2022 in three different habitats: diversified rural (Tzucacab), rural (Tizimín) and urban (Mérida). Molecular analyses had revealed a 5.4% CoV prevalence, with the diversified rural site exhibiting a notably elevated rate (26.3%). Subsequent sequencing and phylogenetic assessment revealed four distinct Alphacoronavirus genotypes, indicating host-specific clustering among Phyllostomidae bats. Notably, one was detected for the first time in a *Sturnira* species. Our findings suggest a reduced likelihood of transmission of these viruses to humans or other species, evidenced by clustering patterns and sequence dissimilarity with known CoVs. We emphasize that maintaining sustained virus surveillance in bats is crucial to understanding viral diversity and identifying potential risks to human and animal health.

1. Introduction

The recent COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the preceding outbreaks of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2009, have prompted research into coronaviruses due to concerns about potential future epidemic outbreaks (Mulabbi et al., 2021; Markov et al., 2023). Coronaviruses (CoVs) are broadly present in nature, classified in four genera: *Alphacoronavirus* (αCoV), *Betacoronavirus* (βCoV), both infecting domestic and wild mammals, and *Gammacoronavirus* (γCoV) and *Deltacoronavirus* (δCoV), primarily detected in birds but also in mammals. Bats harbor the highest diversity of αCoVs and βCoVs, acting as their natural reservoirs (Cui et al., 2019; Shehata et al., 2022).

Evidence based on genetic evolution, pathogenesis and receptor binding data strongly suggests that bats might have played an essential role in the evolution and transmission of CoVs to humans, such as SARS-CoV and MERS-CoV, that spilled over from bats to humans through intermediary hosts (e.g. farmed civets) (Cui et al., 2019; Ruiz-Aravena et al., 2022). Likewise, it is considered that the evolutionary origin of

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SARS-CoV-2 occurred in bats, although the transmission route to humans has not yet been fully demonstrated (Domingo, 2022; Pagani et al., 2023).

The factors contributing to the spillover are complex and dynamic, the primary driver recognized for facilitating the transmission of zoonotic pathogens from wildlife to human populations is human-induced habitat modification and land use changes. Indeed, deforestation for intensive agriculture and cattle raising, as well as urbanization are some of the main anthropogenic drivers of habitat change and loss of biodiversity. This increases the probability of contact between humans and wild animal pathogens (Plowright et al., 2021; Escudero-Pérez et al., 2023; Sánchez-Soto, 2024).

Ongoing research has documented the prevalence of natural CoVs infections in bats across several countries using molecular methods, with a significant concentration of studies in Asia since the SARS-CoV outbreak (Cui et al., 2019; Cohen et al., 2023). In the Americas, the majority of studies have been done in Brazil (Hernández-Aguilar et al., 2021) while in México only three studies have been conducted across seven states (Ciudad de México, Campeche, Chiapas (Anthony et al., 2013); Morelos, Puebla, Hidalgo (Martínez Duque) and Jalisco (Góes et al., 2013). Among the studies conducted in the Americas, only the bat Mex-CoV-9 discovered in Calakmul, Campeche, located in the Yucatán Peninsula, México, exhibits a genetic sequence association with human MERS-CoV (Hernández-Aguilar et al., 2021; Anthony et al., 2013).

The Yucatán Peninsula in southeastern México comprises the states of Yucatán, Quintana Roo and Campeche, a tropical region with a warm subhumid climate, exhibiting significant rich biodiversity of many taxa, including mammal species. Of the approximately 1400 globally recognized bat species, 142 are identified in México, with at least 62 documented in the Yucatan Peninsula (Torres-Castro et al., 2019; Gómez Durán, 2024). In the last 40 years, this region has undergone significant habitat modifications and land use changes, marked by a notable increase in deforestation and expansion of areas dedicated to agriculture and livestock, concurrently with substantial increase in human population (Dupuy Rada, 2020). In Yucatán, over 270,000 ha were deforested between 2001 and 2022 (Biles and Lemberg, 2023; Sistema Nacional de Monitoreo Forestal). Mérida, the most densely populated city in the region, has experienced rapid demographic expansion, swelling from 400,142 residents in 1980 to nearly 1 million by 2020 (Biles and Lemberg, 2023; Sistema Nacional de Monitoreo Forestal). These conditions create an ideal environment for the potential emergence or reemergence of zoonotic diseases (Sánchez-Soto, 2024; Haro et al., 2021). In this context, in this study we aim to further the CoVs surveillance in bats in the Yucatán Peninsula region, specifically focusing on three distinct habitats in the state of Yucatán that have not been previously investigated.

2. Materials and methods

2.1. Study site and sampling

The study focused on three locations in Yucatán state, classified as diversified rural, rural and urban according to data from vegetation cover, land use class (Series VI INEGI) and population census (INEGI, 2020). The first municipality, Tzucacab, is categorized as diversified rural, maintaining up to 40% native vegetation and low population density. Moving northwest, Tizimín, the rural locality, is characterized by over 40% induced vegetation and a population density ranging from low to moderate. In the northwest, the urban site, Mérida, stands out for hosting over 60% urban settlements and the highest population density (Fig. 1A). The characteristics and geographical coordinates of these sites are detailed in S1 Table 1.

Samples were collected during different rainy periods, in Tzucacab during October 2021 and June 2022, Tizimín in October 2021, and Mérida in October 2021 and July 2022. Each bat sampling period encompassed three consecutive nights per location with ten 5 × 12 m mists nets, opened at sunset and remaining open for 5 h consecutively. Morphometric parameters, sex, age and species of captured bats were recorded. Species identification was carried out based on morphological characteristics using established field guides (Medellin et al., 2008), with adjustments based on recent studies (Calahorra-Oliart et al., 2021; Hernández-Canchola and León-Paniagua, 2020). In addition, the geographical region was also considered, as certain species are typically found in specific ecological zones of the Yucatán Peninsula (Medellin et al., 2008). Oral and rectal swabs were collected in tubes with 500uL RNALater (Invitrogen). After sampling, each specimen was released at

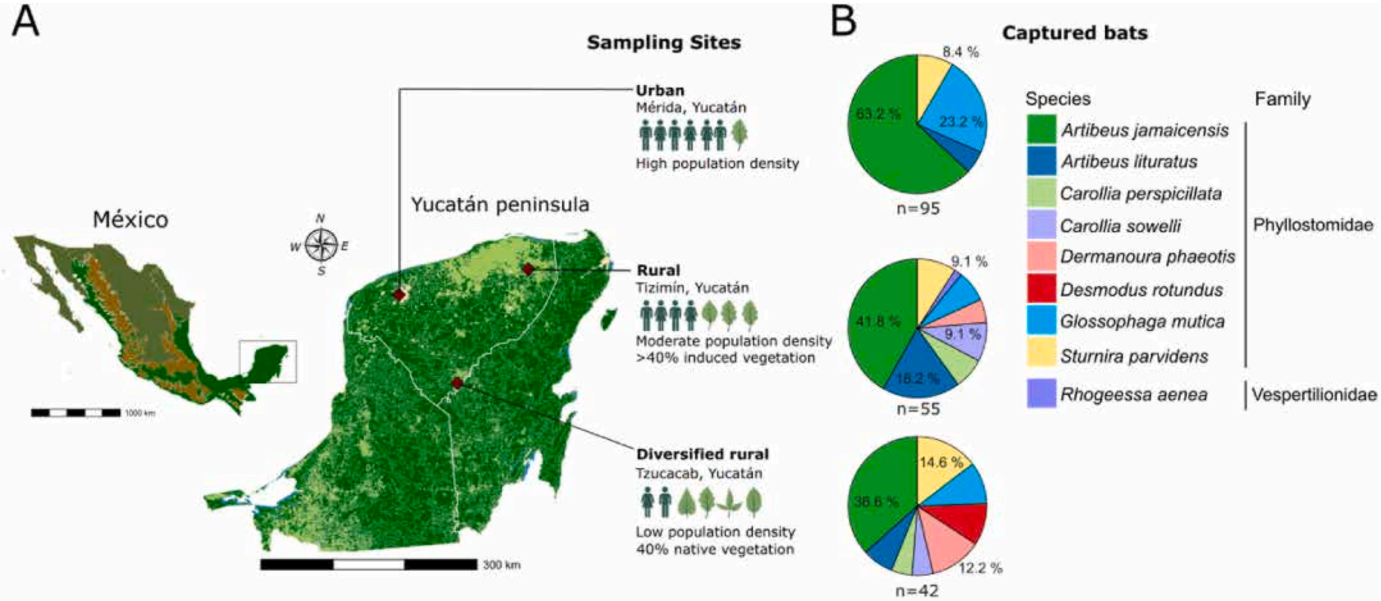

Fig. 1. Study site and bat sampling. A total of 191 bat individuals were captured in Yucatán, México. Sampling locations were classified as diversified rural (Tzucacab), rural (Tizimín) and urban (Mérida) according to vegetation cover, land use class (Series VI INEGI) and population census (INEGI, 2020). Percentages of bat species and families captured per site were shown. Maps were created with the R library ggspatial v1.1.7 (Dunnington and Thorne, 2020), using the mexican biogeographic provinces (Morrone et al., 2017) and land use and vegetation chart (Series VI INEGI) shapefiles.

the capture site. Samples were transported to the laboratory in liquid nitrogen and stored at −80 °C until laboratory processing. To ensure the safety of field workers and prevent potential accidental infections, all team members followed strict biosafety protocols. These included wearing personal protective equipment such as disposable gloves, face shields, and N95 face masks. Field equipment and surfaces were disinfected with 70% ethanol and sodium hypochlorite after each sampling session. Additionally, vaccination against rabies was a prerequisite for all participants involved in direct bat handling. All procedures were conducted by veterinarians according to the American Society of Mammalogists guidelines for use of wild mammal species (Sikes and Animal Care and Use Committee of the American Society of Mammalogists, 2016) and with the corresponding scientific collecting permit from Secretaría del Medio Ambiente y Recursos Naturales (Semarnat-FAUT-0250). The procedures for animal collection and sampling were approved by the ethics committee Institutional Subcommittee for the Care and Use of Experimental Animals (SICUAE) from the Veterinary Medicine and Husbandry Faculty at the Universidad Nacional Autonoma de Mexico (FMVZ-UNAM) permit SICUAE.DC-2022/2-2.

2.2. *CoVs molecular detection*

RNA was extracted from oral and rectal swabs using the QIAamp Viral RNA Mini Kit (QIAGEN), following the manufacturer protocol. Detection of CoVs was carried out using a pan-CoV nested polymerase chain reaction (PCR) assay described by Chu et al. with modifications (Chu et al., 2011), targeting a 440 bp region of the RNA-dependent RNA polymerase (RdRP) gene. cDNA synthesis and the initial PCR were conducted using the OneStep SuperScript III kit (Invitrogen), while the second PCR reaction was performed with HotStarTaq Plus DNA Polymerase (QIAGEN). PCR conditions were in accordance with the published protocol, with the annealing temperature in the second reaction adjusted to 54 °C. Amplicons were visualized on a 2% agarose gel stained with SYBR Safe DNA Gel Stain (Invitrogen). The prevalence of CoVs in each locality was calculated as follows: the number of positive bats (n) in a locality divided by the total number of bats analyzed (N) in that locality, expressed as a percentage (%). Due to logistical challenges during some sampling periods, not all captured bats were sampled. Additionally, individuals that were visibly stressed, pregnant females, and small juveniles were not sampled to minimize potential adverse effects. Despite these limitations, all collected samples were successfully processed.

Positive samples were further processed by a second pan-CoV nested PCR described by Quan et al. (2010). This method amplifies a distinct and non-overlapping region of the RdRP gene of 328 bp, thereby expanding the comparison range with previously reported CoV sequences. Importantly, this protocol was used in two of the three studies conducted in México (Anthony et al., 2013; Martínez Duque). SuperScript III kit (Invitrogen) was used for cDNA synthesis with the random hexamers standard protocol. Both first and second reactions were performed using HotStarTaq Plus DNA Polymerase (QIAGEN). Amplicons of positive samples were purified and Sanger sequenced in Macrogen Europe (Amsterdam, The Netherlands).

2.3. *Sequence and phylogenetic analyses*

Two sets of sequences of 440 bp and 328 bp amplicons were processed in Geneious Prime v. 2023.1.2 (https://www.geneious.com). After the manual trimming of low-quality ends based on electropherograms, the consensus of forward and reverse reads were generated. Subsequently, the consensus sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) to determine their identities with other entries in the database. The CoV sequences identified in this study have Genbank accession numbers PP869944-PP869960. Corresponding metadata for these sequences and BLAST results are provided in S2 Table 1 and 2 For phylogenetic tree construction, the reads with the highest nucleotide identity identified through BLAST were selected, along with CoV sequences of lower sequence identity reported in the same publications, predominantly from neotropical bats. Sequences from both human and veterinary significant CoVs were also incorporated. In addition, we compared the CoV sequences from this study with CoVs from other hosts, including non-neotropical bats and other mammals, whose complete genomes are available in the NCBI Virus database (NCBI Virus). For this analysis, the corresponding RdRP fragments were extracted. The sequence alignments were performed with Clustal omega v.1.2.2 and cured manually in Geneious Prime, followed by phylogenetic tree building using the Neighbor-Joining method with 1000 bootstrap replicates (Sievers et al., 2011). The sequences used for phylogenetic tree construction are included in S2 Tables 3 and 4 The nucleotides and amino acids alignments of the CoV sequences from this study are found in S1 Figs.

3. Results

3.1. *Bat sampling*

A total of 191 bats were captured between 2021 and 2022 in three Yucatán locations categorized according to their land use: 42 bats in Tzucacab (diversified rural), 55 in Tizimín (rural) and 95 in Mérida (urban) (Fig. 1A). Almost all the captured individuals (190/191, 99.5%) belonged to the Phyllostomidae family, except for *Rhogeessa aenea* (Vespertilionidae*)*. The most abundant species recorded were *Artibeus jamaicensis*, *A. lituratus, Sturnira parvidens* and *Glossophaga mutica* (Fig. 1B). These species were present in all three locations, constituting 66, 76, and 100% of the individuals captured in diversified rural, rural and urban sites, respectively.

3.2. *Molecular CoV detection*

From the total of bats captured, we sampled and tested 167 (164 oral and 165 rectal swabs) using pan-CoV PCR. Our findings revealed nine positive bats from three distinct species, indicating a global prevalence of 5.4% and showing variation according to site (Fig. 2A). The greatest rate was found in the rural diversified site (5/19, 26.3%), where we identified two positive individuals of *S. parvidens* and three *A. jamaicensis*. In contrast, rural and urban sites lower prevalence. In the rural site, one positive *A. jamaicensis* was found (1/55, 1.8%), and in Mérida, two *G. mutica* and one *A. jamaicensis* (3/93, 3.2%) tested positive (Fig. 2b). Notably, a higher CoVs detection rate was observed in rectal swab samples (8/9, 88.9%) compared to oral swabs (2/9, 22%). The summarized results of bat captures and CoV detections are presented in Table 1. The database including date, capture site, the taxonomic classification of bats captured, sample type analyzed and CoV detection results are available in S1 Table 2.

3.3. *Sequence and phylogenetic analysis*

Amplicon sequencing of two genomic regions of the RdRP gene allowed us to identify four distinct CoVs genotypes, all classified as αCoV*s* and named here as Yuc_CoV_1a, 1b, 2 and 3. None of these corresponded to novel CoVs sequences, since they are highly similar (>90% sequence identity) to other sequences previously reported according to BLAST analysis.

Likewise, sequences of both regions yielded two phylogenetic trees and expanded our range of comparison of these CoVs with those previously reported in bats from México and other countries. Phylogenetic analysis revealed that these αCoVs sequences show a strong affinity for specific hosts, as they share greater identity (Fig. 3) and clustered with αCoVs sequences previously found in neotropical bats of the same or related genus or species from the Phyllostomidae family, despite being captured in geographically distant locations. In contrast, they are phylogenetically distant from αCoVs found in other bat families, such as

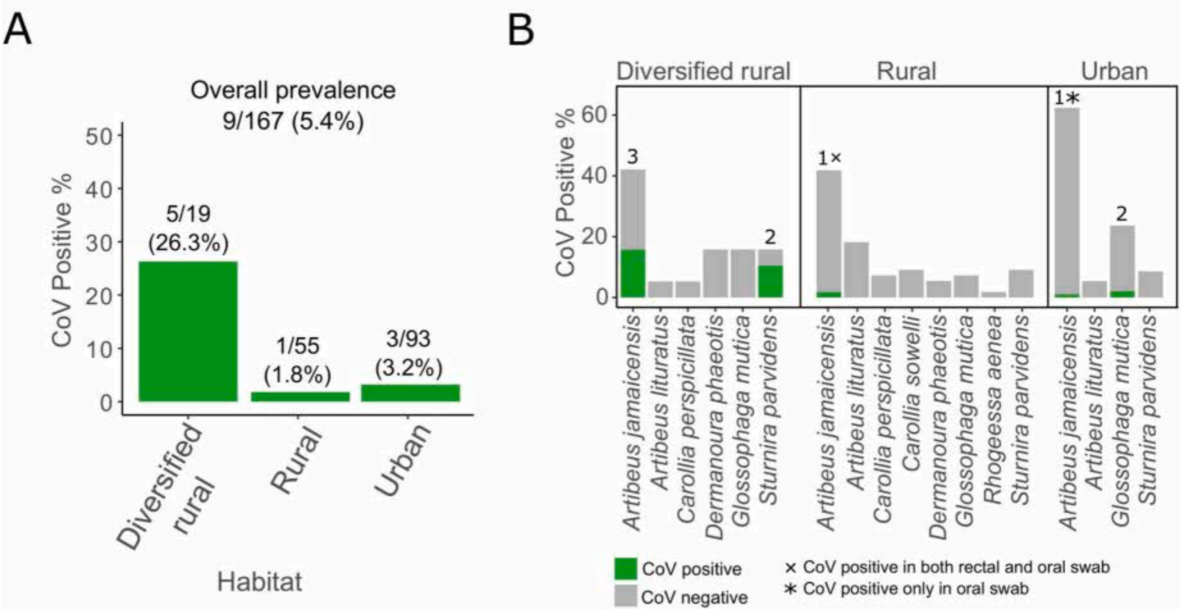

Fig. 2. Coronavirus (CoVs) molecular **detection.** (A) Percentage of overall and site-specific: diversified rural (Tzucacab), rural (Tizimín) and urban (Mérida) prevalence. The number of CoV-positive cases (n) out of the total cases tested (N) expressed as a percentage %. (B) CoV prevalence was categorized by site and bat species tested. The numbers on the bars indicate the total number of individual positives. All CoVs were detected only in rectal swabs, except where indicated.

Molossidae or Vespertilionidae. This pattern is consistent when analyzing both RdRp fragments (Fig. 4, S1). For example, Yuc_CoV-1a was identified in *A. jamaicensis* from both the diversified rural and the rural sites. It shares >99% sequence similarity with Mex_CoV_4 previously found in *A. jamaicensis* in Campeche, México (Anthony et al., 2013), less than 150 km away. Moreover, Yuc_CoV-1a exhibits >98% sequence identity with αCoVs in Panama and Brazil (Corman et al., 2013; Bueno et al., 2022), despite a distance of 3000 km.

Interestingly, a second cluster named Yuc_CoV-1b was found in *S. parvidens* from the diversified rural site. It shares similarities with Yuc_CoV-1a and αCoVs sequences from *Artibeus* but is clustered in distinct subclades in both phylogenetic reconstructions (Fig. 4, S1). Yuc_CoV-1b shows a sequence identity of ~94% and ~96% compared to Yuc_CoV-1a, based on 440 bp and 328 bp fragments, respectively. This is the first report of this CoV lineage in *Sturnira* species, according to BLAST reports.

Similarly, Yuc_CoV-2 was detected in *A. jamaicensis* from both the diversified rural and urban sites, showing the highest sequence identity with αCoVs documented in *A. jamaicensis* from Chiapas (México), Panama and Costa Rica. It is also clustered with αCoVs found in *A. lituratus* and *Phyllostomus discolor* (Phyllostomidae) in Brazil. Likewise, Yuc_CoV_3 was detected in the urban site in *Glossophaga mutica* and may to be restricted to this specific genus, clustering only with αCoVs previously identified in *Glossophaga* sp. from Puebla (México), Trinidad and Tobago, and Brazil (Martínez Duque; Corman et al., 2013; Moreira-Soto et al., 2015; Carrington et al., 2008). The sequence comparisons of the CoVs identified in this study with those from non-neotropical bats and other hosts with available complete genomes are presented in S1 Fig. 3.

4. Discussion

Bats play a crucial role in ecosystems by positively contributing to various ecological functions, including pollination, seed dispersal, and vector control. They are also reservoirs for a range of endemic, emerging and reemerging zoonotic pathogens, including CoVs. The changes in ecosystems caused by human activities increase the interaction between bats and humans (Plowright et al., 2021). Characterizing the transmission of pathogens from wildlife to animals and humans is an ongoing critical challenge. Consequently, it is essential to continuously monitor CoVs in bat populations (Hernández-Aguilar et al., 2021). This surveillance is crucial for establishing measures to control and prevent zoonosis emergence, particularly considering the growing occurrence of such events attributable to human-induced modifications of the environment (Plowright et al., 2021). We here identified αCoVs in bats from three different habitats in the state of Yucatán, México, exhibiting a gradient level of land use.

Most of the bats captured in this study belong to the Phyllostomidae family, typical of Neotropical regions; this result may be associated with the use of mist nets that favor the capture of phyllostomid bats (Moguel-Chin et al., 2023; Plasencia-Vázquez et al., 2020; Pech-Canché et al., 2011). In Mérida, the urban and most densely inhabited region of the Yucatán Peninsula, only four different species were recorded, three frugivorous *Artibeus* (*A. jamaicensis* and *A. lituratus*) and *Sturnira parvidens*, and one nectarivorous, *Glossophaga mutica*. Although phyllostomid bats are sensitive to habitat disturbance by humans, *Artibeus*, *Sturnira*, and *Glossophaga* are considered generalist species that are present in modified habitats and show certain resilience to human activity (Calahorra-Oliart et al., 2021; Plasencia-Vázquez et al., 2020; Diversidad y estructura genética de artibeus jamaicensis (chiroptera: phyllostomidae) en chiapas; García-García et al., 2014). Both Tzucacab and Tizimín, the diversified rural and rural sites, exhibited higher species counts, likely attributed to their higher percentage of conserved habitat and presence of native vegetation in comparison with the urban area (Sánchez-Soto, 2024; Medellín et al., 2000).

We found a 5.4% prevalence of CoVs, which is comparable to the overall values reported in North and South America, 5.64 and 6.52%, respectively (Warmuth et al., 2023). Notably, the detection rate was different in each land use. The diversified rural site showed the highest prevalence of CoVs at 26.3%, being the most conserved site among the three. This contrasts with findings in neotropical bats from Brazil, where higher viral prevalence was observed in deforested areas compared to forested ones within the Atlantic Forest. (Loh et al., 2022). Similarly, in China, higher prevalence of SARS-related CoVs in rhinolophid bats was reported in areas with greater human impact (Rulli et al., 2021). In contrast, another study found no association between CoV prevalence and the degree of habitat disturbance (Seltmann et al., 2017).

These discrepancies may be attributed to the heterogeneity of ecosystems across studies, where diverse environmental variables and bat

Table 1
Summary of bat species recorded and coronavirus (CoV) detection results.

Location	Land use classification	Species	Captured (n)	CoV PCR tested (n)	CoV positive (n (%))
Mérida	Urban	*Artibeus jamaicensis*	60	58	1 (1.7)
		Artibeus lituratus	5	5	0
		Glossophaga mutica	22	22	2
		Sturnira parvidens	8	8	0
Tizimín	Rural	*Artibeus jamaicensis*	23	23	1 (4.3)
		Artibeus lituratus	10	10	0
		Carollia perspicillata	4	4	0
		Carollia sowelli	5	5	0
		Dermanoura phaeotis	3	3	0
		Glossophaga mutica	4	4	0
		Rhogeessa aenea	1	1	0
		Sturnira parvidens	5	5	0
Tzucacab	Diversified rural	*Artibeus jamaicensis*	15	8	3 (37.5)
		Artibeus lituratus	3	1	0
		Carollia perspicillata	2	1	0
		Carollia sowelli	2	0	0
		Dermanoura phaeotis	5	3	0
		Desmodus rotundus	4	0	0
		Glossophaga mutica	4	3	0
		Sturnira parvidens	6	3	2 (66.7)

species traits and functional diversity within specific ecological niches lead to distinct host-pathogen interactions. Additionally, seasonality likely plays a significant role in bat behaviors, such as feeding and breeding, which may affect CoV prevalence. For example, *Glossophaga mutica*, a nectarivorous species, was found in all localities of our study. In Mérida, a higher proportion of *G. mutica* was observed during the early rainy season (June) compared to the end of the rainy season (October) (28% vs. 8%). Higher detection may reflect greater activity during the early rainy season, which is linked to increased food resource availability and its breeding season (John van Dort Composition). This increased activity and mating behaviors during these months may elevate the likelihood of CoV detection and transmission (Loh et al., 2022). Notably, we found two CoV-positive *G. mutica* individuals during this period. Similarly, the breeding seasons of other frugivorous bat species, such as *Artibeus jamaicensis* and *Sturnira parvidens*, both of which yielded CoV-positive samples, differ from one another, further highlighting the role of seasonality (John van Dort Composition; Ortega et al., 2021).

Differences in study designs also contribute to the observed variations. For instance, the Brazilian study primarily sampled during the dry season, whereas our study focused exclusively on the rainy season. Moreover, the Brazilian study reported pooled prevalence of several viruses (e.g., Herpes, Hanta, and Paramyxovirus) without specifying the prevalence of CoVs alone. Additionally, their study sites included both forested and deforested areas of the Atlantic Forest, but did not provide detailed information on human populations in these areas (Loh et al., 2022). In contrast, our study incorporated three sites with varying levels of human density and habitats may not be comparable. Similarly, the study from China focused on insectivorous rhinolophid bats and SARS-related CoVs (Rulli et al., 2021), which may not reflect the dynamics observed in phyllostomid bats in the Yucatán Peninsula. For example, studies from Asia, mainly from China, tend to report CoV prevalence 2-fold higher compared to those observed in neotropical bats (Warmuth et al., 2023). This suggests that the dynamics of CoV transmission and the driving factors may differ geographically, making direct comparisons between regions challenging.

Importantly, the diversified rural site has in fact a highly heterogeneous landscape, where natural and modified habitats intermingle, and with higher density of domestic animals; hence, there is increased interaction between people, domestic animals and wildlife, facilitating the transmission and dispersion of zoonotic pathogens (Sánchez-Soto, 2024; Allen et al., 2017). To confirm our results, further intensive and long-term studies covering both rainy and dry seasons with greater sample size might be developed with the aim to deeply study the effect of land use changes on the diversity of bats and the presence of CoVs. We suggest that prevalence changes of species with viral detection in the three habitats, such as *A. jamaicensis,* can be used as sentinel species for unravel the effect of land use on CoV emergence.

In this study, we identified four different αCoVs genotypes previously reported in México and other Latin American countries (Anthony et al., 2013; Martínez Duque; Góes et al., 2013; Corman et al., 2013; Bueno et al., 2022; Moreira-Soto et al., 2015; Carrington et al., 2008; Góes et al., 2016). Additionally, three out of the four genotypes were found in the diversified rural site, standing out not only for its high prevalence but also as a potential site hosting a diversity of CoVs. Two closely related genotypes, Yuc_CoV_1a and Yuc_CoV_1b, were identified at this locality, each associated with distinct yet related bat species, *A. jamaicensis* and *Sturnira parvidens*, both from the Stenodermatinae subfamily (García-García et al., 2014). The observed slight nucleotide sequence differences (4–6%) between Yuc_CoV_1a and Yuc_CoV_1b indicate independent evolutionary paths, suggesting that these CoVs have adapted to the specific conditions imposed by different host species (Gupta et al., 2021). Both genotypes are associated with Mex_CoV_4 (>95% sequence identity) found in a nearby site (Campeche) described as a forested area (Rico-Chávez et al., 2015). In addition, Yuc_CoV_1a and Yuc_CoV_2 from the diversified rural site were also found in the urban and rural sites, respectively. Yuc_CoV_2 is associated with Mex_CoV_5 (>95% sequence identity) found in fragmented and forested sites of Chiapas, México (Rico-Chávez et al., 2015). The presence of these CoVs in both natural and human-inhabited environments is attributed to the generalist nature of the hosts, allowing them to reside and disperse between habitats that also may facilitate viral spread (Smith and Wang, 2013). This underscores the potential for CoVs to disseminate across various ecological niches within the region.

Remarkably, a higher detection rate of CoVs was observed in rectal compared to oral swabs. This underscores the tropism of these CoVs for the bats gastrointestinal tract and highlights feces as an important transmission source (Hernández-Aguilar et al., 2021). Moreover, this observation suggests that, when prioritizing diagnostic resources, rectal swab samples are preferable for CoV surveillance.

When comparing the nucleotide sequence of αCoVs found in this study with the known αCoVs that infect humans, hCoV-NL63 and hCoV-229E, we observed marked divergence, with less than 76% of sequence identity. Similarly, comparing them to other important αCoVs affecting animal health, such as Porcine Epidemic Diarrhea Virus (PEDV), Swine Acute Diarrhea Syndrome (SADS), and Transmissible Gastroenteritis Virus (TGEV) that infect pigs, there was notable genetic divergence, with less than 75% identity. Comparing with αCoVs from non-neotropical bats and other mammals showed less than 80% sequence identity.

As none of the CoVs from this study were categorized within the βCoV genus, they significantly differ from human-infecting viruses such

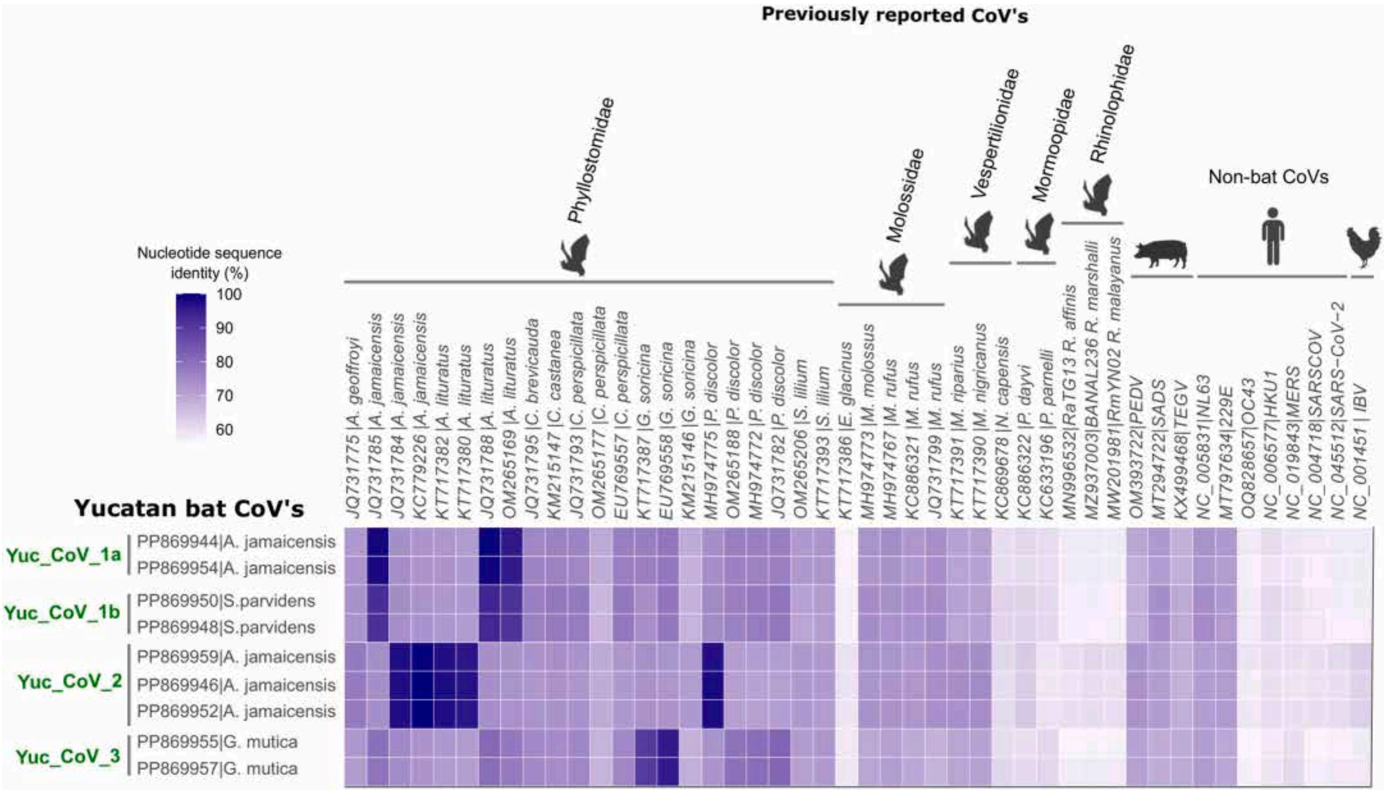

Fig. 3. Heat-map of nucleotide sequence identity of the RdRP fragment (440 bp) of CoVs found in Yucatán compared to those previously reported in bats and other hosts. On the left side, CoVs identified in this study are shown (Genbank Accession numbers and bat species). CoVs derived from previous studies are shown in the upper section. Hosts are depicted with animal silhouettes created with BioRender.com, and respective families, identifying each sequence with their Genbank accession number and bat species. For non-bat host CoVs, the Genbank accession numbers and CoV names are provided.

as MERS-CoV, SARS-CoV, SARS-CoV-2, hCoV-OC43, and hCoV-HKU-1 (Mulabbi et al., 2021; Thakor et al., 2022). To date, there is no evidence that phyllostomid bats naturally harbor SARS-related CoVs, in contrast to the Rhinolophidae and Hipposideridae families found only in the Old World but absent in the Americas. Similarly, MERS-related CoVs have been linked to other bat families like Vespertilionidae, Molossidae, Emballonuridae and Nycteridae (Ruiz-Aravena et al., 2022; Gupta et al., 2021; Tian et al., 2022).

Our sequence identity findings and the fact that these viruses have only been found in related hosts suggest a diminished likelihood of transmission of these bat αCoVs to other species, including humans. This is in agreement with previous reports comparing bat CoVs sequences from México with SARS-CoV-2 (Colunga-Salas and Hernández-Canchola, 2021). It is noteworthy that our study, based on two different fragments of the conserved RdRP gene, yielded consistent results. Furthermore, using both regions also allowed us to expand our comparative spectrum with CoVs sequences from other studies, especially for those lineages whose complete genomes have not yet been reported. Sequencing other CoVs genomic regions, like the spike protein gene, or ideally complete genomes, would provide additional insights for the potential transmission of these viruses to other species (Gonzalez-Isunza et al., 2023).

This study provides initial insights into the significant potential diversity of bat CoVs within the Yucatán state; however, continuous surveillance remains crucial for improving public health efforts and mitigation of future spillover risk. Bat species from various families, including Vespertilionidae, Molossidae, Mormoopidae, Noctilionidae, and Emballonuridae, have been documented in the region (Plasencia-Vázquez et al., 2020; Cafaggi et al., 2024). Additionally, there is a noteworthy precedent regarding the identification of a MERS-related CoV sequence in a molossid bat (*Nyctinomops laticaudatus*) captured in the Campeche state (Anthony et al., 2013). Therefore, it is imperative to intensify sampling efforts to enhance the comprehensiveness of surveillance, and this must extend beyond bats to encompass other wildlife species such as rodents, as well as domestic animals. This effort is justified by their role as intermediate hosts in transmitting bat-borne pathogens to humans, as demonstrated in cases such as SARS-CoV, MERS-CoV, Nipah Virus and Hendra Virus (Ruiz-Aravena et al., 2022; Tian et al., 2022). The high species biodiversity of Yucatán faces significant threats from increasing urbanization, deforestation, landscape modification and fragmentation, expansion of extensive and intensive livestock, highways and railroads. Furthermore, the expansion of the porcine and aviary industry in the region exacerbates these challenges in the context of the risk of emerging zoonotic diseases (Escudero-Pérez et al., 2023; Bautista et al., 2022; Ellis et al., 2017).

5. Conclusion

This first assessment in three previously uninvestigated areas of the Yucatán Peninsula revealed the presence of different CoV genotypes circulating in bats, highlighting the diversified rural site (Tzucacab) as a potential reservoir area for CoVs, jointly with other regions as promising models for the study of the transmission of these viruses in conserved and human-modified environments. Bats play a crucial role in ecosystems; therefore, studying their pathogens not only enhances our comprehension of ecosystem health but also holds significant implications for human well-being, conservation of biodiversity and One Health purposes.

CRediT authorship contribution statement

Marco Antonio Jiménez-Rico: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Ana Laura Vigueras-Galván:**

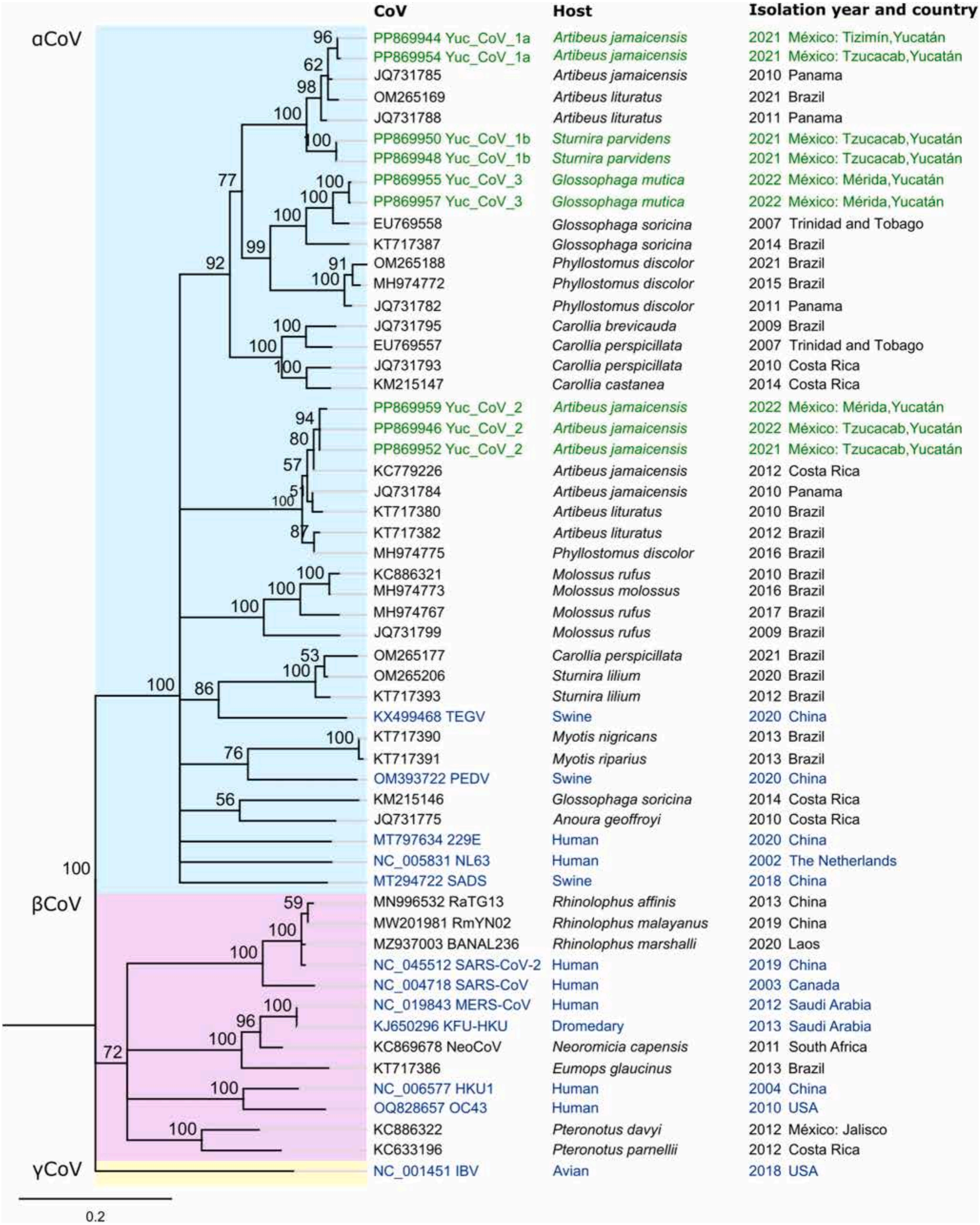

Fig. 4. Phylogenetic tree of a 440 bp fragment of the RdRP gene of coronaviruses. Fifty-six sequences were included, each annotated with its respective GenBank accession number, host, origin country, and sample collection year. Coronaviruses from non-bat hosts are denoted in blue, while those specifically identified in this study are marked in green. αCoVs are shaded blue and βCoVs are purple. Infectious Bronchitis Virus (γCoV) is shaded yellow, used as an outgroup. The phylogenetic tree was constructed using the Neighbor-Joining method with 1000 bootstrap replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Erika N.Hernández-Villegas:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Paola Martínez-Duque:** Writing – review & editing, Investigation, Conceptualization. **David Roiz:** Writing – review & editing, Funding acquisition. **Luisa I. Falcón:** Writing – review & editing, Funding acquisition. **Ella Vázquez-Domínguez:** Writing – review & editing, Funding acquisition. **Osiris Gaona:** Writing – review & editing. **Audrey Arnal:** Writing – review & editing, Investigation. **Benjamin Roche:** Writing – review & editing, Investigation, Funding acquisition. **Rosa ElenaSarmiento-Silva:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Gerardo Suzán:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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