ORIGINAL ARTICLE

Rapid assessment of bat diversity in the biological station 'Las Guacamayas' ('Laguna del Tigre' National Park, Guatemala)

Raphaël Colombo[1,2,*], Audrey Pichard[2], Yann Gager[3]

[1] Asellia Ecologie, 60 chemin de la Nuirie, 04200 Sisteron, France

[2] Association l'Ascalaphe, 460 route de Noyers, 04200 Sisteron, France

[3] Independant researcher, Sebastian-Bach Straße 26, 04109 Leipzig, Germany

*Corresponding author: rcolombo@asellia-ecologie.fr

ABSTRACT

As part of the world biodiversity hotspot 'Meso-America', the country of Guatemala hosts a minimum of 100 bat species. However, the taxonomy and the systematic of Neotropical bats have recently undergone many changes and the knowledge about species diversity and distribution remains patchy. We report here the results of a bat survey conducted in November 2010 inside the biological station 'Las Guacamayas' ('Laguna del Tigre' National Park'). We used three survey methods: roost search, mist net capture as well as ultrasound recording. On the basis of recent taxonomic knowledge, we surveyed a minimum of 25 bat species belonging to seven different families, including two new families for the National Park (Natalidae and Vespertilionidae). We have identified several systematic and taxonomic changes: *Carollia brevicauda* for *C. sowelli*, *Pteronotus parnellii* for *P. mesoamericanus*, *Rhogeesaa tumida* for *R. bickhami / menchuae*, *Sturnira lilium* for *S. parvidens* and *Uroderma bilobatum* for *U. convexum*. Mist net captures and ultrasound recordings resulted in the identification of several new species. By combining the two inventories of the National Park and taking into account the taxonomic changes, we obtained a maximum of 31 species identified so far. Our survey improves the knowledge on bat diversity and distribution in Guatemala, with implications for their conservation.

INTRODUCTION

Located in Central America, Guatemala is part of the world's biodiversity hotspot 'Meso-America' (Myers et al. 2000). Despite its small surface (108.889 km²), the country hosts an important part of the world's bat diversity with a minimum of 100 species identified (Kraker-Castañeda et al. 2016). Despite the fact that the systematics and taxonomy of bats faced numerous changes with the description of several species for the country (Solari & Martínez-Arias 2014), the knowledge on bat diversity in Guatemala remains poorly understood.

Situated in the North of Guatemala, the National Park 'Laguna del Tigre' is of particular interest for biodiversity. With a surface of 289,000 ha, this park represents one of the core areas of the Biosphere Reserve Maya. Wetlands from the Park are recognized as being of international importance according to the Ramsar Convention of 1990 and 1999 (Bestelmeyer & Alonso 2000). Despite this, the park is extremely threatened by human development and colonization linked to oil extraction facilities (causing significant deforestation), unregulated fishing and hunting, and pollution (Bestelmeyer & Alonso 2000). To ensure conservation work, the biological station "Las Guacamayas" (Estación Biológica Las Guacamayas: EBG, 5,050 hectares) was created in the south-eastern area of the Park. It is located in mature and remote lowland tropical forest, but it is also one of the most threatened areas of the Park (Bestelmeyer & Alonso 2000).

Despite the great potential for bat biodiversity studies, little research has been conducted on the diversity of bats in the area. A previous study in five different localities of the 'Laguna del Tigre' National Park (including a locality close to the EBG) reported 20 species caught in mist nets and three species located in roosts (Bestelmeyer & Alonso 2000). One of the major limitations of these two methods lies in the fact that aerial insectivorous species are under-represented among captures (MacSwiney et al. 2008). Acoustic recording is a great complementary method for detecting high-flying species and thus improves the inventories of bats in the Neotropics (Kalko 1998, MacSwiney et al. 2008). Considering the poor knowledge of the bat community in the biological station "Las Guacamayas" and following a request from the managers of EBG, the association 'Ascalaphe' carried out an inventory of the bat diversity within the perimeter of the station. This study is based on three complementary methods: mist net captures, roost search, and ultrasound recordings. The aim of this multi-method inventory was to provide additional information on the specific richness and conservation status of bats within the 'Laguna del Tigre' National Park and more generally in Guatemala on the basis of updated systematic and taxonomic knowledge (Kraker-Castañeda et al. 2016).

MATERIAL AND METHODS

Study site

Located in the forest area of Petén in the north of Guatemala, the biological station is accessible after about 2h 30' off trail to the north-east of the regional capital Santa-Elena / Flores followed by 30' canoe to the east of the traditional community of Paso Caballos. This station is located at the heart of a primary forest, close to the Rio San Pedro but also shows some patches of secondary forest in its vicinity. Established in 1994 by the association ProPetén and Conservation International, this station is administered since 2008 by the association Balam.

The region is mostly at low altitude, with elevations up to 300 m above sea level (CONAP 2006). The dominant habitat is a tropical dry forest (Murphy & Lugo 1995). About 30% of the surface is composed of savannas and flooded swamps, the rest consists of highland forests, some relics of oak forests (*Quercus oleoides*) and riparian vegetation areas, lakes, lagoons and rivers. The region is subject to a wet and warm dominant climate with well-marked seasons. The rainy season lasts from June to December and has an average temperature of 25 ºC. The dry season runs from January to May and has an average temperature of 35 ºC (CONAP 1999). The rainfall for the region averages 1600 mm per year (Bestelmeyer and Alonso 2000).

Sampling

Field sampling was carried out using three methods: i) mist net sampling, ii) roost research, and iii) acoustic surveys using ultrasound detectors. We carried out 16 hours of mist net sampling over eight nights (16-17, 19, 21-25 November 2010), at a rate of two hours per night between 17:30 and 19:30. Seven sampling nights took place near the EBG (border and trails in the primary and secondary forests as well as open landscapes). The eighth night was carried out near the archaeological site 'El Perú – Waka' (trail in the secondary forest, Fig. 1). We used a 6 x 2.4-meter Japanese mesh filament (16 mm mesh; Ecotone, Poland) in undergrowth in order to block forest tracks or potential flight corridors. We also used the same type of mist net as canopy net using 6-meter poles in two sites. Captured specimens were identified to species level using field guides (LaVal & Rodríguez-H 2002, Reid 2009, Rodriguez et al. 2007). To avoid recaptures, all individuals were released at the end of night of sampling. All released individuals were recorded at three meters to obtain reference sequences. A fraction of the sequences (i.e. *Natalus mexicanus*) did not show the required quality and therefore they were discarded. Secondly, diurnal surveys of roosts were carried out in various places (caves, tree cavities, undersides of palm leaves and banana trees) between 16th and 25th of November 2010. Finally, we used ultrasound detectors at capture sites to record over a duration of six hours, spread over six nights (17, 19-22, 23, 25 November 2010) at a rate of one hour per night between 17:45 and 18:45. These passive acoustic inventories were carried out using a Tranquility transect time expansion detector (Courtpan Design Ltd, UK), set to standard gain, recording time of 1.28, and division by 10 in combination with a zoom H2 recorder (Samson

Technologies, USA). Species identification was performed manually using the Syrinx software (John Burt, University of Washington). Identification criteria are based on the association between acoustic call type, call shapes and measurable parameters (pulse duration, inter-pulse interval, bandwidth, terminal frequency and maximum frequency).

RESULTS

Species list

In eight nights of sampling, we caught 84 bats belonging to 17 species and five different families (Table 1 and Supplementary Material Fig. 1). During the six nights using ultrasound detectors, we recorded 12 species of bats representing five families as well as one unidentified species. During the roost search, we also found two species of two different families. By combining the three survey methodologies, we identified 25 species of Chiroptera from 19 genera within the perimeter of the EBG.

Emballonuridae Gervais, 1856

Peropteryx macrotis Wagner, 1843

This species was recorded only once on 23rd of November over Rio San Pedro (Fig. 2A). The species was identified by six pulses with a duration of 6-9 ms in quasi-constant frequency (QCF) around 40 kHz and inter-pulse intervals (IPI) between 80 and 230 ms which are typical of the species. Such short calls with most energy at c. 38-40 kHz are typical of the species (Rydell et al. 2002).

### *Rhynchonycteris naso* Wied-Neuwied, 1820

This small insectivorous species occurs at the edge of the Rio San Pedro in full light, concealed in small groups on rocks or trunks of trees emerging from the water. Two colonies of seven and eight individuals respectively were discovered within one km of the station. Many echolocation calls of the species, characterized by high frequencies (Jung et al. 2007), could also be recorded at the river (e.g. Fig. 2B). These calls comprise a QCF component of high frequency between 90 and 92.5 kHz with inter-pulse interval between 30 and 120 ms. A group of 5-6 individuals was already observed in the Park, on a tree trunk above the water (Bestelmeyer & Alonso 2000).

Saccopteryx bilineata Temminck, 1838

This is a small insectivorous species which is characterized by the presence of two white lines on its back, contrasting with its dark fur. The males have the particularity of being equipped with two small wing sacs under the forearm to store pheromones. Eleven individuals were captured in a forest path leading to the Rio San Pedro. The species was regularly recorded with the detector (e.g. Fig. 2C) but only in mature forest areas. The sounds recorded at release consist of series of 3 to 4 harmonics of QCF, a first harmonic between 23 and 24 kHz of maximum frequency (Fmax) and a measured IPI between 100 and 140 ms. Those measurements are consistent with the values from the Table S1 from MacSwiney et al. (2008). We also identified the

Table 1 - List of the species detected during our survey. Endemism refers to the publication from Kraker-Castañeda et al. (2016). The IUCN status is the status from the IUCN Red List of Threatened Species (Version 2017-3; LC = Least Concern, DD = Data Deficient). The forearm length is given as mean ± SD and number of individuals in brackets.

Family	Species	New for the Park	Endemism	IUCN status	Forearm length
Emballonuridae	*Peropteryx macrotis*	*	no	LC	
	Rhynchonycteris naso		no	LC	
	Saccopteryx bilineata		no	LC	44,3 ± 1 (11)
Mormoopidae	*Pteronotus davyi*	*	no	LC	44 (1)
	Pteronotus mesoamericanus		yes	LC	57,1 ± 1,5 (18)
Molossidae	*Eumops sp.*	*	-	-	
	Molossus rufus		no	LC	
Natalidae	*Natalus mexicanus*	*	no	LC	
Noctilionidae	*Noctilio cf. leporinus*		no	LC	
Phyllostomidae	*Artibeus jamaicensis*		no	LC	60,1 ± 1 (6)
	Artibeus lituratus		no	LC	70,3 ± 1 (3)
	Carollia sowelli		yes	LC	39,0 ± 0,7 (13)
	Carollia perspicillata	*	no	LC	43,3 ±1 (3)
	Dermanura phaeotis		no	LC	38,6 ± 0,9 (5)
	Dermanura watsoni		yes	LC	37,7 ± 0,5 (3)
	Desmodus rotundus		no	LC	56,8 (1)
	Glossophaga cf. commissarisi		no	LC	35,3 ± 0,8 (2)
	Glossophaga cf. soricina	*	no	LC	36,1 ± 0,5 (5)
	Sturnira parvidens		yes	Not yet assessed	38,0 ± 0,4 (2)
	Uroderma cf. convexum		yes	Not yet assessed	43,2 ± 1 (2)
Vespertilionidae	*Eptesicus furinalis*	*	no	LC	
	Lasiurus ega/intermedius	*	no	LC	
	Rhogeessa bickhami/menchuae	*	yes	LC/DD	28,7 ± 0,5 (8)
	Myotis sp.	*	-	-	34,1 (1)
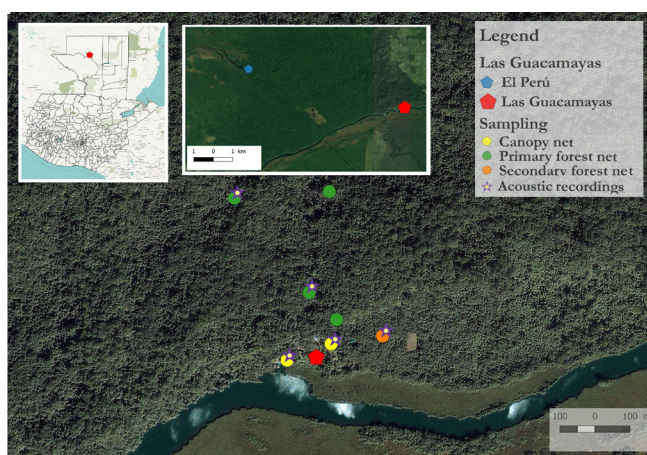
Fig. 1 - Map of the capture and recording sites. The first insert shows the country of Guatemala while the second insert shows the localization of El Perú and Estación Biológica Las Guacamayas (EBG). The main figure is the localization of the different fieldwork sites in the EBG.

pulse alternation which is characteristic of the species (Jung et al. 2007). A group of the species was already observed in a hollow trunk of matapalos (*Ficus* sp.) (Bestelmeyer & Alonso 2000).

Mormoopidae Saussure, 1860

Pteronotus davyi Gray, 1838

An individual was captured eight km from the station on a forest path leading to the Laguna el Perú. This species has a singular peculiarity: its wings are contiguous to its back, giving it a "hairless" look. The lone female captured had a 44 mm forearm, a characteristic measure for the species (43-49 mm, Reid 2009). Furthermore, we recorded many echolocation calls from this species, mainly in the forest alleys around the station (e.g. Fig. 3A). Its echolocation consists of a constant frequency segment (CF) followed by a downwardly modulated frequency segment which terminates with a short, quasi-constant frequency segment. Up to three harmonics were recorded, the second harmonic segments being the most intense in terms of energy. The average frequencies recorded for the constant frequencies of the second harmonic varied between 57-58.5 and 67-70
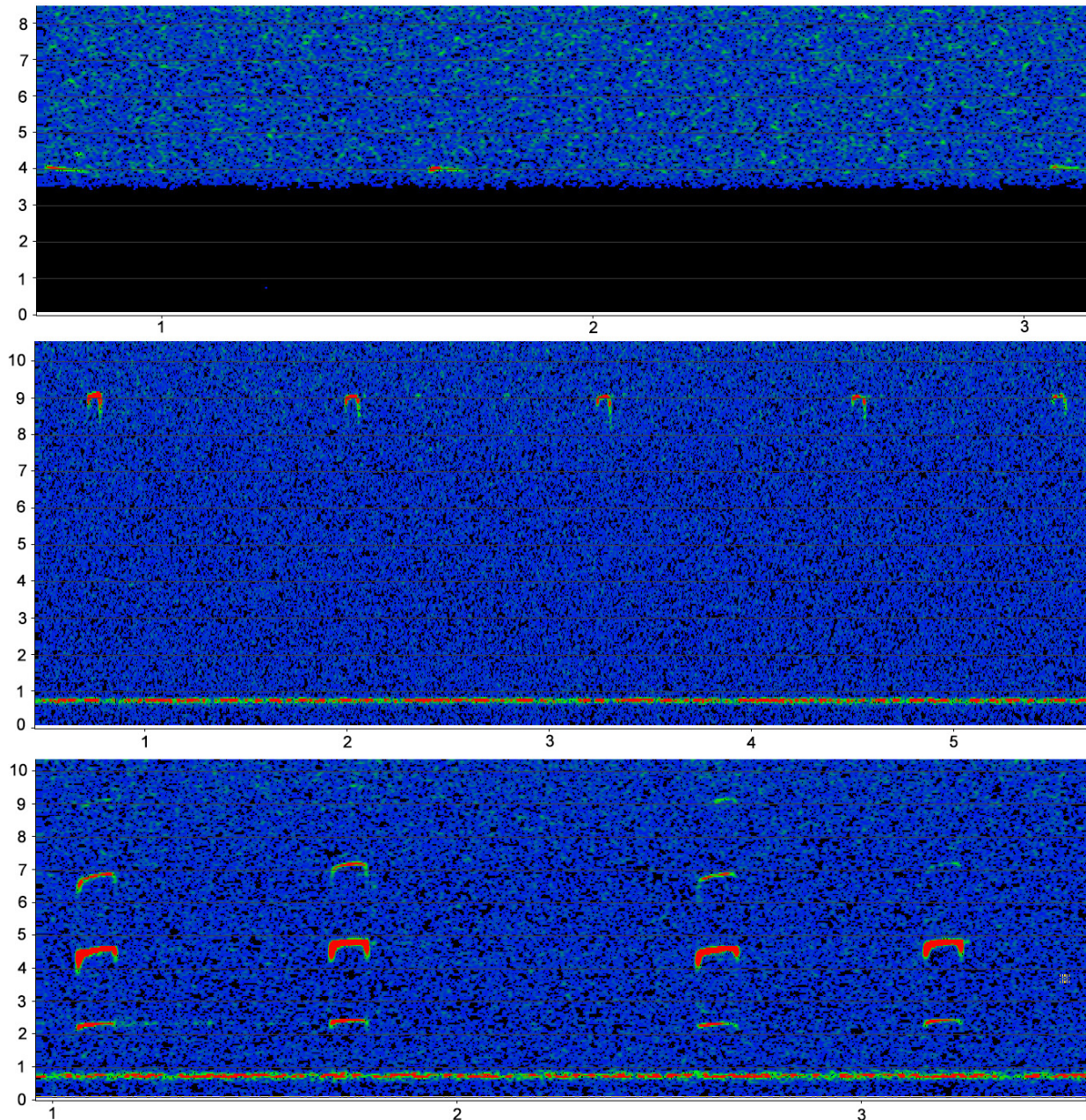
Fig. 2 - A (on top). Ultrasound recording of a free-flying *Peropteryx macrotis* recorded along the Rio San Pedro. B (in the middle). Ultrasound sequence from a hand-released *Rhynchonycteris naso* (time expansion x10). C (at the bottom). Ultrasound sequence from a hand-released *Saccopteryx bilineata* (time expansion x10). x-axis: Time (1 unit = 100 ms), y-axis: Frequency (1 unit = 10 kHz).

kHz for an IPI of 120-130 ms. These values are consistent with the frequencies of 58.0 and 68.0 kHz reported for conspecifics from Panama (Ibánez et al. 1999).

Pteronotus mesoamericanus **Smith, 1972**

Previously considered a species in its own right, *Pteronotus parnellii* is now seen as a complex of species difficult to identify (Lewis-Orrit et al. 2001, Dávalos 2006, Clare et al. 2013, De Thoisy et al. 2014, López-Wilchis et al. 2016, López-Baucells et al. 2017). Captured 18 times (11 males and seven females) and recorded at all capture sites, this species is the most commonly captured bat in the biological station. This species complex was already known for the National Park thanks to the capture of several individuals (Bestelmeyer & Alonso 2000). The forearm measurements of our captured individuals, between 52.2 and 59.2 mm, cannot be used to differentiate species from the complex (Clare et al. 2013). Based on the basis of our ultrasound recordings (30 series analysed and 100 calls measured), the species has a QFC with an average of 63.0 ± 1.7 kHz that varies between 61.8 and 63.8 kHz (e.g. Fig. 3B). Based on the acoustic measurements, we can confirm that we recorded *P. mesoamericanus*, which is known to have an average constant frequency of 62.1 ± 1.91 kHz based on individuals from Central America (Clare et al. 2013). It will be noted however that our individuals appear on average slightly higher in frequency.

Molossidae Gervais, 1856

Eumops **sp.**

We recorded many sequences comprising calls with QCF and downward modulation alternating between 15 and 20
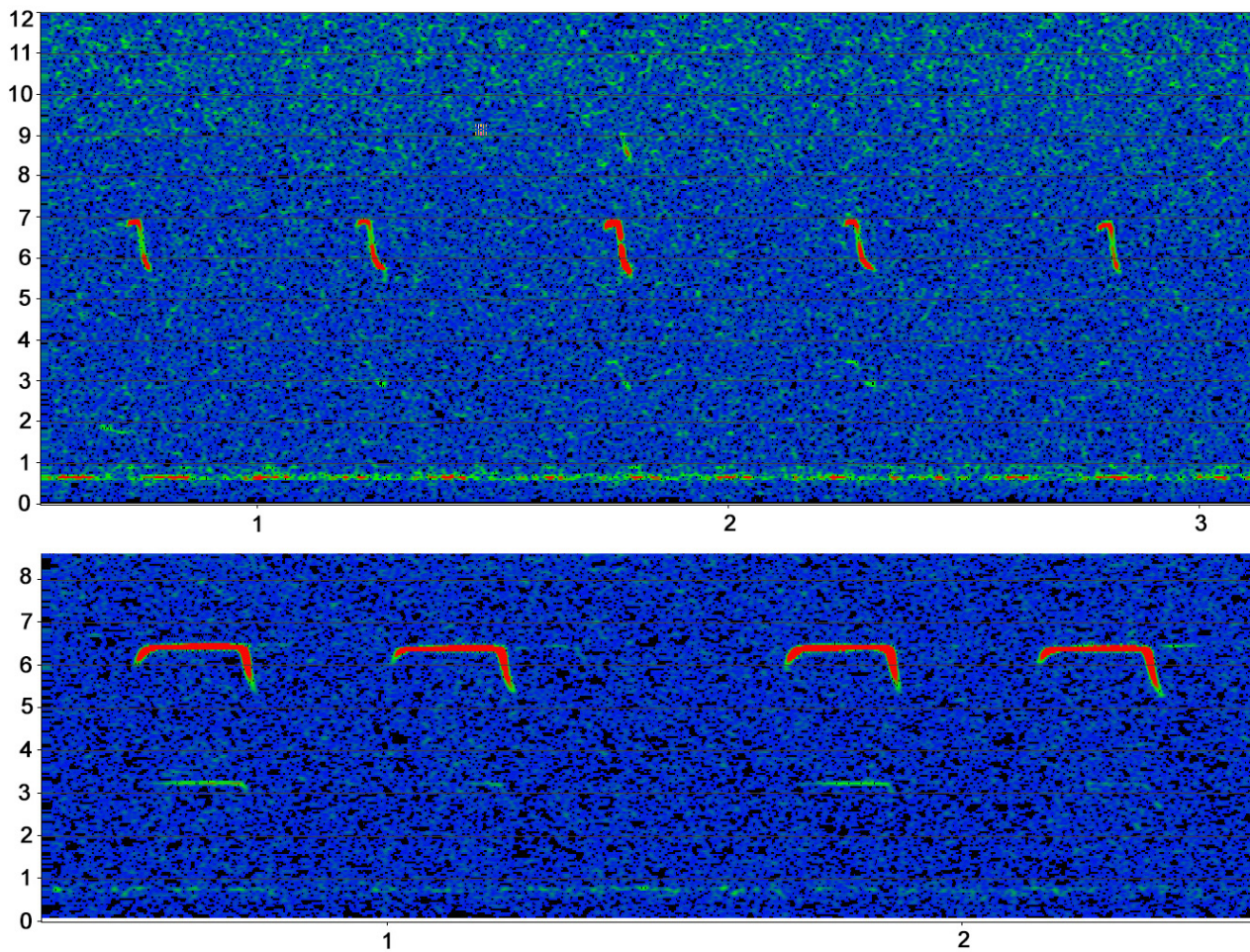
Fig. 3 - A (on top). Ultrasound sequence of a free-flying *Pteronotus davyi* typical of prey capture (time expansion x10). B (at the bottom). Ultrasound sequence of a free-flying *Pteronotus mesoamericanus* typical of foraging (time expansion x10). x-axis: Time (1 unit = 100 ms), y-axis: Frequency (1 unit = 10 kHz).
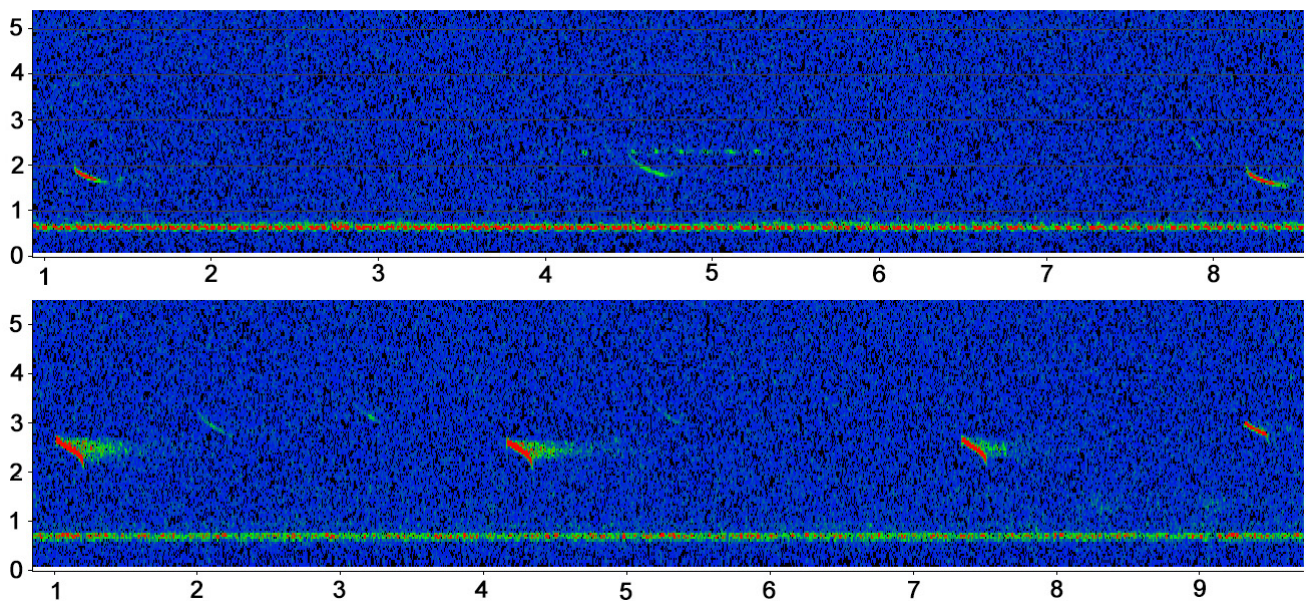
Fig. 4 - A (on top). Ultrasound sequence of a free-flying *Eumops* sp*.* (time expansion x10). B (at the bottom). Ultrasound sequence of a free flying *Molossus rufus* foraging above the Rio San Pedro (time expansion x10). x-axis: Time (1 unit = 100 ms), y-axis: Frequency (1 unit = 10 kHz).

kHz (e.g. Fig. 4A) that are typical of *Eumops* sp. (Jung et al. 2014). Both species of the genus known in Guatemala, *E. auripendulus* and *E. ferox*, have a wide distribution (Kraker-Castañeda et al. 2016). Several other species are also present in neighboring countries (Reid 2009). The scarcity of acoustic data for the genus does not allow us a specific identification.

Molossus rufus Geoffroy, 1805

Many individuals were recorded by the detector, flying over the biological station or foraging above the Rio San Pedro. We recorded calls with QFC and downward modulation between 21 and 27 kHz with pulse alternation (e.g. Fig. 4B) which are typical of the species (Jung et al. 2014). The species was already known for the area with the discovery of an individual under a camping tent of the EBG (Bestelmeyer & Alonso 2000).

Natalidae Gray, 1866

Natalus mexicanus Miller, 1902

An individual was captured early in the night near the station on a forest path leading to the Rio San Pedro. No sound of reference of satisfactory quality could be realized when releasing this individual because this species emit echolocation calls of very low intensity (Rydell et al. 2002). It is actually the only species of Natalidae recorded in Guatemala.

Noctilionidae Gray, 1821

Noctilio cf. *leporinus* Linnaeus, 1758

An individual was found in a tree cavity at the El Perú (Maya site). Since the individual was inaccessible, the forearm length could not be measured. However, its large size suggests that it was *Noctilio* cf. *leporinus* (and not *N. albiventris*). The species was already known for the National Park with the observation of an individual at the 'Laguna el Perú' (Bestelmeyer & Alonso 2000).

Phyllostomidae Gray, 1825

The family of Phyllostomidae, endemic to the new world, represents most of the specific diversity encountered in the biological station with 11 species surveyed. Except for *Glossophaga commissarisi,* we recorded no ultrasounds during the release of captured individuals because Phyllostomidae usually emit low-intensity and high-intensity echolocation calls (Kalko 2004).

Artibeus jamaicensis Leach, 1821

This frugivorous species was captured six times (three males and three females) in different habitats. The measurements of forearm length varied between 58.4 and 61.7 mm, for a specific range between 55 and 67 mm (Reid 2009).

Artibeus lituratus Olfers, 1818

Similar to *A. jamaicensis*, this species has white lines on the rostrum. Three males were caught on the same site, a secondary forest alley. The measurements of forearm length varied between 69.5 and 71.8 mm, when the specific range is 69-78 mm (Reid 2009).

Carollia sowelli Baker, Solari & Hoffman 2002

Individuals of this species were previously attributed to *C. brevicauda*, which is now considered limited to eastern Panama and South America (Wright et al. 1999, Baker et al. 2002, Hoffman & Baker 2003). *C. sowelli* has the same sequence of morphological features as *C. brevicauda* but is slightly larger (Baker et al. 2002). We captured 13 individuals, both in primary and secondary forests. The forearm length of our captured individuals varied between 37.7 and 40 mm, for a specific range known between 37 and 42 mm (Reid 2009). The species was already known for the National Park but reported under the name *C. brevicauda* (Bestelmeyer & Alonso 2000).

Carollia perspicillata Linnaeus, 1758

One male and two females were captured in primary forest alleys. The forearm length of these individuals varied between 40.9 and 44.4 mm, with a known specific range of 41-45 mm (Reid 2009).

Dermanura cf. phaeotis Miller, 1902

The synonym of this species is *Artibeus phaeotis*. Frugivorous species of medium size, it was caught five times on four different forest sites.

Dermanura cf. watsoni Thomas, 1901

The synonym of this species is *Artibeus watsoni*. It has white facial stripes, usually more than in its sister species *D. phaeotis* (Reid 2009). The forearm length, between 35-40 mm for *D. phaeotis* and 35-41 mm for *D. watsoni*, does not allow a specific diagnosis. Three individuals were caught on a forest path.

Desmodus rotundus E. Geoffroy, 1810

This is the only hematophagous species detected in our study, with a male individual captured eight km from the station at the end of the sampling night on a forest path leading to the Laguna el Perú.

Glossophaga cf. *commissarisi* Gardner, 1962

This nectarivorous species was caught twice, in a forest alley at the most isolated site from the station. The forearm length of these individuals varied between 34.5 and 36.1 mm, with a known specific range of 31-38 mm. The lower incisors are small and have a small space between them (López-Baucells et al. 2016). Ultrasound recordings from a hand-released individual show a series of frequency-modulated calls, with initial frequency of 35 kHz, terminal frequency of 12 kHz and IPI of 750 ms (Fig. 5). These pulses show 'zigzags' with a terminal part relatively flattened as well as two very low harmonics.

Glossophaga cf. *soricina* Pallas, 1766

This species was caught five times on a forest path leading to the Rio San Pedro. The forearm length of these individuals varied between 35.4 and 36.8 mm, with a known specific range of 31-40 mm. Unlike its sister species *G. commissarisi*, the lower incisors are not spaced (López-Baucells et al. 2016).

Sturnira parvidens Goldman, 1917

Previously a widely distributed species from Mexico to South America, *Sturnira lilium* (E. Geoffroy 1810) is now considered a species complex (Ditchfield 2000, Velazco & Patterson 2013). *S. lilium* is now restricted to South America (Velazco & Patterson 2013) while subspecies *S. l. parvidens* Goldman 1917 is now ranked as a species distributed in Central America (Velazco & Patterson 2014). This species was captured twice in a forest alley leading to the Rio San Pedro (one male and one female). The forearm length of these individuals varied between 37.7 and 38.4 mm, with a known specific range of 38-41 mm (Velazco & Patterson 2014). The species was already known to the National Park but reported under the name of *S. lilium* (Bestelmeyer & Alonso 2000).

Uroderma cf. *convexum* Baker & McDaniel, 1972

Previously a widely distributed species, *Uroderma bilobatum* (Peters 1866) is now considered a species complex. Two species are found in Central America: *Uroderma davisi* (Baker & McDaniel 1972) distributed along the Pacific coast and *U. convexum* (Lyon 1902) along the Atlantic coast (Mantilla-Meluk 2014). According to the geographical distribution, we have likely captured *Uroderma convexum*. This species was caught twice: in open areas above the canopy net camp (normal mist net pulled up on 6-meter poles) and in a forest alley near the river. The forearm length of the captured individuals varied between 42.3 and 44.2 mm. The species was probably already known for the National Park but reported under the name of *U. bilobatum* (Bestelmeyer & Alonso 2000).

Vespertilionidae Gray, 1821
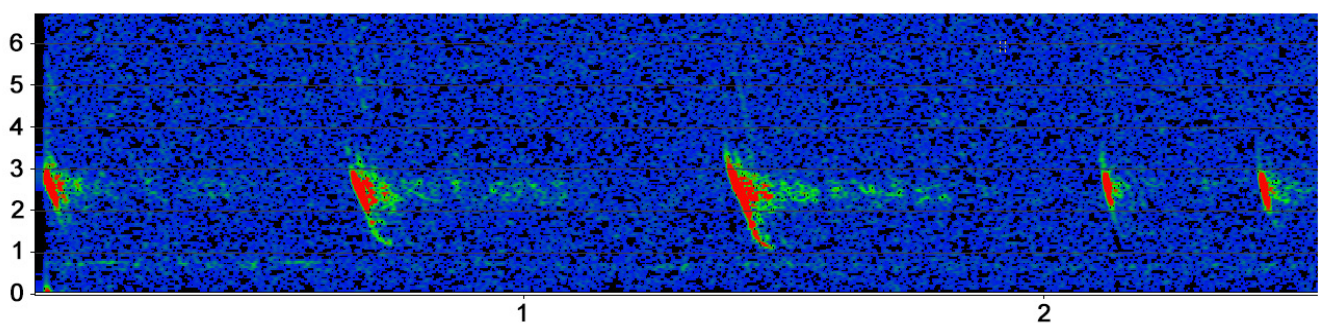
Eptesicus furinalis

High-flying species, *E. furinalis* was detected one time with the ultrasonic detector as it was passing over the camp. The recorded sounds are of the highly modulated type, with a terminal frequency between 36 and 37.5 kHz, an IPI between 120 and 250 ms and a bandwidth between 7 and 20 kHz (e.g. Fig. 6A). These acoustic pulses are in accordance with those described acoustically in the publication by Rydell et al. (2002).

Lasiurus ega/intermedius Gervais, 1856/H. Allen, 1862

This genus was detected with the ultrasonic detector on many occasions (e.g. Fig. 6B). The recorded sounds are of the modulated type, with a terminal frequency between 28.5 and 33 kHz, an IPI between 100 and 250 ms and a bandwidth between 3 and 15 kHz. Sequences with alternating phases could be recorded. The acoustic characteristics of these sounds are characteristic of *Lasiurus ega* or *Lasiurus intermedius* (two species described acoustically in the publication by Rydell et al. 2002). However, the lack of precise study on their acoustic identification does not allow us to make a specific diagnostic.

Rhogeessa bickhami/menchuae Baird et al. 2002

Previously a broadly distributed species, *Rhogeessa tumida* (H. Allen 1866) is now considered a species complex comprising two new species have been identified based on genetic data: *R. bickhami* and *R. menchuae* (Baird et al. 2002). These two species are found in Guatemala: *R. bickhami* in the east of the Motaga Valley and *R. menchuae* at the Rio Vega Grande, Los Amates, Izabal. These two species have no morphological differences except for small differences in the proportions of the skull. We are not in a position here to differentiate these two species. Eight individuals (four females and four males) of *R. bickhami / menchuae* were caught in mature forest alleys during 4 different evenings. The forearm length of these individuals varied between 28.1 and 29.6 mm. The set of sequences recorded at release or in flight has modulated frequencies with a terminal frequency of 47 and 49.5 kHz, an IPI of 80 ms and a bandwidth between 25 and 40 kHz (Fig. 6C).

Fig. 5 - Ultrasound sequence from a hand-released *Glossophaga* cf. *commissarisi*. x-axis: Time (1 unit = 100 ms), y-axis: Frequency (1 unit = 10 kHz).
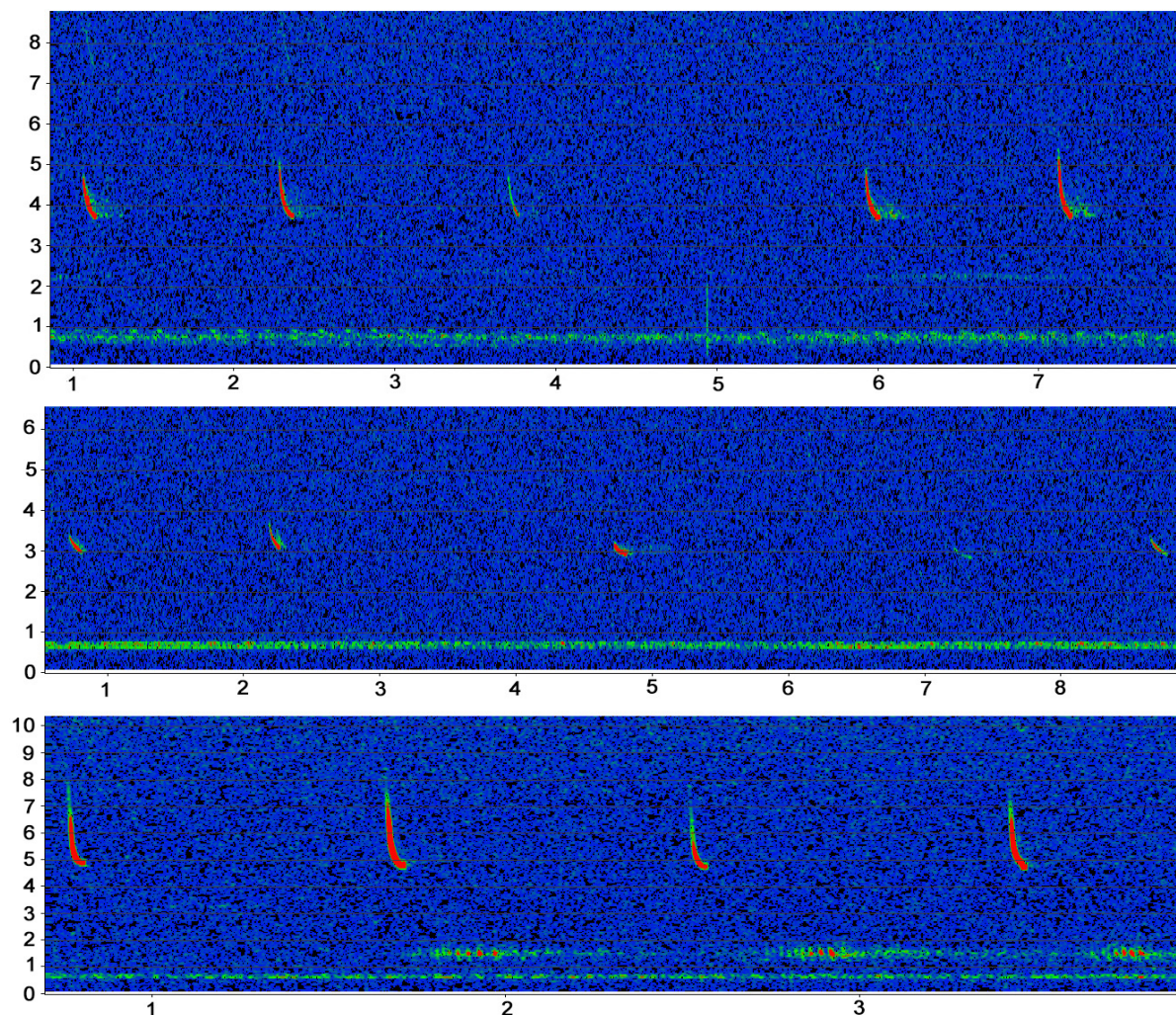
Fig. 6 - A (on top). *Eptesicus furinalis* foraging above the biological station (time expansion x10). B (in the middle). Ultrasound sequences of *Lasiurus sp.* foraging above the biological station (time expansion x10). C (at the bottom). Ultrasound sequence of *Rhogeessa bickhami/ menchuae* (time expansion x10). x-axis: Time (1 unit = 100 ms), y-axis: Frequency (1 unit = 10 kHz).

Myotis sp. Kaup, 1829

This genus was captured only once but recorded several times. An individual was captured early in the night near the station, on a forest path leading to the Rio San Pedro. The forearm length of the female captured measured 34.1 mm, for a specific range known between 32 and 39 mm (Reid 2009). This species appears to show characteristics of *Myotis keaysii.* However, ten species of *Myotis* are known in Guatemala (Kraker-Castañeda et al. 2016). Ultrasounds recorded at release had a terminal frequency between 60 and 65 kHz, an IPI between 20 and 50 ms and a bandwidth of between 30 and 70 kHz (Fig. 7A). In addition, another individual was recorded in a clearing near the station (Fig. 7B). The echolocation calls are highly modulated with a terminal frequency between 61 and 63 kHz, an IPI of 50 ms and a bandwidth between 15 and 30 kHz. The pulses of this second recording appear to be characteristic of *Myotis elegans*, which emits flattened modulated frequencies with terminal frequency between 61 and 62 kHz (O'Farrell & Miller 1999). However, taxonomic uncertainties do not allow us to propose a specific identification. This genus is a new report for the national Park.

Unidentified bats

A short sequence with perfect QFCs with downward modulation at 22 kHz recorded on November 22 above Rio San Pedro (Supplementary material Fig. 2) could not be attributed with certainty to a given species, genus or family. In the current state of knowledge, this sequence could be potentially attributed to *Nyctinomops laticaudatus* but also to *Tadarida brasiliensis* (Jung et al. 2014). Currently, it seems difficult to confirm the identification of this sequence but the future knowledge improvement on the acoustics of the Mesoamerican bats may allow the determination of this sequence.

DISCUSSION

In this study, we present an update of the species list of the 'Laguna del Tigre' National Park, with systematic and taxonomic changes (Kraker-Castañeda et al. 2016) as well as the report of several new species for the National Park. By combining the two inventories of the National Park and taking into account the taxonomic changes, we obtained a maximum of 31 species identified so far.
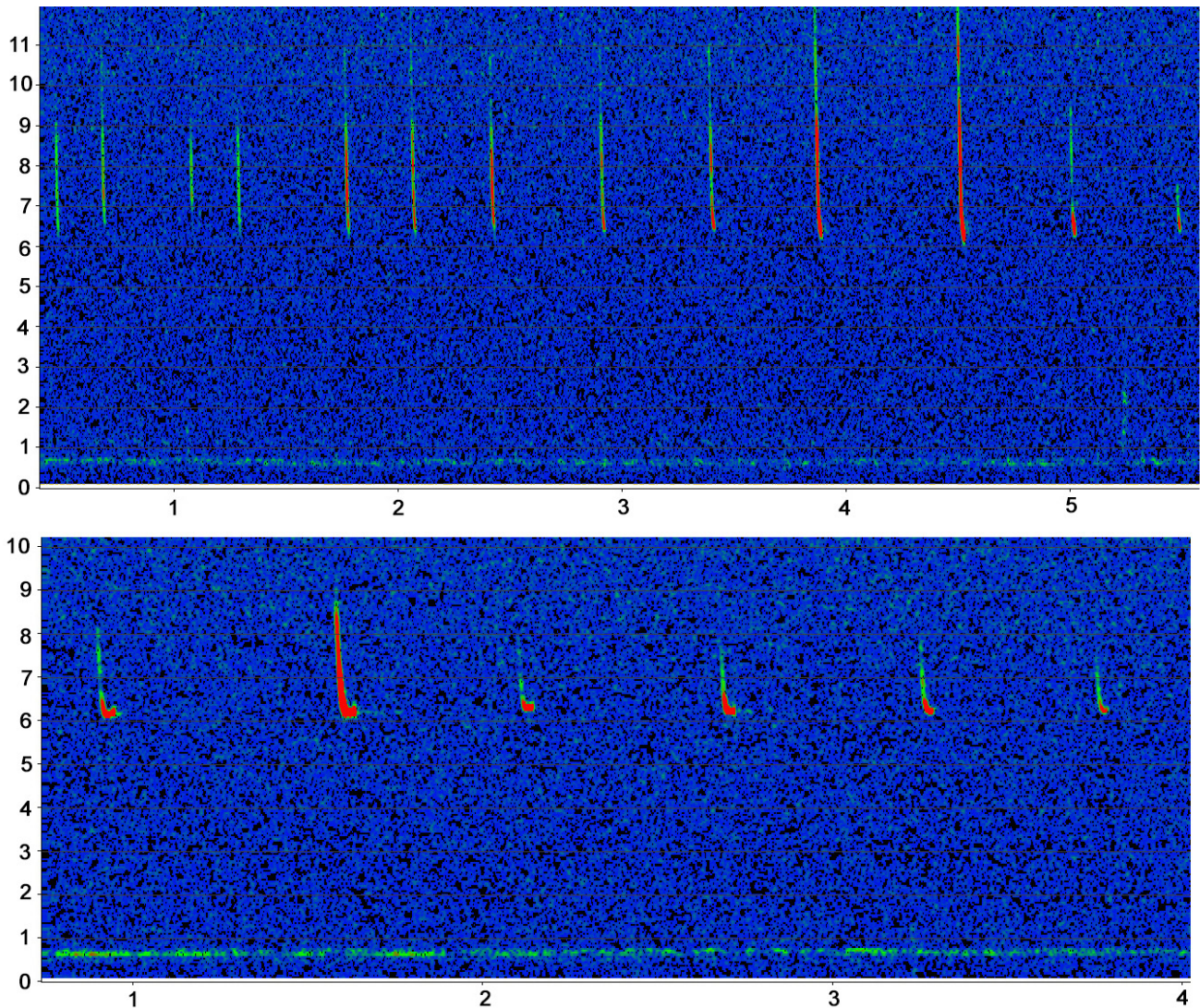
Fig. 7 - A (on top). Ultrasound sequence of the captured individual of *Myotis* cf. *keaysii* at release. B (at the bottom). Ultrasound sequence recorded in flight, potentially of *Myotis* cf. *elegans* (time expansion x10). x-axis: Time (1 unit = 100 ms), y-axis: Frequency (1 unit = 10 kHz).

Specific richness and systematic and taxonomic changes

We have made an inventory totaling 24 species for the EBG. Compared to the inventory of Bestelmeyer & Alonso (2000), we have identified several systematic and taxonomic changes: *Carollia brevicauda* for *C. sowelli*, *Pteronotus parnellii* for *P. mesoamericanus*, *Rhogeesaa tumida* for *R. bickhami / menchuae*, *Sturnira lilium* for *S. parvidens* and *Uroderma bilobatum* for *U. convexum*. Mist net captures and ultrasound recordings resulted in the identification of several new species. The novelty of our inventory resides in finding two new families: the family Vespertilionidae and the family Natalidae. However, several species known from the previous survey of the National Park, six species of Phyllostomidae (*Chrotopterus auritus*, *Mimon bennettii* and *M. crenulatum*, *Micronycteris schmidtorum*, *Tonatia evotis* and *T. brasiliense*) and 1 species of Mormoopidae (*Mormoops megalophylla*), were not recorded in our study.

Mist net surveys

Mist net surveys are a reliable method for capturing many species of bats. In a first inventory, 221 individuals of 20 species were detected at five sampling stations in the National Park 'Laguna del Tigre National Park' with a capture effort of 7,536 N-m / h (Bestelmeyer & Alonso 2000). In this second inventory, almost exclusively focused on the EBG, 84 individuals belonging to 17 species were recorded for a capture pressure of only 96 N-m / h (less than 1.5% of the capture effort of the first study). In both capture surveys, specific richness is clearly biased toward the capture of phyllostomid species. Despite low capture effort and a reduced set of habitat types in our study, we obtained a very similar number of species and a proportionally larger number of individuals. This result can be namely explained by our use of monofilament nets which were new at the time of our study and seldom used in the region. This type of net is much thinner and therefore much less detectable by bats, including insectivorous species such as *Pteronotus mesoamericanus* and *Saccopteryx bilineata* (respectively 18 and 11 individuals captured). Other surveys with mist nets in Guatemala gave different results. Another study surveying coffee shade plantations of Antigua Guatemala reported a lower diversity than our survey with only 12 bat species (Kraker-Castañeda & Pérez-Consuegra 2011). However, two other studies in Guatemala reported higher diversity than

our survey, respectively 20 species of Phyllostomidae in forests of the Tikal National Park (Schulze et al. 2006) and 42 species in 13 sites across Guatemala (Ubico & McLean 1995). The important specific diversity detected in this last study probably results from the high diversity of habitats sampled.

Five of the seven most abundant species of our survey were also part of the most dominant species in a mosaic of forests and arboreal crops in Mexico: *Pteronotus mesoamericanus* (under the name *P. parnelli*), *Carollia sowelli* (under the name *C. brevicauda*), *Artibeus jamaicensis*, *Dermanura cf. phaeotis* and *Glossophaga soricina* (Estrada & Coates-Estrada 2002). Two of these species - *Carollia sowelli* (under the name *C. brevicauda*) and *Artibeus jamaicensis* - were also some of the most abundant species of a rainforest from Mexico (Medellín 1993). The abundance of some species (e.g. *Carollia brevicauda*, Artibeus spp., Dermanura spp. & *Glossophaga soricina*) could potentially result from high ecological flexibility and foraging in various strata of the vegetation (Estrada & Coates-Estrada 2002).

Acoustic surveys

The use of ultrasonic detectors is very effective in complementing the capture inventory, even during a very short period. With only six hours of recording, we detected several species that were not captured. (e.g. the high-flying insectivorous Vespertilionidae *Eptesicus furinalis*). Due to the use of echolocation, these species, that usually forage above the canopy, can easily detect and avoid nets when flying at their level (Berry et al. 2004). Our study reinforces the value of combining ultrasonic recordings to obtain a more complete inventory in a shorter period of time. This combination of methods reduced the effort, time and cost (the ultrasound detector was a loan) and provided a better characterization of bat communities (MacSwiney et al. 2008).

Supplementary inventories

Comprehensive bat inventories in the Neotropics remain very difficult to achieve in the short term because many species are too rare or difficult to capture. By combining three survey methods (mist net in understory, canopy mist nets and roost search), Simmons & Voss (1998) estimated that they sampled only 77% of the specific diversity expected for Paracou in French Guiana. For future surveys at the EBG, we advise the use of canopy mist nets which increase the number of species captured, presumably because of the vertical stratification of species (Kalko & Handley 2001). We also suggest the use of mist nets above water, potentially using a catch net support system (Nelson et al. 2012). This method is ideal to capture bats such as aerial insectivorous species that are attracted to greater abundance of insect-prey than the interior forest (Fukui et al. 2006) or to water bodies for drinking (Russo et al 2012). The repositioning of nets each night is an alternative strategy to capture more species by limiting net avoidance (Weller & Lee 2007, Marques et al. 2013). In complement, sampling throughout the night increases the probability of catching rare species (Simmons & Voss 1998, Esbérard & Bergallo 2005). However, for any bat inventory in the Neotropics, it would be highly preferable to combine capture and ultrasound recording (MacSwiney et al. 2008). Acoustic recording over several nights should allow the detection of further species with lower detection probability as well as the exploration of other environments such as caves and Mayan temples. Last but not least, the democratization of DNA barcoding - despite budget limitation and infrastructure in the Tropics - is highly recommended to enhance the detection of cryptic species (Clare et al. 2011, 2013).

Conservation implications

Species lists are crucial tools that document the biodiversity of a habitat or a geographical area. Many of the species listed in this inventory have a wide distribution across the continent and are therefore not directly threatened. However, several species in our inventory are considered regionally endemic, with a limited distribution between the northern limit of the Tehuantepec Isthmus and northern Colombia (Kraker-Castañeda et al. 2016). Our study shows a strong specific diversity of bats, which is probably still underestimated. Outside the perimeter of the EBG, the National Park 'Laguna del Tigre' is still threatened by human development and colonization related to oil extraction, leading to major deforestation, unregulated fishing and hunting, and pollution (Bestelmeyer & Alonso 2000). Conservation efforts should focus on preserving the primary forest and the Rio which provide shelter and food for many bat species.

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