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Echolocation calls and flight behaviour of the elusive pied butterfly bat (*Glauconycteris superba*), and new data on its morphology and ecology

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The pied butterfly bat, *Glauconycteris superba*, is endemic to the tropical forest zone of Africa, where it was previously known from only five specimens. Here we report the capture of 10 individuals in two localities of the Democratic Republic of the Congo (Mbiye Island and Yoko forest reserve), and we present the first acoustic data of the species recorded using a conventional microphone and a home-made acoustic system for real time 3D localization. Our morphological comparisons show that females are larger and heavier than males, and that the two sexes exhibit the same fur coloration pattern. We found some individual variations concerning the width of the two lateral white stripes on the belly, and the number and extension of white shoulder-spots. The echolocation recordings show evidence for alternation between two call types (A and B), differing in frequency, bandwidth, and duration. The acoustic signals obtained before captures and after releases revealed important variations depending on the trajectories and environmental conditions. Acoustic characteristics, wing measurements, and the unique black and white fur pattern of *G. superba* suggest that it is a canopy species able to fly at high speeds. Our findings will be useful for future ecological studies to provide new data on the range, population size, trend and threats of *G. superba* in order to better assess its conservation status.

Key words: Vespertilionidae, acoustic, flight path reconstruction, frequency alternation, sexual dimorphism, fur colour pattern, canopy

Introduction

One important gap in the knowledge of global biodiversity remains to be deficiency of data pertaining to distribution, as well as population size and trends, and the abundance and ecology of many species (Küper *et al*., 2006). Data deficiency may be a problem in conservation because the extinction risk of species cannot be evaluated. Such species are typically classified as 'Data Deficient' (DD) by the Red List of the International Union for Conservation of Nature, but many of them are also included in the 'Least Concern' (LC) category, despite the lack of data on population trends. The Order Chiroptera is especially subject to this problem, with 200 species (17.5%) classified as 'DD' and 684 considered as 'LC' (60%) by the IUCN (2015). One powerful tool in the study of bats relies on acoustic recordings. In taxonomy, some sibling species can be distinguished on the basis of echolocation calls (e.g., Jacobs *et al*., 2006). In ecology, echolocation calls can be used to study the foraging behaviour of species (Schnitzler *et al*., 2003), the use of several microphones allowing to reconstruct precisely each flight path (Jones and Holderied, 2007). Based on prior knowledge of echolocation calls, surveys can be conducted at large scales to provide valuable information on the distribution and population trends of species (Hughes *et al*., 2010; Barlow *et al*., 2015).

The pied butterfly bat, *Glauconycteris superba* (Hayman, 1939), is an African bat characterized by a black pelage with white spots and stripes on the

head, back and belly. Despite its unique and striking fur pattern, this species was known, up to now, from only five specimens collected in five localities in the Democratic Republic of the Congo (DRC), Ghana, Ivory Coast, and Sudan (Gembu Tungaluna *et al*., 2013; Reeder *et al*., 2013). The natural history of this species is poorly documented. It was considered as 'vulnerable' (VU) in 1996 and 2004 IUCN Red List assessments, but was moved to the 'LC' category by Fahr *et al*. (2008) 'in view of its wide distribution, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category'. Fahr (2013) considered however that a threatened or DD category would be more appropriate for *G. superba*.

In 2013, a field expedition was organized in the Orientale Province of the Democratic Republic of Congo, where the holotype was discovered (Medje — Hayman, 1939), and where a new specimen was recently captured (Mbyie Island — Gembu Tungaluna *et al*., 2013). Following successful capture and acoustic recordings of 10 individuals, we present new information about echolocation calls of the species. We also introduce a light portable 3D acoustic tracking system to observe in real time bat trajectories. Overall, we aim to gather new knowledge on the ecology, sexual dimorphism, fur coloration pattern, and conservation status of the elusive pied butterfly bat.

Materials and Methods

Capture Sites

At the end of the year 2013, a joint bat expedition organized by the University of Kisangani (DRC), Muséum national d'Histoire naturelle (MNHN, France) and Institut Langevin (France) took place in the Orientale province of the Democratic Republic of the Congo. Mist-nets (Ecotone, Gdynia, Poland) were placed near water to capture the bats. In two different sites (Supplementary Fig. S1), a total of 10 individuals of *G. superba* were caught as they were coming close to the ground. Eight individuals were captured on the Mbyie Island (0.44623°N, 25.29287°E) on the 11th and 12th of December 2013, in a stagnant water area located in a relatively young secondary forest, where Gembu Tungaluna *et al*. (2013) collected one specimen previously. Two individuals were also captured in the Yoko forest reserve (0.29410°N, 25.28895°E) on the 16th of December 2013, near a little stream located within a primary forest. All individuals were released at the site of capture, except five specimens, which were collected for museum collections.

Body Measurements

Age and reproductive status of bats were checked directly in the field. A caliper was used to measure the upper tooth row (CM3), tibia (Tib) and forearm (FA, for both wings of each individual). A steel ruler was used to measure length of the third finger (D3), the fifth finger (D5), and the tail. All measurements were collected by a single individual observer (RC) in order to minimize inter-observer error, following the protocol from Dietz and Helversen (2004), except that the fingers were measured with the wrist as detailed in the technical book of the MNHN & Groupe Chiroptères de la SFEPM (2014). Body mass was measured using a balance light line 20g/0.2 (Pesola, Schindellegi, Switzerland).

Wing Shape

Wing parameters of bats are very informative to understand the flight performance, foraging strategy and the ecological niche use in general (Norberg and Rayner, 1987). Wing photos were taken on four specimens kept in the collections of the Muséum national d'Histoire naturelle (Paris, France) (Supplementary Fig. S2). Wing span was taken as the greatest distance from one tip to the other, following Farney and Fleharty (1969). Wing area was measured as the sum of the armwing area and handwing area (not including the uropatagium) following Schmieder *et al*. (2015) and illustrated in Supplementary Fig. S2. Both measurements were estimated using Analyzing Digital Images (Pickle, 2008). They were used to calculate the two following indexes: wing aspect ratio (wing span²/wing area) and wing loading (body mass × gravitational acceleration/wing area).

Analyses of Echolocation Calls

Reference sounds from free-ranging animals were recorded following three procedures. Five individuals were recorded before capture using a SM2 bat detector 384KHz (Wildlife Acoustics, Concord, U.S.A.) placed close to the mist-nets. Five individuals were followed through light tagging protocol and recorded using a D1000X ultrasound detector (Petterson, Uppsala, Sweden). Finally, 10 individuals were monitored before capture or after release with the 3D acoustic real time tracking system described below. With the last procedure, the acoustic recordings can be directly linked to the behaviour of the bat and the flight circumstances. From the two capture sites (Mbyie and Yoko), we selected seven recordings and measured acoustic parameters using the open access softwares Syrinx 2.6 (John Burt) and ScanR 1.6.1 (Binary Acoustic Technology). Signals from 3D acoustic real time tracking system are processed using a homemade MATLAB software (MathWorks). Six parameters of the calls were measured: call duration (D, ms), inter-pulse interval (IPI, ms), maximal frequency (*F*max, kHz), minimal frequency (*F*min, kHz), frequency bandwidth (BW; difference between *F*max and *F*min) and maximal energy frequency (*F*me, kHz).

3D Flight Trajectories

A special system was designed at the Institut Langevin to reconstruct in real time 3D bat flight trajectories. A setup of four microphones (FG 3329, Knowles Acoustics, Itasca, IL USA) arranged in a pyramidal composition (i.e., three in the corners and the last at the top) was chosen as a simple but efficient way to localize an acoustic source in three dimensions. Signals from microphones are pre-amplified by a custom made electronic device of 100 kHz bandwidth and 40 dB gain. An analog to digital converter (Agilent U2542A, Santa Clara, California) is used to

digitize in 16 bits format analogous signals and a portable rugged computer (equipped with Intel Core i7 processor) is used to compute in real time condition 3D positions of bats. The setup is light enough (< 5 kg with motorcycle battery) to be carried everywhere in a backpack. The system was deployed to record bats before capture and after release. The protocol used to convert the acoustic recordings from the four microphones into 3D flight trajectories is detailed in Supplementary Text.

Results

Body Measurements and Fur Colour Pattern

All bats caught were adults, as confirmed by the presence of fully ossified metacarpal phalangeal joints. Two of the four captured females were lactating. Sexual size dimorphism was observed, with females being 5% larger (using FA, tibia, or D5 values) and 10% heavier than males (Table 1). We analyzed wing photos of four *G. superba* (2 ♂♂ and 2 ♀♀). Raw data for each wing parameter measured are summarized in Table 1.

The pelages of the 10 freshly collected bats (Fig. 1) showed that females and males exhibit the same coloration pattern. There are three white markings on the head, including one large band on the top of the muzzle and two lateral bands located on both sides of the face and surrounding the black ears. Ventrally, the large white ruff on the throat is disconnected from the two large white lateral stripes, which start in the pectoral region and merge at the back of sexual organs. On the shoulders, most individuals have two white spots, but the male K13-286 from Mbiye has three white spots, the two anterior ones being larger and partially merged. In the latter individual, all white markings appear more extended. On the back, all individuals have four white stripes, which are much thinner than those that appear on the ventral side: the two anterior stripes are found medially and have a 'lanceolate' or 'boomerang' shape; they start at the base of the neck and terminate in the middle of the back; the two other longer stripes are located on the flanks; starting just before the ends of the medial stripes, they converge posteriorly and terminate at the base of the tail.

Ventral lateral stripes of female K13-266 were found to be yellowish rather than pure white. Similarly, the posterior region around sexual organs also appears yellowish in all other individuals except K13-351 (Fig. 1). We suggest that the 'dirty yellowish' coloration is due to fecal matter, urine, or sexual fluids, as well as milk production in the case of lactating female K13-266. For the five museum specimens, we found that the 'dirty yellowish'

Table 1. Morphological characteristics of the 10 individuals of *G. superba* captured on Mbyie Island and in Yoko forest reserve (two individuals highlighted with an asterisk) (mean of left and right wings)

ID	Sex	Status	Mass (g)	Tail (mm)	FA (mm)	D3 (mm)	D5 (mm)	CM^3 (mm)	Tib (mm)	Wing			
										Span (mm)	Area (mm^2)	Aspect ratio	Loading (Nm^{-2})
K13-266	♀	Lact.	15.5	48.0	47.7	88.5	53.5	5.7	20.5	273.0	937.8	7.95	16.21
K13-287	♀	Lact.	19.0	48.0	45.3	87.5	54.5	5.8	20.2				
K13-289	♀	Nul.	–	–	46.1	87.0	55.0	5.7	20.4	282.1	991.8	8.02	NA
K13-322*	♀	Nul.	14.5	47.0	45.5	87.5	56.0	6.0	20.3				
Mean F			16.3	47.7	46.2	87.6	54.8	5.8	20.3				
K13-262	♂	Ad.	14.5	46.0	42.6	84.5	54.5	5.6	18.8	273.4	926.9	8.06	15.35
K13-263	♂	Ad.	15.0	45.0	44.2	85.5	54.5	5.6	20.0	281.4	909.2	8.71	14.86
K13-264	♂	Ad.	14.5	45.0	43.7	87.0	53.5	5.5	19.7				
K13-265	♂	Ad.	14.0	46.0	43.6	83.5	50.0	5.6	18.8				
K13-286	♂	Ad.	16.5	44.0	44.1	74.5	51.0	5.5	18.8				
K13-351*	♂	Ad.	13.5	37.0	44.5	82.5	52.0	5.4	19.8				
Mean M			14.6	43.8	43.8	82.9	52.6	5.5	19.3				

Abbreviations: Ad. — adult, Lact. — lactating, Nul. — nulliparous, FA — forearm length, D3 — third digit length, D5 — fifth digit length, CM^3 — length of upper tooth row, Tib — tibia length

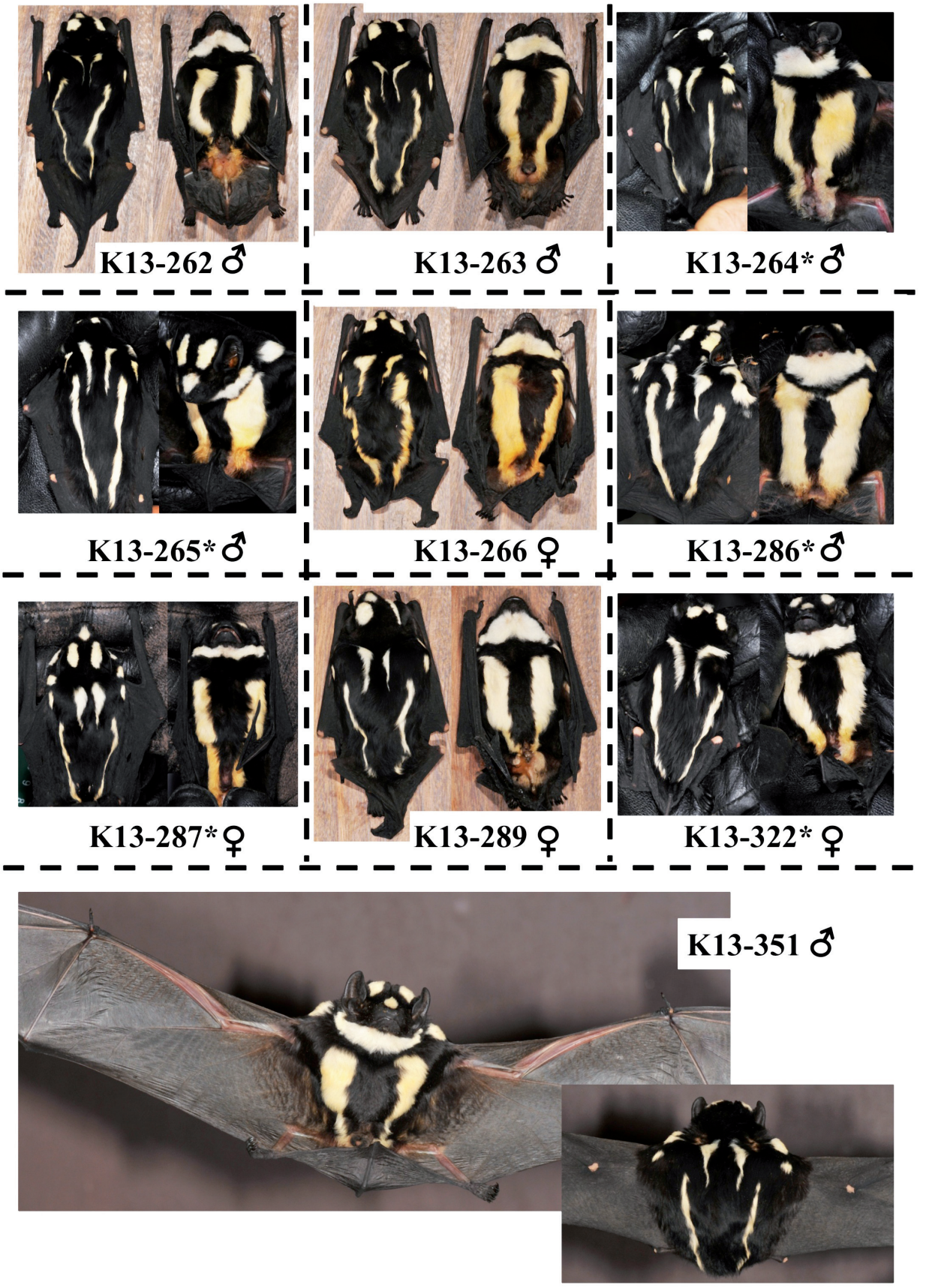

FIG. 1. Dorsal and ventral views of the 10 specimens of *G. superba* captured on Mbyie island (from K13-262 to K13-289) and Yoko forest reserve (K13-322 and K13-351). Released individuals are indicated by an asterisk

colour turned white after fixation and storage in ethanol.

Echolocation Calls

The characteristics of the echolocation calls of *G. superba* are relatively stable. Frequency modulated-quasi constant frequency (FM-QCF) signals alternate between two distinct frequencies: type A with a frequency ranging from 20.5 kHz to 57.0 kHz, and type B with a frequency ranging from 27.3 kHz to 73.3 kHz (Fig. 2 and Table 2). The alternation between the two types of calls was observed in most of recorded sequences. Calls of type A are generally faster than those of type B (5.6 ± 1.9 ms versus 6.4 ± 1.8 ms), and they are characterized by a lower minimal frequency (22.9 ± 1.4 kHz vs 29.8 ± 1.3 kHz), and a lower and less variable bandwidth (13.8 ± 6.3 kHz vs 27.1 ± 13.2 kHz).

3D Flight Trajectories

In general, the accuracy and precision in estimating 3D flight trajectories were dependent on the signal-to-noise ratio of the calls. The trajectories were usable up to a distance of about 10 m if the bat calls are directed toward the 3D acoustic tracking system. As a consequence, the most accurate trajectories were recorded during bat releases and captures because the tracking system was close enough to the animal.

An example of 3D trajectory before the capture of a specimen at Mbyie Island is shown in Fig. 3. At this site, bats can dive for a drink by using two different through-holes in the forest treetop. Mistnets #1 and #2 (9 × 4 m^2) were placed perpendicularly (Supplementary Fig. S1C). The captured specimen came through the hole #1 located on the right side of net #1. It flew along net #1 and then turned right and run along net #2 to be finally caught in it close to the water surface. Most other recorded individuals followed similar trajectories: they avoided net #1, and some of them were caught in net #2.

The 3D trajectory and the spectrograms of some typical echolocation signals during the take-off of a released animal (December 16, 2013 on the soccer field next to the Yoko research center) is shown in Fig. 4. During the first steps of the take-off, the echolocation signal amplitude was strong and second harmonics were clearly visible, whereas the alternate frequency mode did not take place. The flight speed was about 4 m/s when the specimen reached the vertical of the 3D acoustic tracking system. Inter-pulse intervals were short at the beginning of the take-off before increasing. The alternated frequency mode took place when the animal's flight height was stabilized — shortly after the passage above the antenna.

The *G. superba* bats recorded after release and before capture showed highly variable acoustic characteristics, with alternate or fixed frequency modes. When the echolocation pulses alternated in frequency, the difference between the minimal frequencies of call types A and B ranged from 6 to 10 kHz. A video made in Yoko showed that the interpulse interval is equal to the wing beat period, suggesting that the two call types occur at each wing beat. By coupling call emission to wing beat, flying bats can produce more intense echolocation signals at low cost as a by-product of flight (Speakman and Racey, 1991; Wong and Waters, 2001). The alternate frequency mode was recorded when the frequency of wing beat became constant. As shown in Table 2, the frequency bandwidth of type B call was generally greater than that of type A call. When the

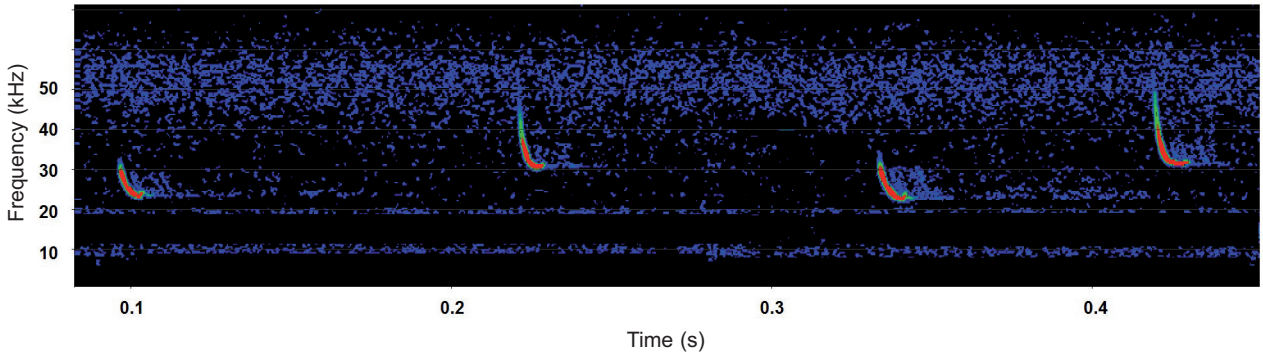

FIG. 2. Echolocation calls of *G. superba* before capture (K13-351). This sequence, which was recorded in Yoko forest reserve, shows a typical alternation between a lower frequency call (type A) and an upper frequency call (type B)

TABLE 2. Acoustic parameters of *G. superba* measured on 52 calls (26 of each type)

Type	IPI (ms)	D (ms)		*F*max (kHz)		*F*min (kHz)		BW (kHz)		*F*me (kHz)	
		A	B	A	B	A	B	A	B	A	B
Min	64	3.1	4.0	29.1	38.8	20.5	27.3	5.9	9.1	22.4	28.8
Max	340	9.6	10.1	57.0	73.3	25.1	31.3	32.6	43.3	30.9	38.2
Mean	148	5.6	6.4	36.8	57.0	22.9	29.8	13.8	27.1	25.7	33.0
SD	73	1.9	1.8	6.6	13.5	1.4	1.3	6.3	13.2	±2.2	±3.0

Abbreviations: IPI — inter-pulse interval between two calls, D — call duration, *F*max — maximal frequency, *F*min — minimal frequency, BW — bandwidth, *F*me — maximal energy frequency

alternate frequency mode was effective, the minimal frequency of calls decreased with the increase of inter-pulse interval (Fig. 5A). The frequency of calls was also shown to decrease with increasing call duration (Fig. 5B).

The bat flight speed was estimated using a total of 239 echolocation calls. The histograms of speed values were computed from successive 3D positions of bats and inter-pulse intervals of calls (Supplementary Fig. S3). For bats released in an open field (soccer field next to the Yoko research center), the flight speeds ranged from 1 m/s to 9 m/s, with a predominant value between 4 m/s and 6 m/s. Before capture at Mbyie Island, an average value of 4–6 m/s was also observed.

DISCUSSION

Evidence for Female-biased Sexual Size Dimorphism

In most species of *Glauconycteris*, sexes are described as being similar in both colour and size (*G. alboguttata*, *G. argentata*, *G. beatrix*, *G. curryae*, and *G. humeralis*), but in the three species *G. poensis*, *G. gleni*, and *G. variegata*, females are slightly larger than males in most body measurements (Happold and Happold, 2013). In *G. superba*, the sex of the holotype remains unknown and no reliable information was available for quantifying sexual dimorphism due to the limited number of collected specimens. By comparing the measurements of 10 individuals captured at Mbiye Island and Yoko, we found clear evidence for sexual dimorphism in *G. superba* (Table 1), the males being smaller and lighter than females. The holotype of *G. superba* was collected at Pawa (DRC), a locality close to Mbiye and Yoko (geodesic distance = 350–400 km). Hayman (1939) described it as 'probably male (the sexual organs were apparently cut away by the collector in opening the body)'. However, the lengths of its forearm (47.5 mm) and tibia (21.3 mm) suggest that it is a female, which is in agreement with the view of Gembu Tungaluna *et al*. (2013), who noted that the nipples of the holotype are much more developed than in the male collected at Matonguiné in Ivory Coast.

Is G. superba *a Canopy Species?*

Based on our wing measurements, we deduced that *G. superba* has high values of aspect ratio, 8.19 ± 0.35 (n = 4), which are similar to the data published by Farney and Fleharty (1969) for the American molossid *Tadarida brasiliensis* (8.05 ± 0.35; n = 39), which often flies at more than 40 km/h (Williams *et al*., 1973). The results indicate therefore that *G.superba* possesses long wings adapted for high-speed flight. In addition, *G. superba* shows high values of wing loading, similar to those found for African molossid species (e.g., *Mops leucostigma* and *Otomops martiensseni*; Taylor *et al*., 2012), suggesting low manoeuvrability. In general, bat species with high aspect ratio and wing loading typically fly in uncluttered or open space, i.e., above the canopy (Norberg and Rayner, 1987), where they catch insects in the aerial mode. This was further confirmed by a video made in Yoko (available at https://www.institut-langevin.espci.fr/IMG/mov/mvi_0377.mov) showing a released individual (marked with a fluorescent capsule on the back) going quickly to the treetops and then flying above the canopy. All these elements indicate therefore that the rarity of *G. superba* records is due to a strong negative bias in capture probabilities. The new description of its peculiar echolocation calls provided in this paper should however increase the recording probability in the future. It must also be noted that all specimens of *G. superba* collected at Mbiye and Yoko, but also in previous studies (Gembu Tungaluna *et al*., 2013; Reeder *et al*., 2013), were captured above the surface of a relatively stagnant pool of water, which may constitute a good place for bats to drink.

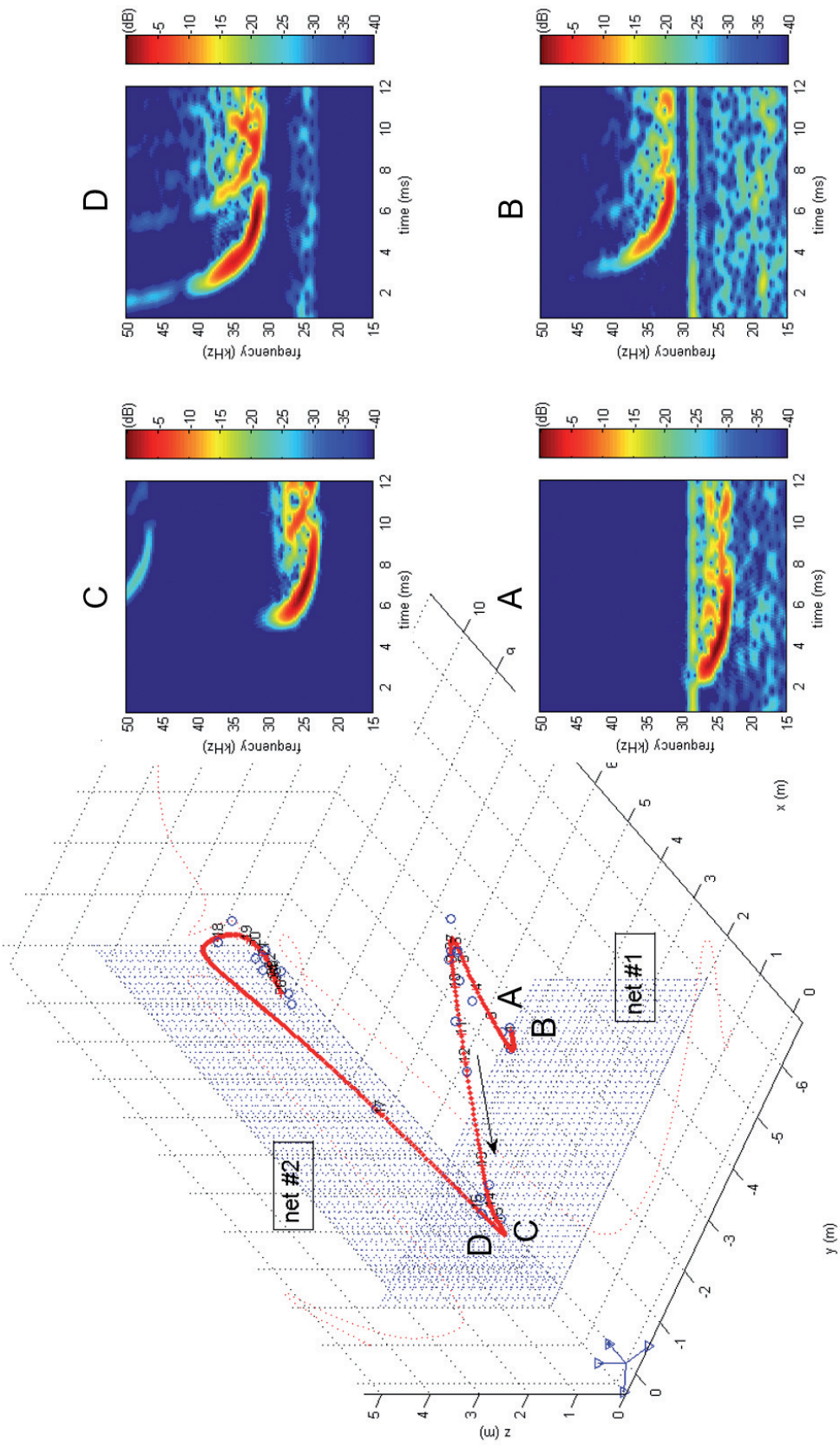

FIG. 3. 3D trajectory of *G. superba* before capture on Mbyie island (K13-287). Spectrograms noted A, B, C, and D shown on the right sides correspond respectively to the calls at positions noted A, B, C. and D on the 3D trajectory representation

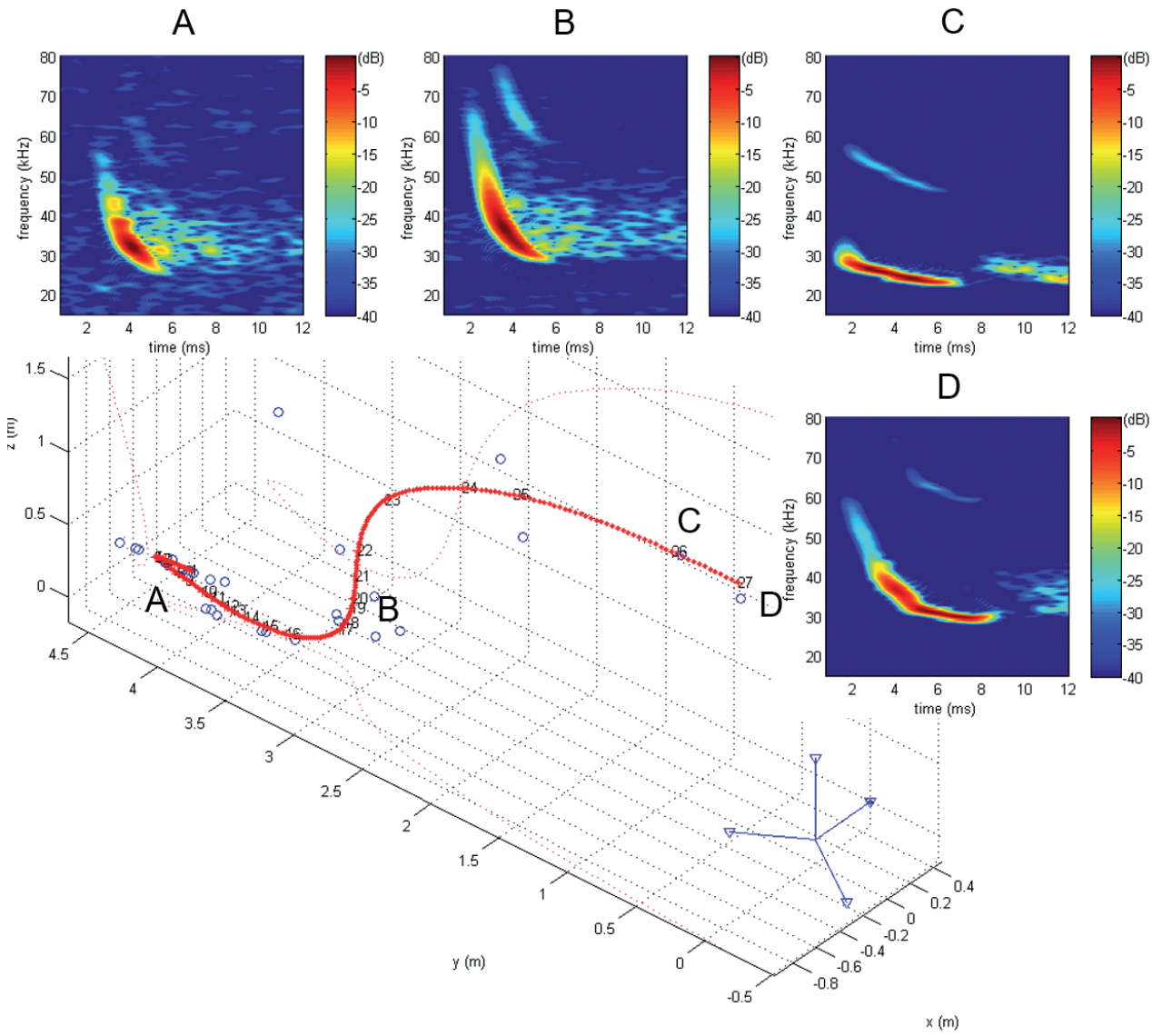

FIG. 4. 3D trajectory of *G. superba* after release (K13-322) on a soccer field close to Yoko research center. Spectrograms noted A, B, C and D correspond respectively to the calls at positions noted A, B, C, and D on the 3D trajectory representation

On the Black and White Pattern of G. superba

The black and white pattern of *G. superba*, which is identical between females and males (Fig. 1), is unique among species of *Glauconycteris*. Although a pair of white shoulder spots and a pair of white dorsal stripes can be found in several species of the genus, such as *G. humeralis* (only spots), *G. egeria* (only dorsal stripes), and *G. alboguttata* (both marking types), the species *G. superba* is exceptional by the presence of many white markings: three on the face, a large white ruff on the throat, four white stripes on the back, and two large lateral white bands on the belly, and generally two spots on the shoulders. The male K13-286 exhibits a slightly different pattern characterized by wider lateral white bands on the belly, as well as three shoulder spots (partially merged) (Fig. 1). Interestingly, this pattern is more similar to that of *G. s. sheila*, a subspecies described by Hayman (1947) on the basis of only one specimen collected in Ghana (BMNH 47.10) (see photos in Gembu Tungaluna *et al*., 2013). The variation in colour pattern here observed for the same population suggests that *G. s. sheila* should be treated as a synonym of the nominate form.

The coloration pattern of *G. superba* has no equivalent among the other species of the order Chiroptera. The most similar pattern is found in another vespertilionid bat, *Scotomanes ornatus* from eastern Asia, in which the orange-brown general

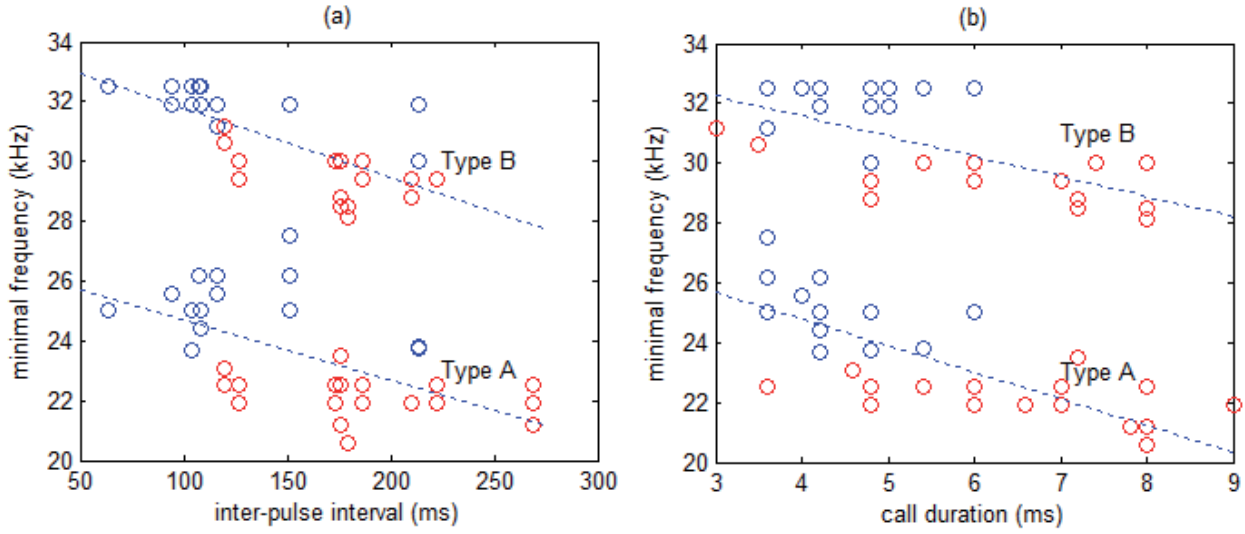

FIG. 5. Minimal frequency versus inter-pulse interval (A) and call duration (B). Released individuals are plotted in red circle and freedom state individuals are plotted in blue circle. The data were recorded when the alternate frequency mode took place

coloration is marked, ventrally, by a white ruff on the throat and two large white lateral stripes, and dorsally, by a narrow median white line and two white spots on the shoulders. Interestingly, *S. ornatus* is a high and fast flying species (Csorba *et al*., 2008), as here assumed for *G. superba* (see above). We suggest therefore that the convergent evolution of ventral white markings in these two species may represent specialized adaptations to the canopy habitat. Indeed, when they fly above the canopy, they can be attacked from below by nocturnal birds of prey, such as owls and hawks. Because of that, we assume that the ventral and dorsal white markings of *G. superba* and *S. ornatus* serve as a disruptive coloration to avoid predation during flight.

The foraging activity of most tropical bats is negatively correlated with moonlight intensity because of greater risk of predation. However, most insectivorous bats are more active at dusk and dawn, because these periods of higher luminosity coincide with the peaks of insect availability (Saldaña-Vázquez and Munguía-Rosas, 2013; Prugh and Golden, 2014). During sunset/sunrise and full moon, bats flying above the canopy are obviously more exposed to avian predators than bats foraging in the understorey and in the subcanopy. Since it has been found that highly contrasting colours enhance the disruptive effect (e.g., Cuthill *et al*., 2005), we propose that the black and white pattern of *G. superba*, which certainly appears more contrasted during twilight and moonlight, has been selected to increase the effectiveness of visual disruption against nocturnal birds of prey.

Alternation of Echolocation Calls

Here, we showed that, except close to mist-nets, *G. superba* alternated two types of calls, which differ in maximal and minimal frequencies, bandwidth and duration (Table 2). This behaviour of alternating calls with little or no overlap in frequencies seems taxonomically widespread, as it was previously described in the families Emballonuridae, Molossidae and Vespertilionidae (Kingston *et al*., 2003; Jung *et al*., 2007, 2014). The significance of call frequency alternation remains unclear and at least six different hypotheses have been put forward: (1) anti-jamming, i.e., to discriminate own echoes from those of conspecifics foraging in the same area (Habersetzer, 1981), (2) optimizing energy expense according to prey detection versus localization needs (Denzinger *et al*., 2001; Kingston *et al*., 2003), (3) increasing obstacle detection radius (Jung *et al*., 2007), (4) countering moth hearing (Goerlitz *et al*., 2010), (5) increasing information update (Ratcliffe *et al*., 2011) and (6) widening prey search angles (Seibert *et al*., 2015). The anti-jamming hypothesis has been already questioned by Jung *et al*. (2007) and Kingston *et al*. (2003). In the case of *G. superba*, it seems particularly unlikely because most individuals alternated calls without conspecifics in the vicinity. According to Jung *et al*. (2007), call alternation with little overlap in frequency allows bats to double

the sound propagation time during which echoes can be interpreted. This means an optimization between information update and echolocation detection radius two times better (Ratcliffe *et al*., 2011), though the increase in detection radius seems not to concern prey detection but only larger objects like obstacles and predators (Jung *et al.*, 2007). Our field data indicate that *G. superba* forages in a particularly complex environment, where it needs to process large amounts of information from various factors, while maintaining a large obstacle detection radius. However, contrary to neotropical emballonurids studied by Jung *et al*. (2007), the two call types of *G. superba* do not differ only in frequency distribution, but also in call duration and sound pressure level. Here, call alternation may also fulfill other purposes, like separate echolocation functions (Denzinger *et al*., 2001). Kingston *et al*. (2003) found this pattern for three of five 'alternating' studied species and inferred that this pattern is linked to a wide spectra in prey size: low frequency signals to detect large distant insects, and higher frequency signals to detect closer and smaller insects. According to our measurements of maximum frequency for both call types of *G. superba*, type B calls may detect prey around five mm long (corresponding to one third of wavelength; Pye, 1993), while type A calls may detect only larger preys. Our recordings close to mist-nets also confirm that this call alternation behaviour is switched off when the bat is close to an obstacle (Kingston *et al*., 2003).

Flight Behaviour Tracking

The real time 3D acoustic tracking system is very useful to analyze bat trajectories in the field, especially when measurements are realized in cluttered environment, where call patterns may change rapidly. In *G. superba*, we showed that the frequency parameters vary in function of call duration and inter-pulse interval, i.e., wing beat, and that the alternate frequency mode generally takes place when the wing beat is regular, whereas high frequencies are emitted during take-off and when the bats face obstacles. In addition, the minimal frequencies of both A and B call types of released bats were found to be systematically lower than those of bats recorded before capture. However, the releases were performed in open areas, whereas the captures were done in complex and cluttered environments (Supplementary Fig. S1). The differences in call frequency may therefore be only explained by environmental differences (Arlettaz *et al*., 2001).

After release in an open area, we detected only type A calls when the bats took off or when they were far from the microphones (Supplementary Fig. S4). During the take-off, the wing beat frequency is anomalously high, which mobilizes a lot of energy. To save energy, some released bats can decide to not emit type B calls. The stress following capture represents an alternative hypothesis. The absence of type B calls in bats flying far from the microphones may be an artefact due to the limited range of the 3D acoustic tracking system, because the type A calls of *G. superba* are generally at least 5 dB more powerful than type B calls. However, we can also propose that *G. superba* does not require a very accurate vision when it flies in open areas. This means that type B calls may be activated in *G. superba* only when it needs to improve its information update, i.e., when it forages in cluttered environments or when it actively searches for preys in open areas.

Our estimation of flight speeds in *G. superba* bats measured either before capture or after release showed evidence for relatively low values (less than 6 m/s). Before capture, the low speeds can be simply explained by the presence of mist-nets and natural obstacles, such as trees, undergrowth, water surface, etc. After release, the low speed values may be due to the limited detection range of the 3D acoustic tracking system (up to 10 m), which does not allow enough time for the animal to reach maximal speeds. The detection range was extended to 20 m in the second version of the tracking system, and the Institut Langevin is currently planning to develop a new long-range system able to reconstruct 3D acoustic trajectories for bats flying at more than 50 m from the source.

Supplementary Information

Contents: Supplementary Text. Protocol used to convert the acoustic recordings from the four microphones into 3D flight trajectories; Supplementary Fig. S1. Capture sites of *G. superba* located at Mbyie Island (A) and Yoko forest reserve (B). Schematic top view of the capture site at Mbyie Island (C); Supplementary Fig. S2. Left wing of a *G. superba* (K13-266) showing areas used to analyze wing shape; Supplementary Fig. S3. Histogram of flight speed values computed from successive 3D positions, after release in an open field next to the Yoko research center (a) and before capture at Mbyie island (b); Supplementary Fig. S4. Spectograms of calls emitted just after takeoff for individuals released in Yoko (A) and Mbyie Island (B). Supplementary Information is available exclusively on BioOne.

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