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Bat diversity across urban sites in the Metropolitan Area of Asunción (Paraguay) and complementarity between mist-netting and acoustic monitoring

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Despite the rapid expansion of urban areas, their role in shaping Neotropical bat assemblages remains poorly understood. In Paraguay, no formal inventory has been conducted for the Metropolitan Area of Asunción. This study aimed to characterize bat diversity and evaluate the complementarity of mist-netting, active and passive acoustic monitoring. Sampling was conducted at 15 sites over 90 nights in the Metropolitan Area of Asunción using a standardized design integrating capture-based and acoustic survey techniques. A total of 26 bat species were recorded, representing 43% of the 60 species currently reported for Paraguay. Acoustic monitoring detected 18 species, whereas mist-netting recorded 15. Sampling completeness was higher for acoustic methods, particularly passive monitoring, while mist-netting underestimated species richness. Each method detected different components of the assemblage, indicating complementarity between capture-based and acoustic approaches. In contrast, acoustic methods showed a high degree of overlap in species composition, differing by only one species, suggesting limited additional benefits of their combined use for species detection. The detections of *Myotis riparius* through both mist-netting and acoustic monitoring, and *Nyctinomops macrotis* exclusively through acoustic data, represent a significant finding, as these species had not previously been reported for the study area. The integration of mist-netting and acoustic monitoring improved the characterization of urban bat assemblages. This work provides the first inventory of bats for Asunción and establishes a baseline for future research and monitoring efforts in Paraguay.

Keywords: bat assemblages, biodiversity assessment, bioacoustic, Chiroptera, species richness, urban ecology.

A pesar de la rápida expansión de las áreas urbanas, su rol en la estructuración de los ensamblajes de murciélagos neotropicales permanece escasamente comprendido. En Paraguay, no se ha realizado un inventario formal para el Área Metropolitana de Asunción. Este estudio tuvo como objetivo caracterizar la diversidad de murciélagos y evaluar la complementariedad entre el uso de redes de niebla, el monitoreo acústico activo y pasivo. El muestreo se llevó a cabo en 15 sitios durante 90 noches en el Área Metropolitana de Asunción utilizando un diseño estandarizado. Se registró un total de 26 especies de murciélagos, lo que representa el 43% de las 60 especies actualmente reportadas para Paraguay. El monitoreo acústico detectó 18 especies, mientras que las redes de niebla registraron 15. La completitud del muestreo fue mayor para los métodos acústicos, particularmente para el monitoreo pasivo, mientras que las redes de niebla subestimaron la riqueza de especies. Cada método detectó diferentes componentes del ensamblaje, lo que evidencia la complementariedad entre los enfoques basados en captura y los acústicos. En contraste, los métodos acústicos mostraron un alto grado de solapamiento en la composición de especies, difiriendo en una sola especie, lo que sugiere beneficios limitados de su uso conjunto en términos de detección de especies. Los registros de *Myotis riparius* mediante redes de niebla y monitoreo acústico, y de *Nyctinomops macrotis* exclusivamente a través de datos acústicos, representan un hallazgo significativo, ya que estas especies no habían sido previamente reportadas para el área de estudio. La integración de redes de niebla y monitoreo acústico permitió mejorar la caracterización de los ensamblajes de murciélagos urbanos. Este trabajo constituye el primer inventario de murciélagos para Asunción y establece una línea de base para futuras investigaciones y programas de monitoreo en Paraguay.

Palabras clave: bioacústica, Chiroptera, ecología urbana, ensamblajes de murciélagos, evaluación de la biodiversidad, riqueza de especies.

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Bats are among the most diverse mammalian groups occurring in urban environments, where assemblages may retain substantial species richness despite extensive habitat modification; however, these assemblages are often dominated by disturbance-tolerant species, while more specialized species tend to decline (Russo and Ancillotto 2015; Jung and Threlfall 2016; Moretto *et al.* 2022). Our knowledge of urban bat diversity remains geographically biased, with most studies concentrated in Europe and North America and comparatively fewer conducted in Neotropical regions, particularly in South America (Ávila-Flores and Fenton 2005; López-Berrizbeitia and Díaz 2013; Moretto *et al.* 2022). Existing research has addressed specific aspects of urban bat ecology, including the use of human-made structures as roosts, variation in bat assemblages along urban–peri-urban gradients, and human bat interactions (Romano *et al.* 1999; López-Berrizbeitia and Díaz 2013; Esbérard *et al.* 2014; Russo and Ancillotto 2015; Jung and Threlfall 2016; Gili *et al.* 2020; Ramírez-Fráncel *et al.* 2021). Consequently, comprehensive inventories based on standardized sampling approaches remain scarce in South American urban environments, leaving important gaps in our understanding of urban bat species diversity and assemblage composition.

In Paraguay, research on bats has traditionally focused on natural and semi-natural systems, including studies on community ecology (Gorresen *et al.* 2005; Stevens *et al.* 2007; Presley *et al.* 2009a; Owen *et al.* 2023), biogeography (López-González 2004; Stevens *et al.* 2007; Presley *et al.* 2009b; Owen *et al.* 2022), parasitology (Dick and Gettinger 2005; Presley and Willig 2008), and conservation (Andelman and Willig 2002; Gorresen and Willig 2004), whereas urban environments remain largely unexplored. Over the past two decades, the combined efforts of local and international researchers have substantially improved knowledge of Paraguayan bat diversity, leading to updated species lists and a better understanding of ecological and biogeographic patterns.

Currently, 60 bat species belonging to six families Emballonuridae, Phyllostomidae, Noctilionidae, Natalidae, Molossidae, and Vespertilionidae—are confirmed for the country (Owen *et al.* 2023; Novaes *et al.* 2025; Owen and Smith 2025; González de Weston *et al.* 2026). Despite this progress, no formal inventory has been published for the Metropolitan Area of Asunción. A review of the available literature, based primarily on records from museum collections and recent field surveys, indicates that 31 bat species have been reported for the area (López-González 2005; Airaldi-Wood *et al.* 2018; Torres *et al.* 2023). The Metropolitan Area of Asunción comprises a predominantly urban landscape associated with the Paraguay River and its wetlands, interspersed with remnant vegetation patches. However, its bat diversity has not been systematically assessed, representing an important gap in the knowledge of urban bat diversity in Paraguay.

A major challenge in characterizing bat assemblages is that species detectability varies substantially among sampling methods. Because bat species differ in flight behavior, foraging strategies, habitat use, and echolocation characteristics, no single method is equally effective for detecting all components of an assemblage (O'Farrell and Gannon 1999; Meyer 2015; Appel *et al.* 2021). This limitation may be particularly relevant in urban environments, where aerial insectivorous bats, including many molossids and vespertilionids, can represent an important component of local assemblages. These fast-flying species typically forage in open spaces and are rarely captured using ground-level mist-nets. Consequently, mist-netting is particularly effective for sampling low-flying and clutter-adapted species, including many phyllostomids, but tends to underestimate aerial insectivorous bats (O'Farrell and Gannon 1999; Meyer 2015; Appel *et al.* 2021).

Acoustic monitoring provides a complementary approach by increasing the detection of fast-flying aerial insectivores, particularly molossids and some vespertilionids that are typically underrepresented in capture-based surveys (Meyer 2015). However, acoustic methods also have important taxonomic limitations. Phyllostomid bats, for example, are often poorly detected and difficult to identify acoustically because many species emit low-intensity echolocation calls (Pinilla-Cortés and Rodríguez-Bolaños 2017; Rivera-García *et al.* 2024; Olmedo *et al.* 2024). Thus, mist-netting and acoustic methods sample different components of bat assemblages, and their combined use can provide more complete estimates of species richness and assemblage composition than either approach alone.

Acoustic surveys can also differ in their ability to document species depending on how detectors are deployed. Active monitoring involves an observer recording bats during defined sampling periods, whereas passive monitoring uses stationary detectors operating autonomously for extended periods. These approaches differ in sampling duration, temporal coverage, observer involvement, and potentially in the probability of detecting species. Evaluating active and passive acoustic monitoring separately, in addition to comparing acoustic methods with mist-netting, can therefore provide a more detailed understanding of the contribution of each technique to species inventories and sampling completeness.

Recent studies in Neotropical ecosystems have demonstrated that combining mist-netting and acoustic monitoring increases species detection and improves sampling completeness. For example, Appel *et al.* (2021) and Carvalho *et al.* (2023) showed that these methods detect complementary components of bat assemblages, with mist-nets being more effective for phyllostomid bats and acoustic monitoring particularly efficient for documenting aerial insectivorous species. However, these studies have been conducted in natural or semi-natural habitats, and comparatively little is known about the complementarity of mist-netting and acoustic monitoring in highly urbanized Neotropical environments, particularly when active and passive acoustic approaches are considered separately.

In this study, we aim to (1) characterize bat species diversity in the Metropolitan Area of Asunción and (2) evaluate the complementarity of mist-netting, active acoustic monitoring, and passive acoustic monitoring for detecting species and assessing sampling completeness. By integrating direct and indirect sampling approaches within a

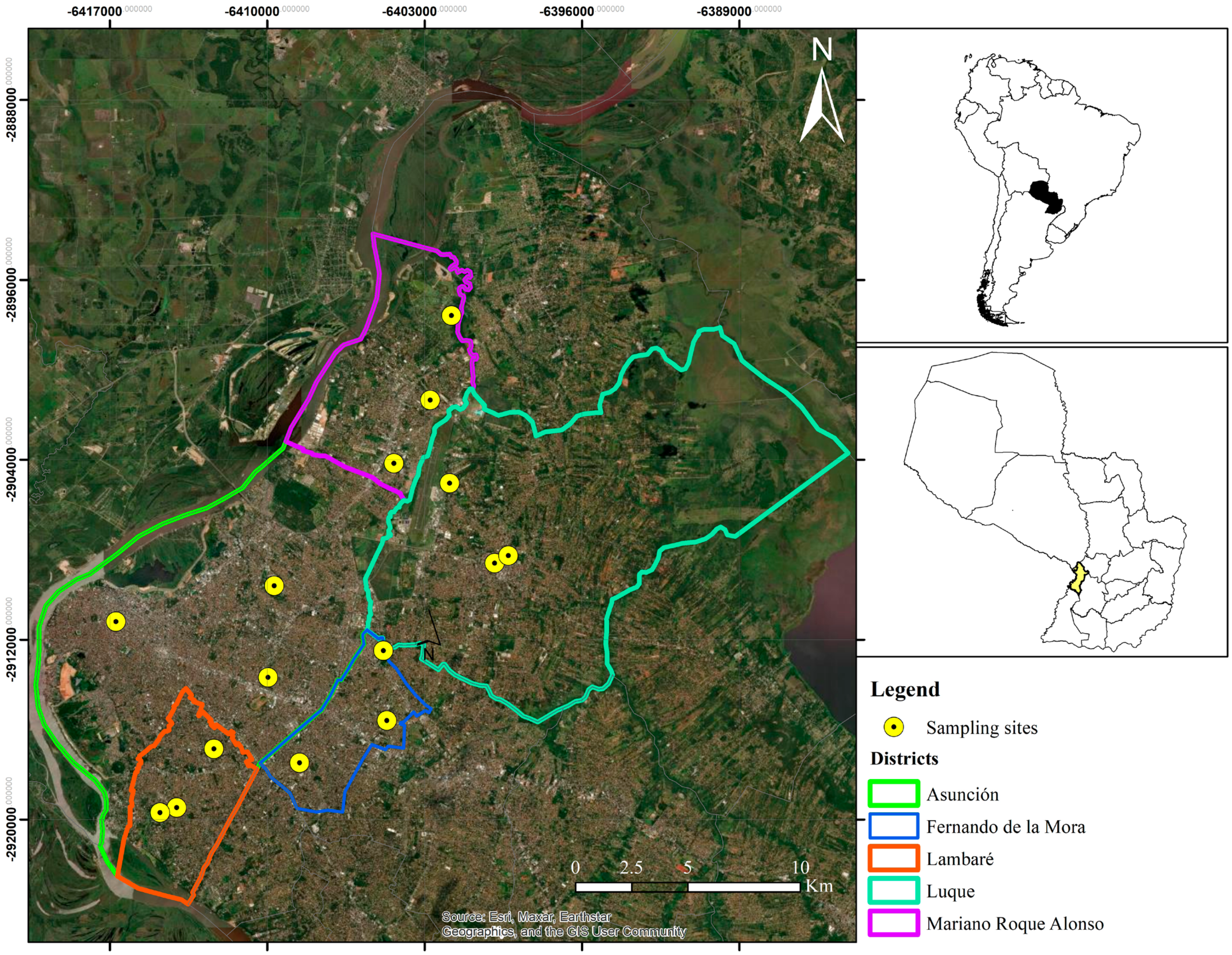

Figure 1. Spatial distribution of sampling sites in urban environments of the Metropolitan Area of Asunción, Paraguay.

standardized survey design, we assess the contribution of each method to documenting the urban bat assemblage and determine the extent to which their combined use improves species detection. This study provides the first formal bat inventory for the Metropolitan Area of Asunción and establishes a methodological and faunistic baseline for future research and monitoring of bats in urban environments in Paraguay.

Materials and methods

Study area. The study was conducted within the Central Litoral ecoregion of Paraguay, a region influenced by the Paraguay River and characterized by extensive floodplains and associated wetlands. Historically, this area represented a transitional zone between the Humid Chaco, Ñeembucú, and Central Forest ecoregions (MADES 2019). Despite intense urbanization, remnant vegetation patches persist and provide potential habitat for bat species.

Sampling was carried out in Asunción, the capital and most populous city of Paraguay, and the adjacent municipalities of Lambaré, Mariano Roque Alonso, Luque, and Fernando de la Mora, which together form the continuous urban core of the Metropolitan Area of Asunción (Figure 1). The surveyed area covers approximately 357.1 km^2 (52% of the total metropolitan area) and consists of a heterogeneous urban landscape including residential neighborhoods, industrial areas, urban parks, the Paraguay River, Asunción Bay, and a network of streams and wetlands.

The region has a humid subtropical climate, with warm, humid summers and mild winters. Mean monthly temperatures range from approximately 17–19 °C in July to 28 °C in January. Rainfall is distributed throughout the year, peaking during spring and summer, with November among the wettest months, and reaching its lowest levels in winter, particularly during July and August (Dirección de Meteorología e Hidrología 2023).

According to the 2022 National Population and Housing Census, the five municipalities included in this study have a combined population of approximately 1.12 million inhabitants (Instituto Nacional de Estadística 2024).

Sampling design. A total of 15 sampling sites were established across the five municipalities previously

Figure 2. Examples of sampling sites in the Metropolitan Area of Asunción: A) Private residence, Luque; B) Property of the Asociación Rural del Paraguay, Mariano Roque Alonso; C) Private residence, Fernando de la Mora; D) Public Park, Mariano Roque Alonso; E) Public Park, Fernando de la Mora; F) Public Park, Asunción.

mentioned. Site selection was based on accessibility and the availability of permits, with the aim of prioritizing fully urban environments where human activities are concentrated. Accordingly, sampling sites encompassed a range of urban settings, including private residences, industrial properties, and public parks (Figure 2). Each site was sampled for six nights, with each night considered an independent sampling unit. In total, sampling effort comprised 90 nights conducted between March 2022 and September 2023. Sampling was conducted only under environmental conditions favorable for bat activity, including absence of precipitation, low wind, and a minimum sunset temperature of ≥ 14 °C, as adverse weather conditions are known to significantly reduce bat flight activity (Erickson and West 2002; Barros *et al.* 2014; Bat Conservation Trust 2024; Rodríguez-San Pedro *et al.* 2024). Bats were surveyed using a combination of mist-netting and acoustic monitoring to maximize detection of species with different ecological and behavioral traits (Ávila-Flores and Fenton 2005; Pech-Canché *et al.* 2010; Voigt and Kingston 2016).

Mist-netting. Five mist-nets (two 9 m and three 6 m long × 3 m high, with a 36-mm mesh size) were set up 1 m above

Figure 3. Photographic records of bat species recorded exclusively by mist-netting during field surveys in urban environments of the Metropolitan Area of Asunción, Paraguay. A) *Artibeus lituratus*, B) *Sturnira lilium*, C) *Platyrrhinus lineatus*, D) *Artibeus planirostris*, E) *Glossophaga soricina*, F) *Artibeus fimbriatus*.

ground level at suitable locations within the urban matrix, along potential bat flyways such as trails, vegetation edges, open areas, and near bodies of water. Mist-nets were open from 18:00 to 00:00 h during spring-summer and from 17:00 to 23:00 h during autumn-winter and were checked every 20 min to minimize stress in captured individuals.

Captured bats were placed in cloth bags for processing. Standard external measurements (Total length [TL], Head and body length [HBL], Tail length [TaL], Hind foot length [HFL], Ear length [EL], Forearm length [FA]), sex, age (determined by the degree of epiphyseal fusion), reproductive condition, and body mass, were recorded. To prevent recapture bias, released individuals were temporarily marked by trimming the fur in the interscapular region. Individuals were identified in the field using morphological keys (Díaz *et al.* 2016, 2021). Specimens with uncertain identification or representing unusual records were collected as vouchers and deposited in the Zoological Collection of the Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Asunción (CZCEN). Uncertain identifications were subsequently confirmed through comparison with reference specimens housed in scientific collections and specialized taxonomic literature.

All sampling procedures, handling, and specimen collection were conducted under the scientific collecting permits No. 006/2022 and No. 005/2023 issued by the Ministerio del Ambiente y Desarrollo Sostenible (MADES 2019). During fieldwork, the Biosecurity Protocol established by the Red Latinoamericana y del Caribe para la Conservación de los Murciélagos (RELCOM) was followed (Suárez-Álvarez and López-Berrizbeitia 2020). All procedures involving animals were conducted in accordance with the guidelines of the American Society of Mammalogists for the use of wild mammals in research and education (Sikes *et al.* 2016).

Acoustic monitoring. Acoustic monitoring was conducted using two complementary approaches: active and passive acoustic monitoring.

Active acoustic monitoring. Echolocation calls were recorded using an Echo Meter Touch Pro 2 ultrasonic detector (Wildlife Acoustics Inc.) connected to a mobile device. The operator moved around the fixed sampling point within the accessible area surrounding each site during the first two hours after sunset, directing the microphone toward potential flight paths or areas of bat activity. The distance covered was not standardized due varying the spatial configuration, physical barriers (e.g., buildings, fences, streets, vegetation, and other infrastructure), together with routine human activity (e.g., pedestrians, children using public spaces, and vehicular traffic). Consequently, the survey route was adapted to local site conditions while maintaining a standardized survey duration across all sampling sites. Recordings were obtained using a sampling rate of 256 kHz, with a frequency range of 8-128 kHz and a resolution of 16 bits. The trigger window was set to 15 s with medium sensitivity, and files were stored in WAV format.

To standardize sampling effort between methods and reduce potential bias associated with differences in recording duration, a subsampling protocol was applied to acoustic data. For active acoustic monitoring, recordings obtained during the first 15 minutes of each of the first two hours after sunset were analyzed.

Passive acoustic monitoring. An autonomous Song Meter Mini BAT detector (Wildlife Acoustics Inc.) was used, configured to record continuously from sunset to sunrise. The device was installed at a minimum height of 2 m above ground level at each sampling site, where it remained all consecutive nights without human intervention. Recordings were obtained at a sampling rate of 384 kHz and 16-bit resolution, and files were stored in WAV format. For acoustic monitoring, one continuous five-minute segment was analyzed per hour throughout the night. The position of the selected segment advanced progressively across successive hourly intervals (e.g., 19:00–19:05 during the first hour, 20:06–20:11 during the second hour, and 21:12–21:17 during the third hour, continuing through sunrise).

Acoustic data processing and call identification. Acoustic recordings obtained from both active and passive monitoring were processed using Kaleidoscope Pro software (Wildlife Acoustics Inc.). An automated classifier for Neotropical bats was initially applied, followed by manual validation of acoustic sequences through visual inspection of spectrograms. This inspection consisted of comparing call structure and acoustic parameters (e.g., frequency, duration, and interpulse interval) with published reference descriptions from the regional literature used for manual validation, as detailed in Supplementary data SD1, in order to reduce potential misclassification errors associated with automated identification (Russo and Voigt 2016).

Only sequences with an adequate signal-to-noise ratio and clearly distinguishable temporal structure were included in the analysis. The acoustic detection unit was defined as a bat pass, understood as a sequence of echolocation calls recorded within the effective detection range of the microphone and separated from other sequences by a time interval of ≥ 1 s, consistent with thresholds of 1–2 s commonly used in previous studies (Loeb *et al.* 2015; Rodrigues *et al.* 2018). Because multiple bat passes may correspond to the same individual, acoustic records were interpreted as independent activity events rather than direct estimates of population abundance (Russo and Voigt 2016). For species of the genus *Molossus*, whose echolocation pulses are typically emitted in pairs, detections were aggregated per audio file (WAV) file to avoid overestimation of activity events resulting from the automated detection of individual pulses (Zamora-Gutiérrez *et al.* 2016).

The sampling unit was defined as night per site for each acoustic monitoring method. Based on the identified bat passes, incidence matrices (presence/absence data) were constructed. The following acoustic parameters were measured: maximum frequency (Fmax), minimum frequency (Fmin), frequency of maximum energy (FME), pulse duration (Dur), and inter-pulse interval (IPI). These parameters were selected due to their relative intraspecific stability across the geographic distribution of species and their usefulness for the systematic identification of insectivorous bats using bioacoustic approaches (Zamora-Gutiérrez *et al.* 2016).

Statistical analysis. The complementarity of mist-nets, active acoustic monitoring, and passive acoustic monitoring was assessed using presence/absence data, following the approach proposed by Colwell and Coddington (1994) and Moreno (2001). According to this framework, complementarity ranges from zero, when two methods share an identical species assemblage, to one, when they detect entirely distinct sets of species. Rarefaction and extrapolation curves were constructed using the iNEXT package (Chao and Jost 2012; Chao *et al.* 2014; Hsieh *et al.* 2022) in R (R Core Team 2024), based on incidence data. Incidence-based richness estimators (Chao2, Jackknife 1, and Jackknife 2) were calculated using EstimateS (Colwell 2013).

Results

Combining mist-net captures and acoustic records, this study documented a total of 26 bat species from four families (Table 1). Acoustic monitoring recorded bat passes corresponding to 18 species, whereas mist-net sampling resulted in the capture of 314 individuals representing 15 species. The total sampling effort comprised 90 sampling nights, including a mist-netting effort of 19,440 m·h (Medellín 1993) and 2,385 acoustic recordings, of which 2,097 met the quality criteria described in the methodology.

Species identified based on the analysis of echolocation calls (Supplementary data SD1) were largely shared between active and passive acoustic monitoring. Only one species was detected exclusively through active acoustic monitoring, whereas all others were also recorded by passive method. Seven species were consistently identified

Table 1. Bat species captured with mist-nets and species/sonotypes registered by acoustic recorders in the Metropolitan Area of Asunción, Paraguay.

		Methods		
Family	**Species**	**Active**	**Passive**	**Mist-nets**
Phyllostomidae	*Artibeus fimbriatus*	-	-	X
Phyllostomidae	*Artibeus lituratus*	-	-	X
Phyllostomidae	*Artibeus planirostris*	-	-	X
Phyllostomidae	*Glossophaga soricina*	-	-	X
Phyllostomidae	*Platyrrhinus lineatus*	-	-	X
Phyllostomidae	*Sturnira lilium*	-	-	X
Noctilionidae	*Noctilio albiventris*	X	X	X
Molossidae	*Cynomops abrasus*	X	-	-
Molossidae	*Eumops perotis*	X	X	-
Molossidae	*Eumops* sp.	X	X	-
Molossidae	*Molossops temminckii*	X	X	X
Molossidae	*Molossus fluminensis*	X	X	X
Molossidae	*Molossus molossus*	X	X	X
Molossidae	*Molossus* sp.	-	-	X
Molossidae	*Nyctinomops laticaudatus*	X	X	-
Molossidae	*Nyctinomops macrotis*	X	X	-
Molossidae	*Promops centralis*	X	X	-
Molossidae	*Tadarida brasiliensis*	X	X	-
Vespertilionidae	*Aeorestes villosissimus*	X	X	-
Vespertilionidae	*Dasypterus ega*	X	X	-
Vespertilionidae	*Lasiurus blossevillii*	X	X	-
Vespertilionidae	*Myotis nigricans*	X	X	X
Vespertilionidae	*Myotis riparius*	X	X	X
Vespertilionidae	*Myotis* sp.	-	-	X
Vespertilionidae	*Neoeptesicus brasiliensis*	X	X	-
Vespertilionidae	*Neoeptesicus furinalis*	X	X	X

across all sampling methods (Table 1), highlighting a core assemblage detectable regardless of method. In contrast, eight species were recorded exclusively by mist-netting (Figure 3), reflecting the limitations of acoustic detection for certain taxa (Table 1).

At the family level, Molossidae was the most represented group with 11 species (42.30%), followed by Vespertilionidae with eight species (30.76%), Phyllostomidae with six species (23.10%), and Noctilionidae with one species (3.85%). Two captured individuals of *Molossus* could not be assigned to any described species and were collected as voucher specimens and they are currently under systematic evaluation. Molecular analyses indicate that these specimens likely represent an undescribed species; however, they are conservatively treated here as *Molossus* sp. pending formal description (González de Weston, unpublished data). Additionally, four *Myotis* individuals corresponded to lactating females that were released in the field without collection, which prevented the detailed morphological examination required for species-level identification (Table 1). *Eumops* sp. was recorded acoustically but could not be identified to the species level because its echolocation calls are consistent with both *Eumops auripendulus* and *Eumops glaucinus*. Although only *E. auripendulus* has previously been recorded in the Metropolitan Area of Asunción, the occurrence of *E. glaucinus* cannot be excluded given its broad geographic distribution and the limited bat sampling conducted in the region. Therefore, the record is conservatively reported as *Eumops* sp. Of the 26 species recorded, two (*Myotis riparius* and *Nyctinomops macrotis*) represent the first records for the Metropolitan Area of Asunción. *Myotis riparius* was detected through both mist-netting and acoustic monitoring, whereas *Nyctinomops macrotis* was recorded exclusively through acoustic monitoring.

Rarefaction curves showed a rapid initial increase in species richness followed by a gradual asymptotic trend for all sampling methods (Figure 4). However, differences were observed among methods in both observed richness and sampling completeness. Active acoustic monitoring recorded the highest observed richness (18 species), with extrapolated richness approaching approximately 20-21 species (Chao2 ≈ 20; Jackknife 1 ≈ 20; Jackknife 2 ≈ 21), suggesting that a small number of additional species may remain undetected despite the tendency toward stabilization. Passive acoustic monitoring recorded 17 species and exhibited the fastest convergence toward an asymptote, with minimal increase under extrapolation and estimator values closely aligned with observed richness (Chao2 ≈ 18; Jackknife 1 ≈ 18; Jackknife 2 ≈ 18), indicating high sampling completeness. In contrast, mist-net sampling recorded the lowest observed richness (15 species) and showed a slower approach to asymptote, characterized by a pronounced increase under extrapolation and consistently higher estimator values (Chao2 ≈ 17-18 species; Jackknife 1 ≈ 18; Jackknife 2 ≈ 19), suggesting lower sampling completeness and that several species present in the assemblage were likely not detected by this method.

Estimates of sampling completeness, defined as the proportion of observed to estimated richness, further supported these patterns. Active acoustic monitoring achieved approximately 85-90% completeness, whereas passive acoustic monitoring showed the highest completeness (approximately 95-100%). In contrast, mist-net sampling exhibited lower completeness (approximately 75-85%), indicating that a proportion of species remained undetected by this method.

Complementarity values between active and passive acoustic methods were very low (0.06), indicating a near-complete overlap in species composition and minimal compositional dissimilarity between both techniques. In contrast, comparisons between acoustic approaches and mist-netting yielded substantially higher complementarity values (0.73 for active vs. mist-nets; 0.72 for passive vs. mist-nets), reflecting a greater degree of species turnover.

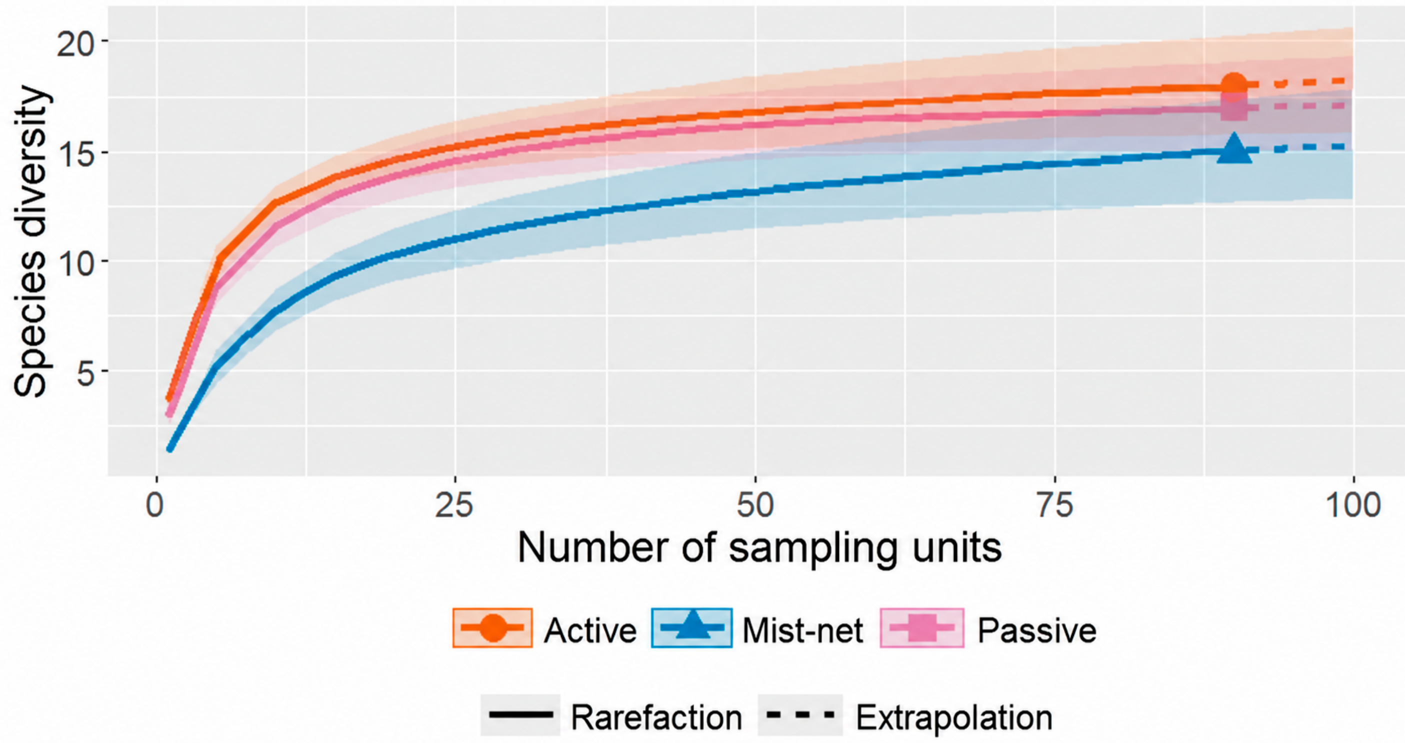

Figure 4. Rarefaction (solid lines) and extrapolation (dashed lines) curves based on incidence data (presence/absence) for the three sampling methods (active acoustic monitoring, passive acoustic monitoring, and mist-netting) in the Metropolitan Area of Asunción, Paraguay. Shaded areas represent 95% confidence intervals. Diversity is expressed as Hill numbers of order q = 0 (species richness).

Discussion

This study provides the first comprehensive assessment of bat diversity in the Metropolitan Area of Asunción, documenting 26 species, including two recorded for the first time in the study area (*Myotis riparius* and *Nyctinomops macrotis*). By combining mist-netting with active and passive acoustic monitoring, we detected substantially more species than would have been recorded using either approach alone, demonstrating the strong complementarity between these survey methods. This integrated sampling strategy resulted in high inventory completeness and revealed marked differences in species detectability among methods, highlighting the importance of integrating multiple survey techniques to obtain a more complete characterization of urban bat assemblages. Together, these findings provide an important baseline for future ecological studies and conservation planning in the Metropolitan Area of Asunción.

Complementarity analysis (Colwell and Coddington 1994; Moreno 2001) highlighted the strong redundancy between active and passive acoustic monitoring and the complementary role of mist-netting in characterizing bat assemblages. The high overlap observed between active and passive acoustic approaches suggests that the simultaneous use of both methods may offer limited additional benefits in terms of species composition, and that either method alone could be sufficient under similar sampling conditions. Indeed, differences in observed species richness between the two acoustic methods were modest, suggesting that both approaches contributed comparably to documenting the assemblage despite their inherent methodological biases.

Observed patterns are consistent with previous studies showing that mist-nets are inherently selective and tend to underrepresent aerial insectivorous species, such as Molossidae and Vespertilionidae, which forage at higher flight heights or are able to detect and avoid nets (MacSwiney *et al.* 2008; Appel *et al.* 2021; Mancini *et al.* 2022; Soares and da Rocha 2026). In contrast, mist-netting is more effective for capturing species that forage in cluttered environments, particularly many phyllostomid bats, due to their flight behavior and reduced ability to detect nets (Soares and da Rocha 2026). Together, these results reinforce that neither method alone is sufficient to fully characterize bat assemblages, and that their combined use is necessary to reduce sampling bias and improve inventory completeness.

The systematic composition recorded by each method reflects differences in detectability among ecological guilds. Molossidae were predominantly detected through acoustic monitoring, consistent with their adaptation to open-space foraging and the emission of high-intensity echolocation calls that facilitate long-distance detection (Oliveira *et al.* 2018). Similarly, vespertilionid species were more frequently detected acoustically, whereas phyllostomid bats were primarily recorded through mist-netting. This pattern reflects the low-intensity, broadband echolocation calls characteristic of most phyllostomids, which are adapted for navigation in cluttered environments and attenuate rapidly, substantially reducing their detectability by acoustic detectors. Consequently, the absence of ultrasonic detections does not necessarily indicate species absence but may instead reflect limitations in acoustic detectability. Such differences suggest that urban bat assemblages are structured not only by habitat availability but also by species-specific detection probabilities, which may bias interpretations of community composition if a single method is used.

Differences in detectability among ecological guilds have important implications for understanding urban bat assemblages. Urban environments may favor species adapted to open-space foraging, such as molossids, while simultaneously supporting clutter-adapted species associated with vegetation patches and green areas. As a result, assessments based on a single method may provide a biased representation of community structure, potentially underestimating the contribution of certain functional groups.

Beyond detectability patterns, the integration of capture and acoustic data is essential for improving species identification, particularly in regions where acoustic reference libraries remain incomplete. Recordings obtained from captured individuals provide validated call parameters that can refine acoustic classifications and reduce misidentification (Arias-Aguilar *et al.* 2018). Thus, mist-netting plays a critical role not only in documenting species presence but also in strengthening the reliability of acoustic datasets.

Studies conducted in urban environments across Latin America have shown variable patterns in the relative performance of sampling methods. In many cases, acoustic monitoring substantially increases species richness compared to mist-netting, particularly by detecting aerial insectivores that are rarely captured (Pech-Canche *et al.* 2010; Rodrigues-Silva and Bernard 2017; Silva de Sales *et al.* 2025). For example, Martínez-Rodríguez *et al.* (2026) reported a markedly higher number of species detected through acoustic monitoring than through capture-based methods in a suburban environment. Similarly, Cabrera-Pantoja *et al.* (2026) found stronger differences between sampling approaches in an arid ecosystem. Together, these studies suggest that the degree of complementarity and methodological bias may vary among ecosystems, likely reflecting differences in habitat structure and bat assemblage composition. In contrast, the relatively small differences observed in the present study suggest a more balanced contribution of sampling methods. This pattern may reflect the heterogeneous urban landscape of the Metropolitan Area of Asunción, whose mosaic of built areas, green spaces, and water bodies supports species with diverse ecological traits and may reduce differences in detectability among methods.

At the national level, bat research in Paraguay has largely relied on capture-based methods, primarily focused on natural and semi-natural systems (Willig *et al.* 2000; López-González 2005; Airaldi-Wood *et al.* 2018; Gamarra de Fox *et al.* 2019). As a result, aerial insectivorous species may have been underrepresented in previous inventories due to methodological biases associated with mist-net sampling (MacSwiney *et al.* 2008; Rodrigues-Silva and Bernard 2017). Moreover, recent records and taxonomic studies indicate that bat diversity in the country remains incompletely documented, with new species records continuing to emerge (Moratelli *et al.* 2015; Airaldi-Wood *et al.* 2018; Torres *et al.* 2023). In this context, the incorporation of acoustic monitoring represents a significant methodological advancement, allowing the detection of species that are typically overlooked in traditional surveys and contributing to a more comprehensive understanding of bat assemblages.

Recent taxonomic advances, particularly in molecular systematics, have substantially refined the understanding of bat diversity in the region. Notably, the description of the cryptic species *Myotis guarani*, associated with Chaco, Pantanal, and Cerrado environments, highlights the relevance of Paraguay within a broader biogeographic context and underscores the importance of biological collections as reservoirs of hidden diversity (Novaes *et al.* 2025). Additional studies have emphasized the role of Paraguay as a contact zone between Amazonian and Chacoan lineages and as a refuge for populations at the southern limits of distribution of many Neotropical species (Owen and Smith 2025).

Furthermore, recent field-based studies have contributed to increasing the number of species known for the country, incorporating new departmental records, and improving the understanding of patterns of distribution, abundance, and activity in bat assemblages (Torres *et al.* 2023; González de Weston *et al.* 2024; Smith *et al.* 2024). As sampling efforts expand into previously unexplored or poorly studied areas, additional records and taxonomic novelties are likely to continue emerging, reinforcing the notion that bat diversity in Paraguay remains incompletely documented.

From a conservation perspective, maintaining heterogeneous urban landscapes, including remnant vegetation patches and water-associated environments, is essential to support diverse bat assemblages in metropolitan areas (Threlfall *et al.* 2012; Voigt and Kingston 2016). Moreover, the integration of complementary survey methods can improve biodiversity assessments and provide a more robust basis for conservation planning and management in rapidly expanding urban environments (Russo and Voigt 2016; Silva de Sales *et al.* 2025).

Despite the strengths of the integrative approach, some limitations should be acknowledged. Acoustic identification in the Neotropics remains constrained by incomplete reference libraries, which may affect species-level assignments and lead to conservative classifications. In addition, mist-net sampling may have underestimated species that forage above the canopy or are able to detect and avoid nets. Finally, temporal and seasonal constraints may have influenced detectability patterns, particularly in urban environments where bat activity can vary with climatic conditions.

This study represents the first comprehensive assessment of bat diversity in the Metropolitan Area of Asunción and the first in Paraguay to explicitly integrate complementary sampling approaches. The detection of *Myotis riparius* through both mist-netting and acoustic monitoring, and *Nyctinomops macrotis* exclusively through acoustic data, is particularly relevant, as these species had not been previously reported for the study area (López-González, 2005; Airaldi-Wood *et al.* 2018; Torres *et al.* 2023). Such records highlight the contribution of acoustic methods to improving species detectability and refining our understanding of urban bat assemblages in South America. Collectively, our findings underscore the value of integrative sampling strategies for minimizing methodological biases and improving characterization of biodiversity in urban environments, particularly those that remain understudied.

Conclusions

We recorded a total of 26 bat species, representing 43% of the 60 species currently reported for Paraguay, using three complementary methodologies: active and passive acoustic monitoring, and mist-netting. This study represents the first inventory of bats for Asunción and its metropolitan area, as well as the first in Paraguay to explicitly integrate complementary methods for assessing chiropteran diversity.

Our results highlight the importance of combining mist-netting with acoustic monitoring to adequately sample Neotropical bat assemblages in urban environments. Each method detected different components of the community, confirming that reliance on a single technique may lead to biased assessments, either toward low-flying species commonly captured in nets or toward aerial insectivores more readily detected acoustically.

By integrating these complementary approaches, we provide a more complete representation of the urban chiropteran assemblage and reveal a high species richness within the study area. These findings underscore the need for an integrative framework to improve the accuracy of biodiversity assessments and monitoring programs.

Finally, we emphasize the importance of expanding regional echolocation call libraries and strengthening bioacoustic research in the Neotropics, as these tools are essential for improving species identification and enhancing the effectiveness of future studies and monitoring efforts in urban environments.

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Declaration of Artificial Intelligence use

The authors declare that ChatGPT (GPT-4, OpenAI) was used to review spelling, grammar, and syntax in English.

Author contributions

Gloria González de Weston: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - Original Draft Preparation; Karen Chavez-Duré: Conceptualization, Investigation, Visualization, Writing - Original Draft Preparation, Writing - Review and Editing; Karina Núñez: formal analysis, Writing-Review and Editing; Mónica Díaz: Conceptualization, Supervision. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Supplementary data

SD1. Echolocation call parameters of the species analyzed in this study. Acoustic parameters were measured from manually validated echolocation calls and include maximum frequency (Fmax), minimum frequency (Fmin), frequency of maximum energy (FME), pulse duration (Dur), inter-pulse interval (IPI), number of bat passes (N) and, pulse. References correspond to published studies used to support taxonomic identification based on echolocation call characteristics.

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