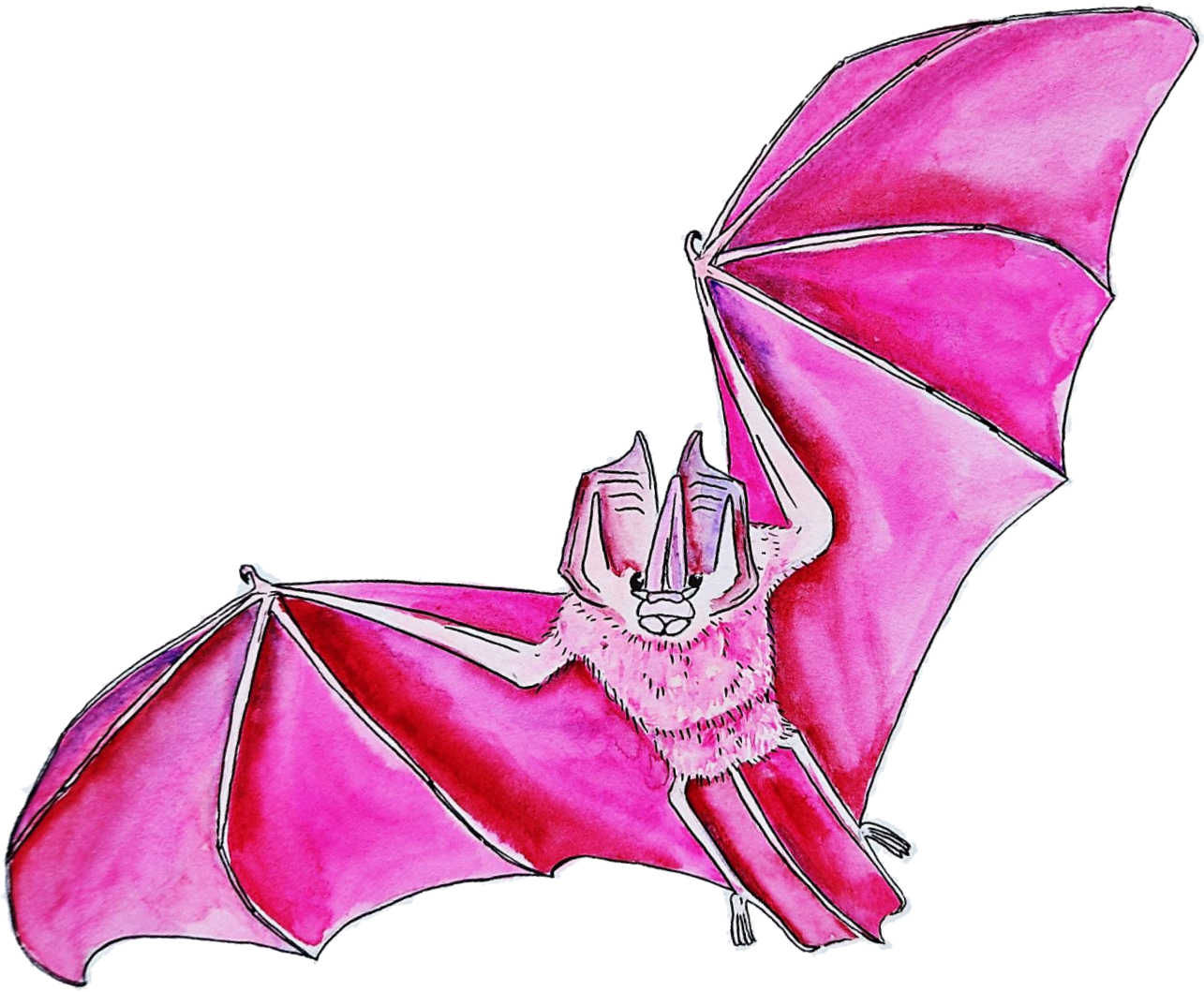
On the sensory ecology of phyllostomid bats



Dissertation

Gloria Gessinger

On the sensory ecology of phyllostomid bats

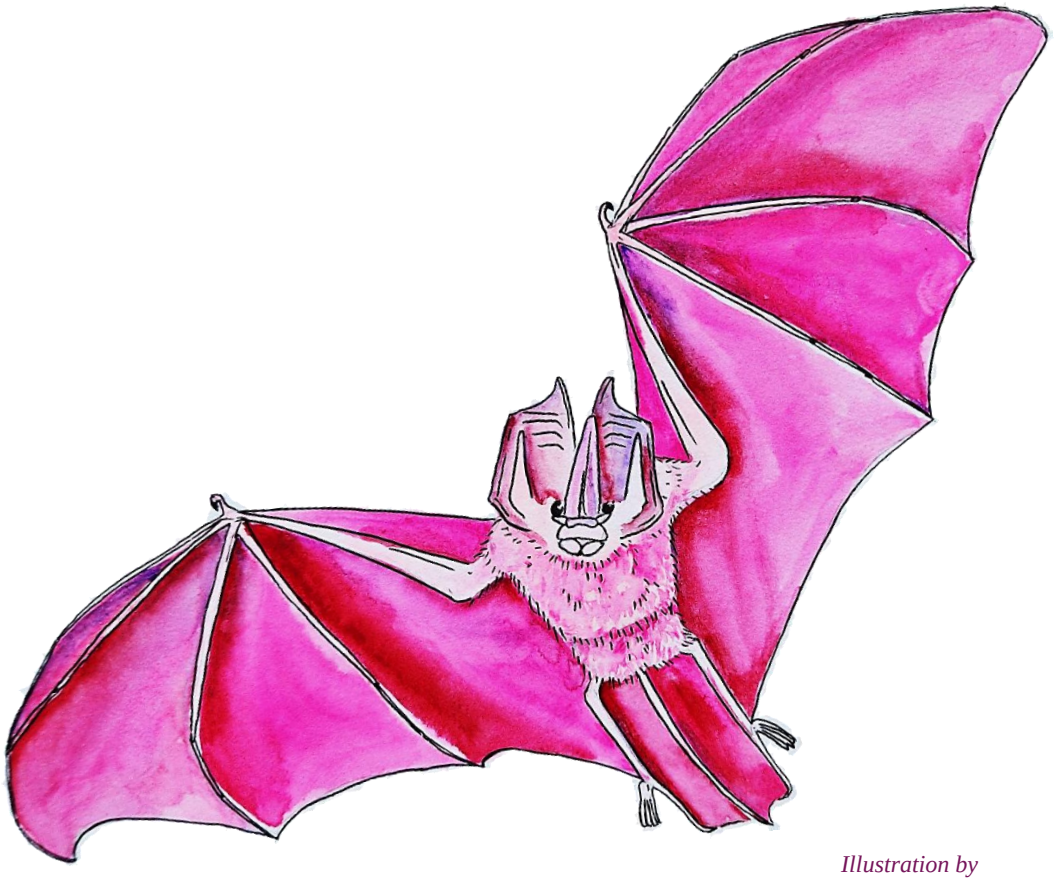


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Dissertation

zur Erlangung des Doktorgrades Dr.rer.nat. der Fakultät für Naturwissenschaften der Universität Ulm

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On the sensory ecology of phyllostomid bats

Summary

General introduction

Sensory ecology

Animals face a multitude of challenges when interacting with their biotic and abiotic environments. These include orientation, obstacle avoidance, acquisition of food, finding mates and roosts as well as predator and stress avoidance. Obtaining sufficient information about the environment allows for better decisions in dealing with these challenges. Mammals use vision, hearing, smell, taste and touch to assess their surroundings. Depending on the situation and the environment involved, some senses are more useful than others. Star-nosed moles forage in the darkness of the ground and rely on touch to identify prey (e.g., Catania, 2020). Monkeys orientate to odorous fruit trees after sniffing for their scent (e.g., Melin et al., 2019). As humans, we may detect both danger and food over long distances through visual perception. Bats successfully avoid obstacles in pitch-black darkness in a very specialized way using their sense of hearing (e.g., Sändig et al., 2014).

Bat hearing is highly sensitive over a high frequency range, ranging from low frequencies up to very high frequencies (Lattenkamp et al., 2021). Bats have evolved to use their ears for more than passively listening to environmental sounds.

Most species use their auditory system also for echolocation (Teeling et al., 2016). They emit high-pitched ultrasonic calls that bounce off of surfaces, prey and obstacles, and use these echoes to add yet another layer of spatial orientation to their sensory system, in addition to using smell, touch, taste and vision (Griffin, 1958). Therefore, the echolocation system enables bats to successfully orient and forage in environments with little to no light at all (e.g., Grinnell et al., 2016; Mac Arthur, 2000). The echolocation system allows access to information on distance to or relative speed of prey, obstacle size, surface structure, and even wing beat frequency (e.g., Geipel et al., 2013; Mora et al., 2013; Schnitzler and Denzinger, 2011).

The echolocation system varies among different bat families in terms of emitter design (nose and mouth), echolocation behavior, and receiver design (ears). With one exception, all laryngeally echolocating bat families feed almost exclusively on insects (Wilson and Mittermeier, 2019). Species from these families use echolocation to localize prey (e.g., *Pipistrellus pipistrellus*, *Nyctalus noctula*, Denzinger et al., 2016). During the final approach to prey, they often emit so-called feeding buzzes, a sequence of many short calls with an extremely high repetition rate. Feeding buzzes provide a fast update of the prey's position, which is essential for a capture attempt (Griffin, 1958; Griffin et al., 1960; Schnitzler and Kalko, 2001). Design of echolocation search calls is also influenced by phylogeny, but especially by the hunting habitat and prey type. Bats that forage for insects in open space, far away from

vegetation, use long echolocation calls with lower frequency, whereas bats that forage in dense forests use short echolocation calls that are generally higher (Denzinger and Schnitzler, 2013). Some insectivorous species use long constant frequency echolocation calls and are able to obtain information on the wing beat frequency of their prey, which could be used for prey classification (Schnitzler and Denzinger, 2011). Search calls within a bat family are rather similar but may vary, for example, in the dominant frequency, call design, and call duration (e.g., Jung et al., 2014, 2007; Yoh et al., 2020).

Echolocation calls are usually emitted through the nostrils (e.g., Rhinolophidae) or the mouth (e.g., Vespertilionidae, Metzner and Müller, 2016; Pedersen, 1993). In some families the nostrils are surrounded by fleshy structures, so called noseleaves, which vary in shape, size and structure (Figure 2), and may have an influence on the emission characteristics of a call, such as the width of the emitted sound, the so-called beam width (e.g., Brinkløv et al., 2011; Vanderelst et al., 2013; Webala et al., 2019; Wilson and Mittermeier, 2019, Figure 1*i*). Some bats that emit echolocation calls through the mouth have been reported to adjust their mouth gape during flight, which also affects the emission characteristics, especially the beam width (Arditi et al., 2015; Kounitsky et al., 2015). Generally, the beam width is larger for sound emitted through a small sound-emitting structure, such as a nostril, compared to sound emitted through a larger structure, such as the mouth (Kounitsky et al., 2015). However, interference phenomena could occur if the sound is emitted through more than one structure, e.g., two nostrils, and these phenomena could narrow the beam in the horizontal plane (Gessinger et al., 2021; Jakobsen et al., 2018).

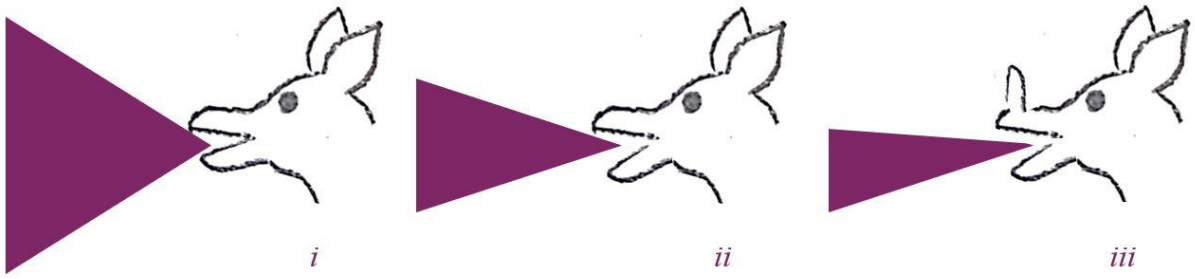


Figure 1: Schematic and simplified beam width of a bat emitting sound through i. a small mouth gape, ii. a wide mouth gape, iii. a wide mouth gape in the presence of a noseleaf.

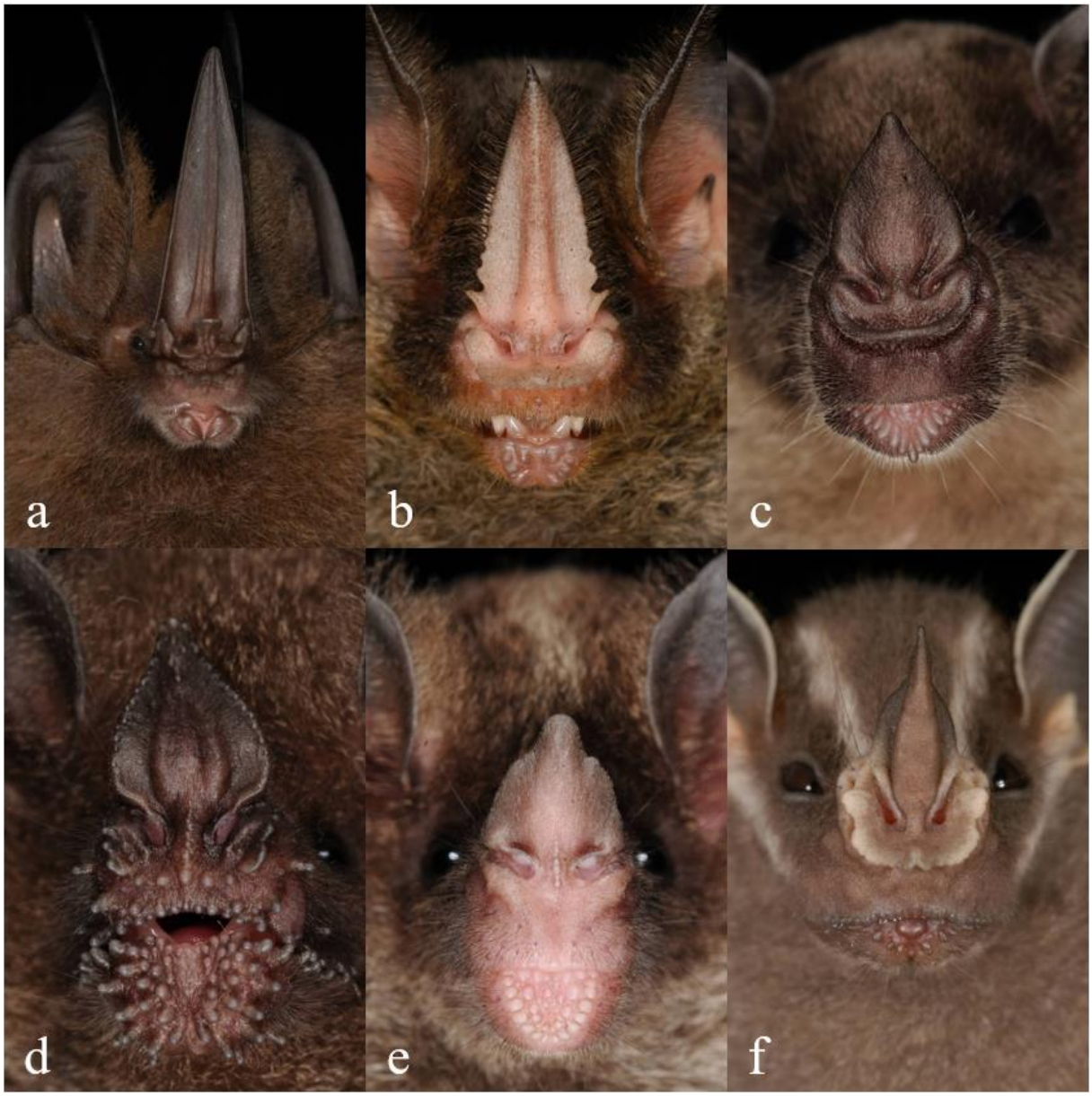


Figure 2: Examples of nose structures in phyllostomid bat species: a Lonchorhina aurita, b Gardnerycteris crenulatum, c Phyllostomus discolor, d Trachops cirrhosus, e Tonatia saurophila, f Uroderma bilobatum. Photographs by Marco Tschapka with permission.

The New World leaf-nosed bats (Phyllostomidae)

Phyllostomidae is the bat family with the most diverse food sources and correspondingly many different dietary preferences: frugivory, insectivory, sanguivory, nectarivory and carnivory are all found within this outstanding family (Giannini and Kalko, 2004). Most of them can be considered narrow space foragers, foraging in dense forests (Denzinger et al., 2018). In narrow space, echolocating bats are challenged by echoes being reflected not only from food items, but also from the close background (Denzinger and Schnitzler, 2013). Therefore, most phyllostomid bats use also alternative strategies for finding prey, such as olfaction, e.g., fig-eating bats, such as the common fruit bat *Artibeus jamaicensis*, (Hodgkison et al., 2013) or the Caribbean white tent-making bat *Ectophylla alba* (Ripperger et al., 2019), or passive listening for prey-generated cues (= mating sounds, rustling sounds, and others), e.g., the frog-eating bat *Trachops cirrhosus* (Denzinger and Schnitzler, 2013; Page and Ryan, 2008; Tuttle and Ryan, 1981). Most animalivorous phyllostomid bats belong to the narrow space gleaning foragers that collect insects from branches, leaves or from the ground using prey-generated cues to detect prey (Denzinger and Schnitzler, 2013). They often have very large ears with a high hearing sensitivity for low frequencies (Geipel et al., 2021). Echolocation is mainly used for obstacle avoidance (Page and Ryan, 2008).

Bats with obvious structures around the nostrils, such as noseleaves, are generally considered to be so-called nasal emitters, who emit their echolocation calls through the nostrils (Brokaw and Smotherman, 2020; Hartley and Suthers, 1989; Matsuta et al., 2013). This is also the case for the phyllostomid bat family. Almost all species in this family have prominent noseleaves (Arita, 1990; Bogdanowicz et al., 1997; Leiser-Miller and Santana, 2020). However, photographs of flying phyllostomids often show individuals in flight with a distinctly opened mouth (e.g., *Micronycteris microtis in* López-Baucells et al., 2016). Yet, emission behavior had not been systematically investigated. A systematic phylogenetic overview of the emission behavior, such as mouth posture while echolocating, could provide insight into the evolutionary aspects of different emission types.

The common sword-nosed bat *Lonchorhina aurita*

Phyllostomid echolocation calls are mostly multiharmonic, frequency-modulated and very short (<2ms) (Gessinger, 2016; Kalko, 2004; Yoh et al., 2020, Figure 4). However, bats of the genus *Lonchorhina* have a very unique echolocation call design, that differs in several aspects from the standard phyllostomid calls (Yoh et al., 2020). The genus also shows the largest noseleaves among phyllostomids (Wilson and Mittermeier, 2019, Figure 3).

The echolocation search calls of the common sword-nosed bat *Lonchorhina aurita* are characterized by a constant frequency onset followed by a frequency modulated downsweep. With a duration of approximately 6 ms duration, they are about three times as long as standard phyllostomid echolocation calls (Gessinger et al., 2019, Figure 4). The frequency modulated component is often omitted in search phase. Earlier work from our workgroup suggests that *L. aurita*, unlike most other phyllostomid bats

that are mainly gleaners, hunts flying moths in the forest using an aerial hunting mode (Gessinger et al., 2019). We observed also a buzz-like call group, that may be used by the bats during the final approach to the prey, with very short calls and very short intervals (Gessinger et al., 2019). So-called feeding buzzes are known from many insectivorous bat families (e.g., Molossidae, Vespertilionidae, Emballunoridae), but are very atypical for animalivorous phyllostomid bats. While there are plenty of insect-eating species within the Phyllostomidae, only one species has been observed to use a feeding-buzz-like terminal group. The long-legged bat *Macrophyllum macrophyllum* hunts insects above water bodies and is considered an edge space trawling forager (Denzinger and Schnitzler, 2013; Weinbeer and Kalko, 2007). Trawling insects from smooth water surfaces is a comparably easy echolocation task, because the water acts like a mirror, reflecting most of the bat-produced sounds away from the bat, thereby reducing background echoes, while amplifying target echoes (Greif and Siemers, 2010; Siemers et al., 2005). Additionally, *M. macrophyllum* hunts aerial prey using feeding buzzes (Weinbeer et al., 2013). Perhaps the echolocation call sequences of *L. aurita* observed in the forest could also be an adaptation to hunting aerial prey. Together with the forest as their preferred hunting habitat this could classify *L. aurita* as a so-called edge space aerial forager. Edge space aerial insectivores hunt flying prey close to vegetation and show several behavioral adaptations (Denzinger and Schnitzler, 2013).

Fecal analysis has shown that *Lonchorhina aurita* from a population in Panama predominantly consume moths (Kalbantner, 2018). It is therefore obvious that *L. aurita* can somehow discriminate moths from other insects. However, the prey cues they used for discrimination are so far unknown. Furthermore, a flying insect is a rather difficult target to detect by echolocation in the forest, as it can easily be masked by the echoes from the background (Denzinger and Schnitzler, 2013). Nevertheless, the conspicuous echolocation call design of *L. aurita*, with a long constant frequency component, suggests that they use echolocation for prey detection (Gessinger et al., 2019, Figure 4). Additionally, the low frequency sound of a flying moth, the preferred prey of *L. aurita*, is a rather difficult target to detect by passive listening, simply because many noise sources in a nocturnal forest are in similar frequency ranges and are much louder (Anderson and Racey, 1993; Riede, 1993). Still, the large ears of *L. aurita* are similar to those of other phyllostomid gleaning bats (Wilson and Mittermeier, 2019), which often utilize prey-generated sounds to find prey by passive listening (Falk et al., 2015; Mantilla-Meluk and Montenegro, 2016; Wilson and Mittermeier, 2019). Knowing the cues used for prey detection and prey classification by the potentially only edge-space aerial insectivore phyllostomid *L. aurita* will be another step in studying the evolution of this unique hunting strategy, as well as the evolution of the associated unusual echolocation behavior and morphological structures for sound emission and reception.



Figure 3: The common sword-nosed bat Lonchorhina aurita. Illustration by Gloria Gessinger. Photograph by Marco Tschapka with permission.

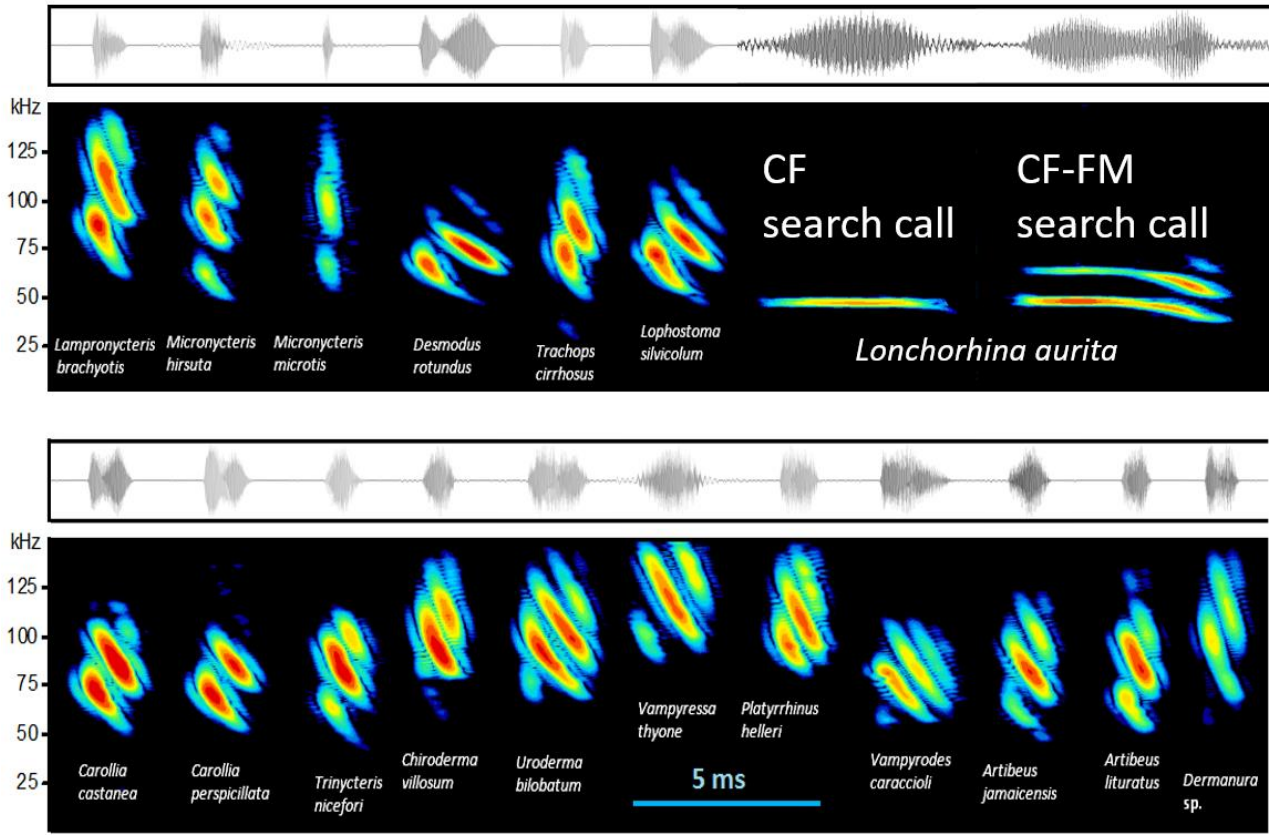


Figure 4: Examples of echolocation calls from phyllostomid bats. Based on Gessinger (2016) with permission.

Aim of the study

The aim of my thesis was to advance the knowledge on sensory ecology of phyllostomid bats with a main focus on their echolocation and hunting behavior.

Chapter 1: The often conspicuous noseleaves of phyllostomid bats led to the assumption that they emit their echolocation calls exclusively through their nostrils, such as in families with similar structures, e.g., the Rhinolophidae or the Hipposideridae. However, this has never been systematically tested for. I wanted to investigate this hypothesis further by investigating whether published photographs of phyllostomid bats flying with their mouths open are just random snapshots of single individuals and do not reflect the general call emission behavior of that certain species. I predicted that phyllostomid bats with prominent noseleaves should usually fly with their mouths closed.

Chapter 2: After observing the unique echolocation call design in *Lonchorhina aurita*, I wanted to determine the associated foraging niche. I assumed that the search call design is related to preying on moths in an aerial hunting mode, and hypothesized further adaptations in echolocation behavior such as the use of a feeding buzz. Using this information, I aimed to identify the foraging niche of *L. aurita* in comparison to other bat families.

Chapter 3: The diet of *Lonchorhina aurita* is specialized on moths. This specialization relies on a successful target detection (*is there anything?*) and classification (*is it food?*). Focusing on the bats sense of hearing, flying moths could be detected either through passive listening to the moth's wing beat or through echolocation. The sound of their wing beats is rather silent compared to the noisy forest hunting habitat of *L. aurita.* The wing beat cue is not necessary for detection through echolocation, since any object in the air will produce an 'acoustic image' (echo) when ensonified with echolocation calls. However, adaptations in the echolocation call design could increase the detection success for fluttering insects. Additionally, the wing beat could be used for prey classification. Therefore, I wanted to determine which prey cues *L. aurita* uses to successfully detect and classify prey.

Study area and general methods

The bat species studied in the first chapter were captured in tropical rainforests in Panama and Peru between 2015 and 2019. The focus species of the second and third chapter, *Lonchorhina aurita*, was captured near known flight paths and a roost in Soberanía National Park, Panama. Standardized releases and hunting experiments were conducted either in a large tent or in a large outdoor flight cage. After the flight cage experiments, the bats were marked and released.

Summary of chapters

Chapter 1: Phylogenetic patterns in mouth posture and echolocation emission behavior of phyllostomid bats.

The presence of noseleaves and similar structures around the nostrils of bats is considered to be a sign that echolocation calls are emitted through the nostrils (Brokaw and Smotherman, 2020; Hartley and Suthers, 1987; Jakobsen et al., 2018; Matsuta et al., 2013). The emission structure effects several call characteristics, such as the width of the emitted sound, the so-called beam width (Figure 1). A broader beam is not favorable within the forest, as it can result in more echoes from the background. While the beam width of a call is broader when emitted through a smaller structure (e.g., nostril < mouth, Kounitsky et al., 2015), noseleaves could be involved in narrowing of the beam width in the vertical plane (Hartley and Suthers, 1987). This could be used by nasally emitting phyllostomid bats to narrow their beam width. However, in spite of the prominent noseleaves present in most phyllostomid bats, there are many photographs of individuals from different species flying with their mouths open, indicating an oral emission (e.g., *in* López-Baucells et al., 2016, Figure 5). We assumed that this behavior is random and that phyllostomids with prominent noseleaves are consistent nasal emitters, flying generally with their mouths closed. We further hypothesized, that they point their nostrils in the direction of flight. For studying this behavior, we observed 40 different species in a standardized release flight situation in a flight cage. The bats were filmed from the side with a high-speed camera while their echolocation was recorded with ultrasound microphones. Surprisingly, an open mouth during flight was not, as predicted, a random individual behavior, but largely species-specific. About half of the species consistently flew with their mouths open (47.5 % open mouth emitters) and others maintained always a closed mouth (40.0 % nasal emitters). An ancestral state analysis showed a distinct phylogenetic signal for the emission behavior (open mouth emission vs. nasal emission), with the open mouth mode being the ancestral state. In many families, one emission mode was dominant. Exclusive nasal emission evolved at least twice, within the frugivorous subfamily Stenodermatinae and the omnivorous genus *Phyllostomus*, here, all species have prominent noseleaves. While species with closed mouths did indeed point their nostrils in flight direction and emit their echolocation calls nasally, open mouth emitters pointed both nostrils and mouth in flight direction. The emission mode for open mouth emitters is less clear. There are four different possibilities of sound emission in open moth emitters: *i*. nasally, through the nostrils, *ii*. through the mouth, *iii*. alternately through mouth and nostrils, *iv*. synchronously through mouth and nostrils. While we cannot conclusively resolve how open mouth emitters emit their echolocation calls our findings lead to a paradigmatic shift in how we think about the echolocation call emission in phyllostomid bats.

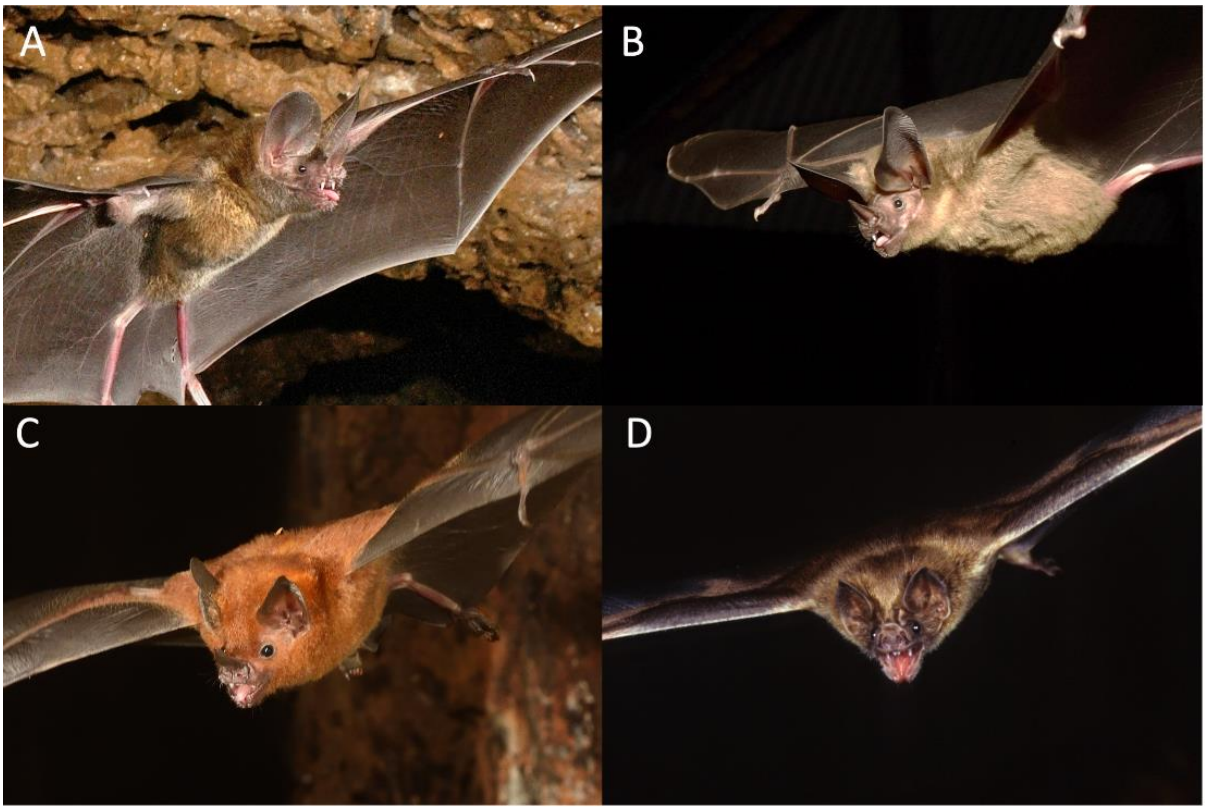


Figure 5: Phyllostomid bats photographed in flight with open mouths. Photos by Marco Tschapka. Published in Gessinger et al., 2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/.

Chapter 2: Aerial insectivory in a phyllostomid bat: *Lonchorhina aurita* uses unique multiple pulse echolocation to catch airborne prey.

One of the most unique echolocation call designs in phyllostomid bats is found in the genus *Lonchorhina* (Gessinger et al., 2019). Additionally, dietary analysis has revealed a diet specialized on moths (Kalbantner, 2018). Further, *Lonchorhina aurita* has been reported to forage for prey in the forest (Gessinger et al., 2019). Therefore, we investigated the hunting behavior of *L. aurita* to test whether the species can be considered an edge space aerial insectivore. Edge space aerial insectivores hunt flying prey close to vegetation. They use echolocation calls emitted at temporal intervals of 90-100 ms, which often consist of shallowly modulated quasi constant frequency components and a steeply modulated component (Denzinger and Schnitzler, 2013; Schnitzler et al., 2003). Just like open space bats, edge space aerial insectivores show adaptations to aerial prey in their echolocation behavior, so called feeding buzzes (Griffin, 1958; Griffin et al., 1960; Schnitzler and Kalko, 2001). We recorded *L. aurita* hunting live moths in an 25m^2 flight cage using infrared cameras and ultrasonic microphones. The size of the flight cage was similar to small gaps in the forest, the presumed hunting habitat of *L. aurita*. We were able to observe behavioral similarities to typical edge space aerial insectivores (e.g., capture behavior of some Vespertilionidae, Kalko and Schnitzler, 1989; Melcón et al., 2007; Surlykke and Moss, 2000), and echolocation call design of Noctilionidae, Kalko et al., 1998). In the confined space of the flight cage bats readily caught moths in mid-air using a feeding buzz and showed great maneuverability in flight (Figure 6). With search calls emitted at 47 kHz they are within the expected frequency range of edge space foragers (Denzinger and Schnitzler, 2013; Jung et al., 2007; Mora et al., 2013). Our analysis also revealed a typical decrease in both pulse interval (temporal distance between echolocation calls) and pulse duration during the approach to aerial prey through the use of both multi pulses and feeding buzzes. The typical capture behavior, the feeding buzzes, as well as the echolocation call frequency and design exhibit many similarities to edge space aerial species from different families, such as the greater sac-winged bat *Saccopteryx bilineata* or Thomas's shaggy bat *Centronycteris centralis* (both Emballonuridae, Denzinger and Schnitzler, 2013; Jung et al., 2007), that we observed in the same hunting habitat as *L. aurita*. Therefore, we conclude, that *L. aurita* is the first phyllostomid bat reported to occupy the niche of an edge space aerial insectivore.

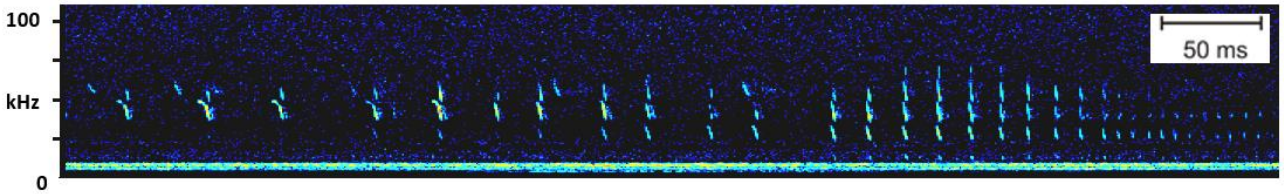


Figure 6: Example of a short feeding buzz emitted by Lonchorhina aurita.

Additionally, we found unique groups of echolocation calls, so called multi pulses, during approach sequences that have never been shown in any other bat species (Figure 7). These call groups contained an initial CF-FM search call followed by one to many FM calls. They were only observed in the context of prey and were never used by the bats during search flight or in landing maneuvers. They often resembled short, simple feeding buzzes but it remains unclear, whether their role is related to prey localization, as in feeding buzzes, or prey classification.

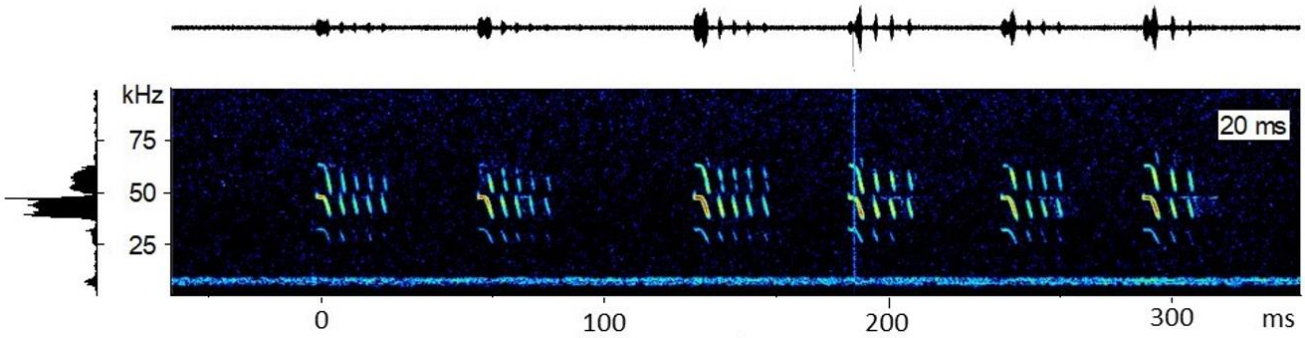


Figure 7: Multi pulse echolocation sequence of Lonchorhina aurita. Chapter 2.

Chapter 3: Prey movement and wing beat as cues during prey captures of the phyllostomid bat *Lonchorhina aurita*.

In experiments we were able to confirm that *Lonchorhina aurita* captures free-flying prey (Chapter 2), and consequently we were interested in the prey cues they use during hunting. Free-flying moths constantly change their position in the air and flap their wings. Therefore, the prey cues we investigated were prey movement and wing beat. We offered different prey items to bats in captivity and analyzed their behavior. While each bat was flying freely in a large flight cage, the prey was tied to the ceiling and offered in the center of the flight cage. In a multi-choice experiment, we offered the bat three types of prey simultaneously: a stationary live moth, that was flapping its wings, a stationary control, that was a freshly killed dead moth with its wings outstretched, and a manually moved freshly killed moth. While the bats were able to detect all prey items and consequently approached them, they did not attack the different items with equal frequency. The prey type that was clearly attacked the most was the wing beat target (Figure 8), followed by the manually moved moth. The control was not attacked during the choice experiment. We conclude, that movement alone is sufficient for prey identification and triggers the approach of the bat. However, the perception of the wing beat is by far the most important cue for the bat, even in the absence of movement.



Figure 8: Snapshots of Lonchorhina aurita hunting a tethered moth, that was flapping its wings. Snapshots by Gloria Gessinger. Chapter 3.

General conclusion

The New World leaf-nosed bats (Phyllostomidae) are one of the ecologically most diverse bat families. The diets of many species have been well studied and show a broad resource use, ranging from fruit, nectar, insects and other animals to blood (Fenton, 1992; Giannini and Kalko, 2004). Despite this, uniformity within the family has long been assumed for many other traits of their sensory ecology such as:

- search call design (short, frequency-modulated, multi-harmonic calls: Kalko, 2004; Yoh et al., 2020),
- call emission (nasal: Brokaw and Smotherman, 2020; Hartley and Suthers, 1987; Jakobsen et al., 2018; Matsuta et al., 2013) and
- foraging guilds (mostly narrow space gleaning foragers; Denzinger et al., 2016; Denzinger and Schnitzler, 2013).

However, the use of different resources also requires different adaptations in the sensory system of phyllostomid bats. Vampire bats may use special thermoreceptors embedded in their face to localize warm blooded animals and select promising biting sites on the animal's body (e.g., Kürten and Schmidt, 1982). Fruit eating bats are able to discriminate ripe fruits from unripe fruits using the fruit odour (e.g., Hodgkison et al., 2013; Korine and Kalko, 2005; Thies et al., 1998). Animalivorous gleaning bats often use prey-generated sounds, such as rustling and mating sounds for prey detection and localization (= passive listening, e.g., Jones et al., 2016; Page and Ryan, 2008).

We were previously able to show that the assumption of a total uniformity in search call design within the family has to be rejected, as the genus *Lonchorhina* uses very different echolocation calls compared to other phyllostomid bats. Only one (the third) harmonic is present during search flight (Gessinger et al., 2019). Similarly, we were able to show that *Centurio senex* uses single harmonic echolocation calls (first harmonic, Rodríguez-Herrera et al., 2020). Free flying *Desmodus rotundus* use a quasi-constant call onset (Rodríguez-San Pedro and Allendes, 2017) comparable to the constant frequency component of *Lonchorhina aurita*.

Furthermore, contrary to our prediction, we were able to show that the general assumption of nasal emission as the standard emission behavior in phyllostomid bats needs to be re-evaluated. While nasal emission is the case in many species, about half of the species tested, used an open mouth emission mode, which is the ancestral state (Chapter 1). We observed a distinct phylogenetic pattern of the emission types and almost all phyllostomid subfamilies had one dominant emission type. Together with the presence of the noseleaves, a synchronous sound emission through nostrils and mouth could aid in narrowing the beam width (Hartley and Suthers, 1987; Jakobsen et al., 2018; Kounitsky et al., 2015). A narrow beam width could be especially useful for animalivorous gleaning bats, who face a dilemma between calling and listening to prey cues (Jones et al., 2016), and may reduce irrelevant background

echoes. Together with observed head angles, mouth and nostrils pointing in the directing of flight, we suggest a synchronous emission of echolocation calls through mouth and nostrils in open mouth emitters. Further research is needed to evaluate the drivers of the evolution from open mouth emission to nasal emission. Nasal emission could have evolved as a tradeoff between carrying large fruits and using a narrow beam width. Therefore, a comparison between the beam width of open mouth emitters and nasal emitters could be very interesting and could perhaps shed light on the function of open mouth emission and nasal emission for different sensory tasks.

By analyzing the hunting behavior of *Lonchorhina aurita* we were able to confirm, that the species captures aerial prey. Like other aerial insectivores *L. aurita* used its tail membrane to scoop moths – it's main diet – out of the air and ate them during flight (e.g., Kalko and Schnitzler, 1989; Melcón et al., 2007; Surlykke and Moss, 2000). *L. aurita* catches flying moths using a feeding buzz (Chapter 2). Additionally, it uses call groups that, to our knowledge, have never been described for any bat species before, the so-called multi pulses. These call groups contained a normal *Lonchorhina* search call (long, with a constant frequency onset) followed by several short frequency modulated calls emitted at very low pulse intervals and a high repetition rate. Yes, there are some animalivorous phyllostomid bats that emit echolocation calls during prey approach. For example, the frog-eating bat *Trachops cirrhosus* gleans frogs from the ground or from leaves. But, while it emits echolocation calls throughout the attack, these are not grouped in the way feeding buzzes or our newly described multi pulses are grouped and the interval between the calls is much longer (Barclay et al., 1981; Simmons et al., 1979). Like the echolocation calls of *Trachops cirrhosus* throughout the attack, the described multi pulses of *Lonchorhina aurita* are clearly associated with prey and we suppose that they are used to pre-assess the current position and flight direction of a moth. The groups could also potentially be used to roughly classify prey, increasing the probability of perceiving information from the wing beat of the moth (Denzinger et al., 2018). This hunting behavior is very different from the majority of phyllostomid species who use a narrow space gleaning foraging strategy (Denzinger et al., 2016; Denzinger and Schnitzler, 2013) and raises the question why other edge space aerial insectivores have not been described to use such multi pulses when foraging. Further investigation of the function of multi pulse echolocation is therefore needed.

The feeding buzzes of *Lonchorhina aurita* are similar to feeding buzzes of other insectivorous families hunting aerial prey, especially regarding the low pulse interval (Griffin et al., 1960; Jakobsen et al., 2015; Melcón et al., 2007; Schnitzler and Kalko, 2001). Only two other phyllostomid species have been shown to use a similar echolocation behavior. First, the nectarivorous lesser long-nosed bat *Leptonycteris yerbabuenae* uses a feeding buzz-like sequence of echolocation calls when approaching cactus flowers and should therefore be considered a narrow space passive/active gleaning bat (Gonzalez-Terrazas et al., 2016). Second, the long-legged bat *Macrophyllum macrophyllum* is an edge space trawling forager and uses a feeding buzz when hunting insects over water bodies (Weinbeer and Kalko, 2007). When the trawling bat *Macrophyllum macrophyllum* hunts over water, the mirror effect reduces

the amount of background echoes (Greif and Siemers, 2010; Siemers et al., 2005). *L. aurita* search for prey in their forest habitat, where echoes from the forest vegetation can overlap and mask prey echoes, reducing the probability of prey detection (Denzinger and Schnitzler, 2013). As a result of our study, *L. aurita* is the first phyllostomid species to be added to the guild of the edge space aerial foragers (Chapter 2).

Lonchorhina aurita seems to be more sensitive to moving than to stationary targets, just like other aerial hawking bats (Anderson and Racey, 1993; Jensen et al., 2001) and is able to extract prey 3D-information from successive echoes (Jensen et al., 2001). In our experiment, this was sufficient for finding and localizing the manually moved moth target, and in the forest it could help to differentiate between moving prey objects and motionless obstacles, such as small leaves caught in spider webs (Chapter 3). While the manually moved moth was only occasionally attacked, the critical prey cue for *L. aurita* seems to be the wing beat of the moth (Chapter 3). Wing movements of a moth produce low frequency sounds and are potentially perceptible by passive listening (Anderson and Racey, 1993; Coles et al., 1989). The hearing of some gleaning bat species is well adapted to these low frequency wing sounds (Anderson and Racey, 1993; Coles et al., 1989; Geipel et al., 2021). However, in a loud and noisy environment such as a nocturnal tropical rainforest we consider the rather silent wing sounds an unlikely prey cue (Chapter 3). We suggest, that the wing beat cue is mainly perceived through echolocation. It has been suggested for other bats using similar echolocation calls that so-called ‘acoustic glints’ (small amplitude and frequency modulations in successive calls) could be perceived (Emde and Schnitzler, 1986). Overall, these results expand the diversity of phyllostomid bats in terms of foraging guilds.

In my dissertation I was able to show, that the sensory ecology of New World leaf-nosed bats is much more diverse than previously thought. Besides nasal emission of echolocation calls, I was able to show an open mouth emission mode, which seems to be the ancestral echolocation behavior of this family. Based on echolocation and behavioral observations I was able to assemble convincing arguments for assigning the common sword-nosed bat (*Lonchorhina aurita*) as the first phyllostomid bat to the guild of edge space aerial insectivores. I found that prey wing beat is the most important cue triggering attack behavior in hunting *L. aurita*, and that they use not only unique echolocation calls but also unique multi pulse echolocation during hunting. All in all, my dissertation work demonstrates additional levels of diversity in the ecological niche of New World leaf-nosed bats in terms of echolocation call emission, foraging niche, and hunting behavior.

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Chapter 1



Illustration by Gloria Gessinger

Chapter 1: Phylogenetic patterns in mouth posture and echolocation emission behavior of phyllostomid bats.

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Abstract

While phyllostomid bats show an impressive range of feeding habits, most of them emit highly similar echolocation calls. Due to the presence of an often prominent noseleaf, it has long been assumed that all phyllostomids emit echolocation calls exclusively through the nostrils rather than through the mouth. However, photo evidence documents also phyllostomid bats flying with an opened mouth. We hypothesized that all phyllostomid species emit echolocation calls only through the nostrils and therefore fly consistently with a closed mouth, and that observations of an open mouth should be a rare and random behavior among individuals and species. Using a high-speed camera and standardized conditions in a flight cage, we screened 40 phyllostomid species. Behavior varied distinctly among the species and mouth posture shows a significant phylogenetic signal. Bats of the frugivorous subfamilies Rhinophyllinae and Carolliinae, the nectarivorous subfamilies Glossophaginae and Lonchophyllinae, and the sanguivorous subfamily Desmodontinae all flew consistently with open mouths. So did the animalivorous subfamilies Glyphonycterinae, Micronycterinae and Phyllostominae, with the notable exception of species in the omnivorous genus *Phyllostomus*, which consistently flew with mouths closed. Bats from the frugivorous subfamily Stenodermatinae also flew exclusively with closed mouths with the single exception of the genus *Sturnira*, which is the sister clade to all other stenodermatine species. Further, head position angles differed significantly between bats echolocating with their mouth closed and those echolocating with their mouths opened, with closed-mouth phyllostomids pointing only the nostrils in the direction of flight and open-mouth phyllostomids pointing both the nostrils and mouth gape in the direction of flight. Ancestral trait reconstruction showed that the open mouth mode is the ancestral state within the Phyllostomidae. Based on the observed behavioral differences, we suggest that phyllostomid bats are not all nasal emitters as previously thought and discuss possible reasons. Further experiments, such as selectively obstructing sound emission through nostrils or mouth, respectively, will be necessary to clarify the actual source, plasticity and ecological relevance of sound emission of phyllostomid bats flying with their mouths open.

Introduction

The majority of bats depend on echolocation for both orientation and foraging. Echolocation is a highly complex behavior and can be adjusted to the specific ecological needs of a species in many ways (Denzinger et al., 2018). Several different echolocation call designs have evolved that vary widely in temporal and spectral parameters, call intensity, and beam width (e.g., Brinkløv et al., 2009; Heller and Helversen, 1989; Jung et al., 2014; Surlykke et al., 2013).

Echolocation calls can be emitted orally or nasally (Metzner and Müller, 2016). Families considered to be oral emitters are, e.g., Emballonuridae, Mormoopidae, Noctilionidae, Molossidae, and most Vespertilionidae (Pedersen, 1993). Individuals from these groups emit calls from the mouth, keeping the mouth widely open during flight, as seen in photographs of flying individuals, e.g., *Noctilio leporinus* (Noctilionidae), *Eumops glaucinus* (Molossidae, Taylor and Tuttle, 2019), and *Hypsugo bodenheimeri* (Vespertilionidae, Kounitsky et al., 2015). On the other hand, bats in several other families are known to echolocate through the nostrils, e.g., Rhinolophidae and Hipposideridae (Metzner and Müller, 2016). These species usually keep their mouths closed during flight (e.g., *Rhinolophus hildebrandtii*; *Hipposideros ruber*, Taylor and Tuttle, 2019). In addition, they often have prominent structures surrounding their nostrils, including so-called noseleaves (e.g., Vanderelst et al., 2013; Webala et al., 2019; Wilson and Mittermeier, 2019). Both Hipposideridae and Rhinolophidae – two families with often very elaborate noseleaves – are exclusively insectivorous and hunt in narrow space habitats (Bell and Fenton, 1984; Bontadina et al., 2002; Denzinger and Schnitzler, 2013; Neuweiler et al., 1987). To be able to differentiate prey from the background they use glint detection and narrow space flutter detection, respectively (reviewed by Schnitzler and Denzinger, 2011). A third family with very prominent noseleaves are the Phyllostomidae (Arita, 1990; Bogdanowicz et al., 1997; Leiser-Miller and Santana, 2020). This exclusively Neotropical family includes currently 217 species (Wilson and Mittermeier, 2019) and is highly diverse in foraging behavior, diet and habitat use (Ferrarezzi and Gimenez, 1996; Giannini and Kalko, 2004; Kalko et al., 1996; Surlykke et al., 2014).

Similar to Hipposideridae and Rhinolophidae, many phyllostomid bats are narrow space foragers that hunt in dense forest (Denzinger et al., 2018). In contrast to the mainly insectivorous hipposiderids and rhinolophids, phyllostomid bats feed on a huge variety of resources with diet specificity roughly reflected by the systematic classification into subfamilies. Animalivorous species feed mostly on insects, but some include small vertebrates (e.g., Micronycterinae, Glyphonycterinae, and most Phyllostominae, Giannini and Kalko, 2004). Other phyllostomids are phytophagous, predominantly consuming fruits or pollen and nectar (e.g., Stenodermatinae, Carolliinae, Glossophaginae, Lonchophyllinae, Giannini and Kalko, 2004). Furthermore, there are three phyllostomid species that feed exclusively on the blood of vertebrates (Desmodontinae, Fenton, 1992).

Generally, echolocation is shaped by the respective ecological niche (Jones and Holderied, 2007; Schnitzler and Kalko, 2001), so we expect differences between species with differing foraging

behaviors. However, on first glance most phyllostomid bat species use very similar echolocation calls, which are usually short (< 2 ms), multiharmonic, frequency-modulated (FM), and cover a broad bandwidth (Yoh et al., 2020; Gessinger, 2016; Weinbeer and Kalko, 2007; Korine and Kalko, 2005; Kalko, 2004). The free-standing noseleaves found in almost all phyllostomid bats are thought to play an essential role in the emission of these echolocation calls (Arita, 1990; Hartley and Suthers, 1987; Vanderelst et al., 2010). Reduced noseleaves are only found in the two subfamilies Desmodontinae and Brachyphyllinae (Wilson and Mittermeier, 2019). Because of the ubiquitous noseleaves, phyllostomid bats are generally considered to be nasal emitters (Brokaw and Smotherman, 2020; Hartley and Suthers, 1987; Jakobsen et al., 2018; Matsuta et al., 2013). Additionally, anatomical features of the skull indicate that phyllostomid bat echolocation is optimized for emission through the nostrils. Phyllostomids have straight air flow from the larynx to the nostrils, whereas sound emitted through the mouth has to travel a longer and less direct path (Pedersen, 1998, 1993).

Phyllostomid bats in flight can be seen in many photographs with their mouths closed, as would be expected for nasal emitters, e.g., *Mesophylla macconnelli*, *Phyllostomus discolor* (López-Baucells et al., 2016). However, a closer inspection of own and published photographs revealed also bats flying with a partially opened mouth, e.g., *Trachops cirrhosus, Lophostoma silvicolum, Carollia perspicillata,* and *Desmodus rotundus* (Figure 1), *Carollia castanea* (Taylor and Tuttle, 2019), *Hsunycteris* (*Lonchophylla*) *thomasi*, *Artibeus gnomus*, *Phylloderma stenops*, *Lophostoma carrikeri*, *Micronycteris microtis* (López-Baucells et al., 2016). This behavior has also been commented on by Fenton (2013).

We therefore asked whether these open-mouth observations reflect only random and occasional behavior of individuals, or whether specific behavioral patterns exist among the phyllostomid species that can be related to the emission mode of echolocation calls.

Specifically, we focused on the following hypotheses, based on the general view that the noseleaf is an adaptation for nasal echolocation:

1. Phyllostomid bat species with free-standing noseleaves are consistent nasal emitters and fly with the mouth closed.
2. The head position enables the emitter (nostrils) to point in the main direction of flight.

To investigate these hypotheses, we recorded high-speed video footage of the behavior of 40 phyllostomid species in a flight cage under standardized conditions and quantitatively assessed the head position and degree of mouth opening (measured as gape angle).

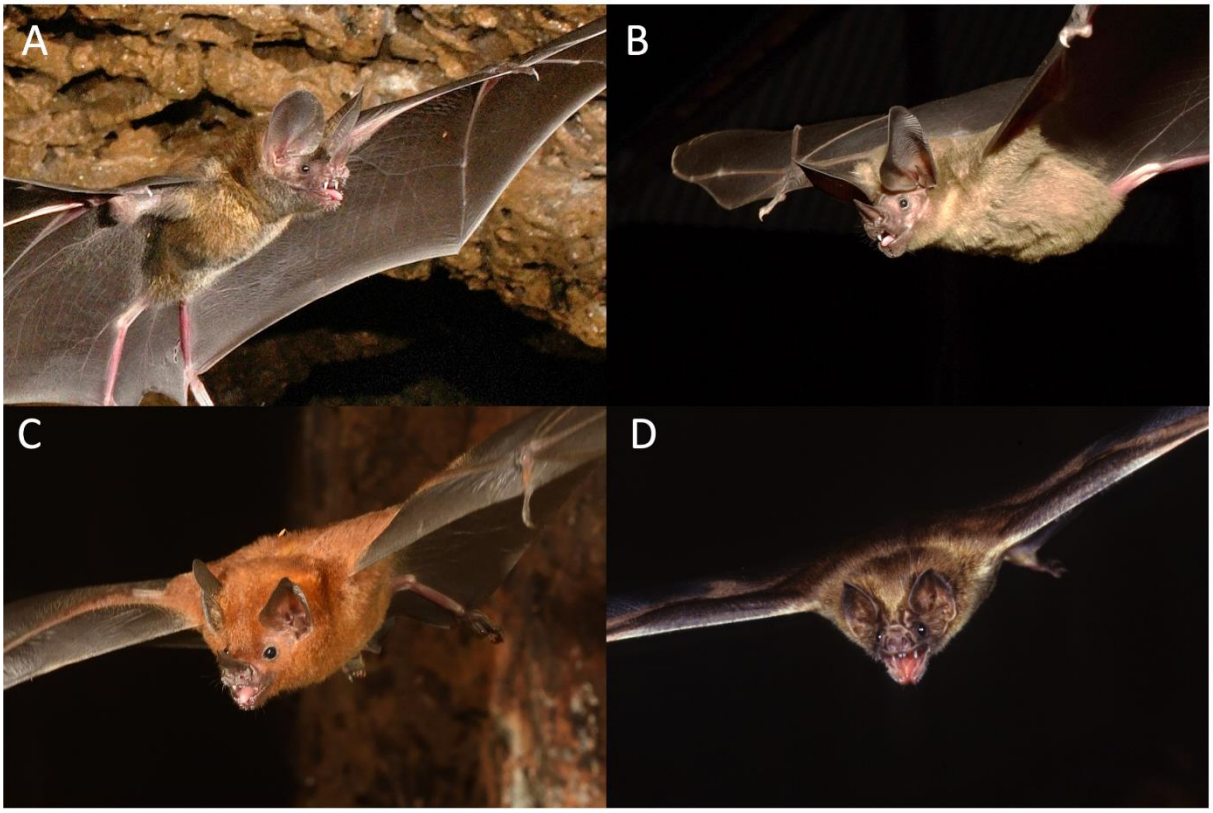


Figure 1: Phyllostomid bats photographed in flight with open mouths. (A). Trachops cirrhosus, (B). Lophostoma silvicolum, (C). Carollia perspicillata, (D). Desmodus rotundus. (Photos by MT). Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/

Material and methods

Study sites

Bats were captured at five different Neotropical field sites over a five-year period (2015–2019) in Panamá and Perú: 1. Field station Barro Colorado Island (BCI) of the Smithsonian Tropical Research Institute (STRI) and the surrounding Barro Colorado Nature Monument in Panamá (9° 9′ 0″ N, 79° 51′ 0″ W) between March and July 2015. 2. Field station Gamboa of the Smithsonian Tropical Research Institute and nearby Soberanía National Park, Panamá (9°07'11.5"N 79°42'18.2"W), June to July 2016, and April 2019. 3. Las Pavas, Panamá (9°05'21.1"N 79°52'37.5"W), July 2015 and June 2016. 4. Sachavacayoc Center at the river Rio Tambopata, south-east of Puerto Maldonado, Madre de Dios, Perú (12°51’11.9 S, 69°22’2.3 W), September 2017. 5. Allpahuayo-Mishana National Reserve, Iquitos, Perú (3°56'39.0"S 73°36'23.4"W), August 2018. Individuals in Panamá were partly captured for the study of Brändel et al. (2020). Individuals from Peru were partly captured during a bat course organized in 2018 by CEBIO (Centro de Biodiversidad).

Studied bats

Phyllostomid bats of 40 species were captured with mistnets. Individuals were from both sexes and belonged to nine subfamilies: Micronycterinae, Desmodontinae, Phyllostominae, Lonchorhininae, Glossophaginae, Lonchophyllinae, Carolliinae, Glyphonycterinae and Stenodermatinae (Rojas et al., 2016). We used the phylogeny of Noctilionoidea by Rojas et al. (2016). To identify species, we used specific identification literature for Panamá (Handley et al., unpublished) and Perú (Díaz et al., 2011; López-Baucells et al., 2016; Reid, unpublished), respectively. A characteristic feature of the phyllostomid bats is a fleshy appendage on the nose, the so-called noseleaf (Wilson & Mittermeier, 2019). Most species within the family, including our study species have a free-standing noseleaf that is mostly taller than wide. The subfamily with the consistently smallest noseleaves are the Desmodontinae or vampire bats, where the nasal structures are reduced to low folds or ridges. Following Wilson and Mittermeier (2019) we therefore classified *Desmodus rotundus* as the only species in our data set with a reduced noseleaf.

High speed videos

In order to score whether the bats flew with their mouths open or closed, we hand-released each individual following a standardized protocol in a flight cage (Panamá, permanent outdoor flight cage, approx. 360cm x 250cm x 230cm (length x width x height); Perú, EUREKA screen house (hexagon), approx. 395cm x 420cm x 235cm (length x width x height)) while filming with a high-speed video camera (Optronis CR600 x 2, 8 GB memory) at resolutions between 1280 x 800 pixels and 1280 x 1024 pixels. The camera was placed at a distance of ca. 40 cm from the releaser at a 90° angle to the anticipated, ideal flight path of the bats on a parallel line at ca. 30 cm to the right (Supplementary Figure 1). For most species we used a 20 mm lens (Sigma, 1:1.8) at an aperture of 4 - 5.6, which covers approximately 40 cm of the flight path. For small species we occasionally used a 50 mm lens (Nikkor:

1:1.2) at an aperture of 4 - 5.6, which covered approximately 20 cm of the flight path. We recorded at 500 frames per second at an exposure time of 1/2000 or 1/3000 seconds, using a pre- and post-trigger period of 2 seconds each. The recordings were triggered manually with a handheld trigger as soon as the bat started flying. Bats were illuminated with infrared spotlights (Panamá: Uniflood 500 Strahler, 230 V, 220 W, 730 nm; Perú: AEGIS UFLED Intelligent-IR Strahler 20-8BD, BOSCH, 230 V, 45 W, 850 nm). To verify echolocating behavior, we monitored the ultrasound range (Avisoft Bioacoustics UltraSoundGate 416H; microphone: Avisoft-Bioacoustics, CM16, sampling rates 300 and 500 kHz) and found that bats were consistently echolocating during all flight sequences. After the recording session, we released the bats at the site of capture, usually within the same night.

We obtained 331 sequences of 177 individuals of 40 species to determine whether the mouth was open or closed during flight. From these, we selected 1 to 8 representative videos for each species for further measurements. We used one recording per individual, selecting the recording with the straightest flight path and best video quality, for measurements of gape angle (degree of mouth opening) and head position angle on 2 to 5 representative frames. Occasionally, individuals exhibited both conditions – mouth opened and closed – in consecutive flights. In this case we selected one video from each mode, resulting in two videos of this individual. For measuring gape angle, we drew two axes following the upper (palate) and lower (dentary) jawbone and measured the angle in between (Supplementary Figure 2A). Additionally, to measure the head position angle, we drew a line along the main body axis and crossed it with a second line drawn centered between the upper and lower jawbone (Supplementary Figure 2B). We used the angle tool of the software ImageJ (http://rsb.info.nih.gov/ij), measured every angle three times (digitalization error 2° per 3 measurements, Supplementary Data 2) and calculated the mean value. Hereafter, we refer to bats flying with the mouth open (gape angle > 0°) as "open-mouth bats" and those flying with the mouth closed as "closed-mouth bats".

Roost emergence photographs

For *Lonchorhina aurita*, in addition to flight cage observations described above, we also took photographs during roost emergence in Soberanía National Park, Panamá. We used a Nikon D810 with a 20-70 mm zoom lens with an open shutter, while the flash (Nikon SB900, set to 1/8) was triggered by the emerging bat interrupting a light beam. We obtained 75 photographs in which the mouth was clearly visible. Whenever teeth were visible, the individual was scored as an 'open-mouth bat', otherwise as a 'closed-mouth bat'.

Phylogenetic analysis

To test for a phylogenetic signal in the mouth posture trait, we constructed a phylogeny of the species included in this study based on the sequences provided in Rojas et al. (2016). In total, we were able to include 39 taxa; the Genbank IDs of sequences are indicated in supplementary data 3. The subspecies of *L. silvicolum* used in this study (*L. silvicolum silvicolum*) was not included in Rojas et al. (2016), we therefore used the genetically well-characterized *L. silivicolum laephotis*. For the newly described

Sturnira giannae, we used species-specific sequences (pers. comm. P. Velazco, Supplementary Data 3). We had to exclude *Artibeus ravus* because sequences are unavailable for this species.

We used Beast 1.10.4 to reconstruct a phylogenetic tree. The available sequence data consist of up to 9 nuclear and mitochondrial sequence fragments, including both coding and non-coding sequences; we curtailed fragments so they either only contained coding or non-coding sequences. We used the SRD06 model of sequence evolution (Shapiro et al., 2006) as a nucleotide substitution model for the coding sequences. For the non-coding sequences, we used the same parameters (substitution model: HKY, estimated base frequencies, gamma-distributed site heterogeneity with 4 gamma categories), but without partitioning the sequence into codon positions. For the substitution model, we linked the priors in four groups: nuclear coding fragments (*atp7, bdnf, ttn6, rag2*); nuclear non-coding fragments (*plcb4, stat5a, thy*); mitochondrial coding fragments (*cox1, cytb*) and mitochondrial non-coding fragments (*12s*). We used the relaxed lognormal clock as a clock model, linking priors into two groups (nuclear and mitochondrial) without time calibration. We used the flexible GMRF skyride as a demographic tree prior (Minin et al., 2008), linking priors across all fragments. All models were run long enough to obtain effective sample sizes >200 for all parameters with a 10% burn-in, and checked for convergence in Tracer (v1.7.1). We used TreeAnnotator v.1.10.4 to produce Maximum Clade Credibility (MCC) trees. To reconstruct the ancestral states of mouth posture, we performed a discrete trait analysis in Beast 1.10.4 with an asymmetric substitution model for mouth posture. The tree was visualized in FigTree v.1.4.4.

We then used the MCC tree generated in Beast (without the discrete trait) and the fitDiscrete function in the geiger package in R to calculate Pagel's lambda. We generated a null model (no phylogentic signal, lambda = 0) and a model assuming Brownian motion evolution (lambda = 1) and compared models with a log-likelihood test.

Statistical analysis

We used R (version 2.6.2, 2008-02-08) for all statistical analyses. To compare the two groups, 'open-mouth emitters' and 'nasal emitters' we used the Wilcoxon rank sum test. As we found a significant phylogenetic signal for mouth posture, we additionally performed a phylogenetic analysis using the phylANOVA function in the phytools package in R to test whether mouth posture (three states: open, closed and 'both') significantly affects mean head position angle; the drop.tip function was used to remove the tip for *Glossophaga soricina*, for which no head angle data was available. For species in which both open and closed mouth occurred, we used an exact binominal test to determine which was the more common behavior. All statistical analyses were performed using a significance level of $\alpha = 0.05$.

Results

Mouth posture

We obtained 331 flight sequences from 177 individuals of 40 phyllostomid bat species (Table 1, Figure 2, Supplementary Video 1). Sixteen species consistently flew with the mouth closed. Five species exhibited both closed and open mouths in flight. Nineteen species consistently flew with the mouth open. These patterns were not distributed randomly across the phylogenetic tree; rather they reflect the phylogenetic relationships of these species as shown by reconstructing ancestral traits with a discrete trait analysis in Beast 1.10.4 (Figure 3). An open mouth posture was reconstructed as the ancestral state of the Phyllostomidae. The closed mouth posture has evolved at least in two independent clades, within the Stenodermatinae and the genus *Phyllostomus*. Mouth posture shows a significant phylogenetic signal (Pagel's lambda $\lambda = 0.799$, log-likelihood-test, $p < 0.001$); λ did not significantly differ from Brownian motion evolution ($p = 0.13$).

Most bats from the Stenodermatinae, except two species of the genus *Sturnira*, consistently flew with the mouth closed and we therefore consider them closed-mouth species. Additionally, all three scored species of the genus *Phyllostomus* (Phyllostominae) were closed-mouth species (Table 1).

Within a species most individuals showed the same behavior and maintained the mouth either consistently open or consistently closed (Table 1). Inconsistent behavior occurred within the Stenodermatinae, Lonchorhininae and Phyllostominae. In two species, the behavior varied across individuals. Of the six individual *Lonchorhina aurita* recorded in the flight cage, four individuals flew with mouths consistently open, while two flew with mouths consistently closed. Similarly, of the four *Chrotopterus auritus* recorded, three flew with the mouth consistently open, while one individual always flew with the mouth closed. Six individuals (3.4 %) from three species out of our entire sample (n=177 individuals) switched between open-mouth and closed-mouth mode in consecutive flights. Of the 16 individual *Artibeus jamaicensis* recorded, three individuals flew with the mouth slightly open in one out of two sequences. Two of these three had the mouth closed in the first sequence and open in a second sequence. One individual had the mouth open in the first sequence, closed in the second, and reopened in the third sequence. In addition, one of the five *Artibeus planirostris* individuals recorded had the mouth opened in the first out of three sequences. Individuals of *Gardnerycteris crenulatum* showed the greatest behavioral variation. One of the four individuals used open-mouth mode in the first and third trial, and closed- mouth mode during the second and fourth trial. A second individual kept the mouth open during the first flight and closed during the next four flights. The last two individuals were only recorded using closed-mouth mode (in one and two flights, respectively).

Desmodus rotundus (Desmodontinae), the only species in our data set with a reduced noseleaf, consistently flew with an opened mouth. All species of the subfamilies Micronycterinae, Glossophaginae, Carolliinae, Lonchophyllinae and Glyphonycterinae were also consistently open-mouth species. Additionally, certain species within both Phyllostominae (*Lophostoma silvicolum*,

Tonatia saurophila and *Trachops cirrhosus*) and Stenodermatinae (*Artibeus gnomus, Sturnira tildae* and *Sturnira giannae*) were open-mouth species (Table 1).

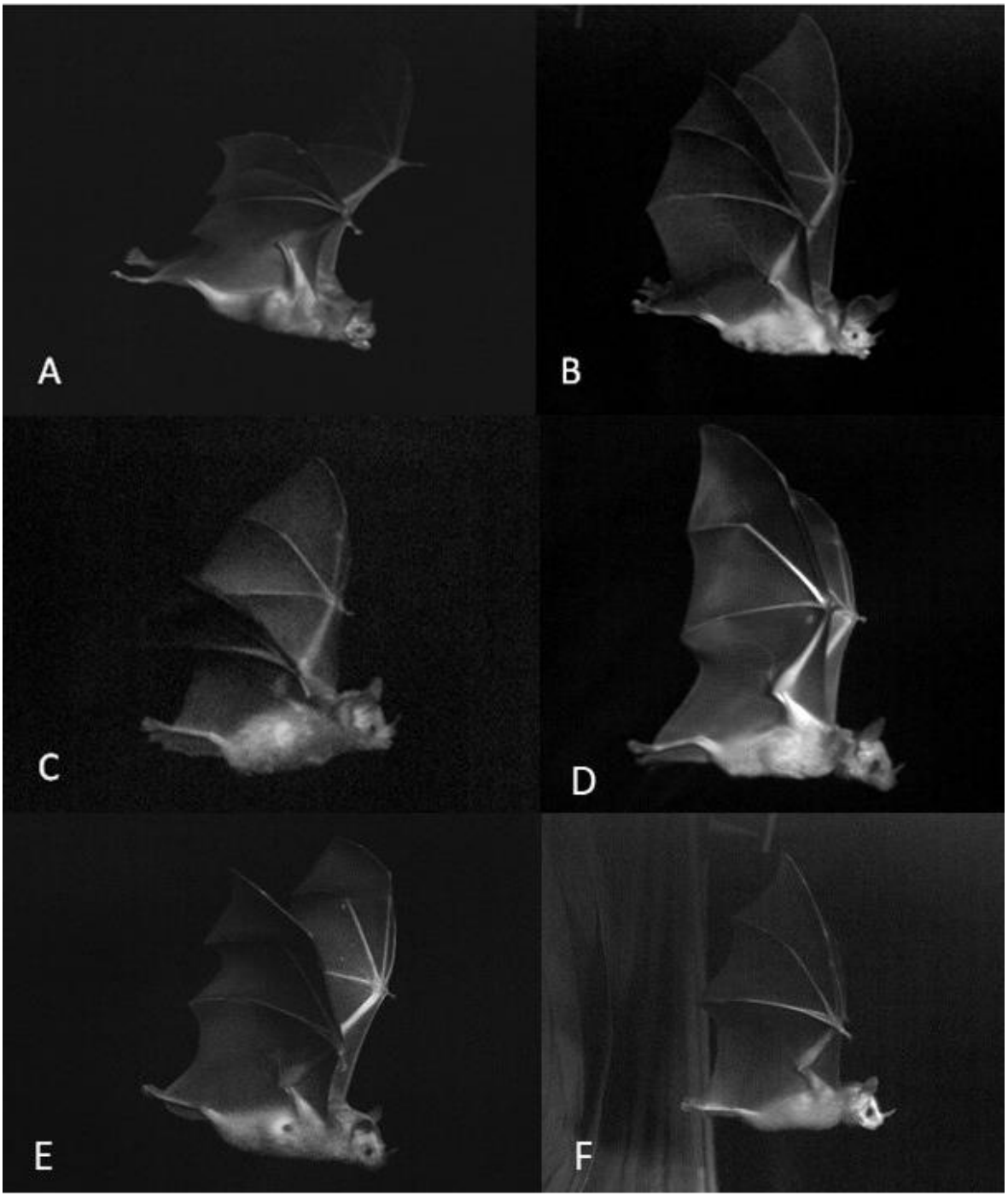


Figure 2: Examples of still shots extracted from the high-speed videos. Open-mouth phyllostomids (gape angle > 0°): (A) Desmodus rotundus, (B) Lophostoma silvicolum, (C) Carollia castanea. Nasal emitters: (D) Phyllostomus discolor, (E) Artibeus jamaicensis, (F) Vampyrodes major. Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/

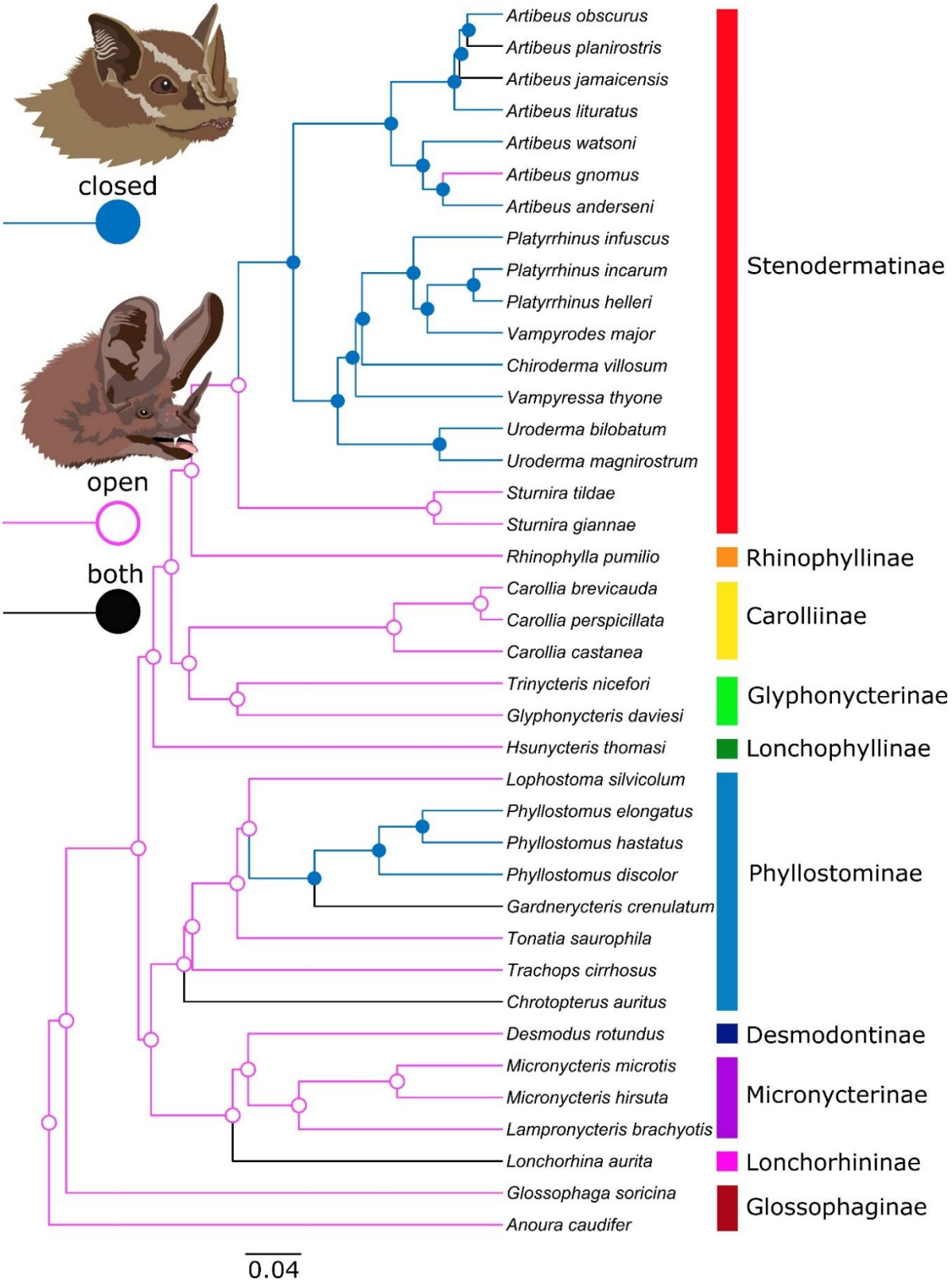


Figure 3: Ancestral state reconstruction of mouth posture. The MCC tree was constructed with TreeAnnotator v.1.10.4 on Beast v1.10.4 runs using sequence data provided in Rojas et al. (2016) (see supplementary data 3). Mouth posture was reconstructed as a discrete trait with an asymmetric substitution rate. Colors and node shapes indicate the mouth posture state (pink/light circle = open-mouth species; blue/filled circle = closed-mouth species; black = "both"). Posterior support is shown on the tree. Subfamilies are indicated by rainbow colors. (Illustrations by D. Kyllo). Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/

Table 1: Scoring of the mouth posture of 40 phyllostomid species during flight (mean number of sequences scored per individual: 2). "Closed" indicates a closed mouth during flight, "open" means open during flight. 'Both' indicates that individuals switched between behavior types. "Open/closed" indicates that both behaviors were present within a species. We used an exact binominal test (ebt) to compare numbers from "open" and "closed". In most species only one behavior type was observed. When both closed and open mouths were observed within the same species, p-values below 0.05 indicate, that the more common behavior occurred significantly more out of the two possibilities 'open' and 'closed'. Based on Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/

subfamily	species	mouth posture / # of individuals			behavior: mouth	ebt
		open	closed	both		p
Stenodermatinae	*Artibeus obscurus*		3		closed	0.25
	Artibeus jamaicensis		13	3	open/closed	< **0.001**
	Artibeus planirostris		4	1	open/closed	0.125
	Artibeus lituratus		8		closed	< **0.01**
	Artibeus watsoni		6		closed	< **0.05**
	Artibeus ravus		5		closed	0.063
	Artibeus anderseni		1		closed	1
	Artibeus gnomus	1			open	1
	Platyrrhinus infuscus		4		closed	0.125
	Platyrrhinus incarum		3		closed	0.25
	Platyrrhinus helleri		2		closed	0.5
	Vampyrodes major		3		closed	0.25
	Chiroderma villosum		2		closed	0.5
	Vampyressa thyone		1		closed	1
	Uroderma bilobatum		5		closed	0.063
	Uroderma magnirostrum		1		closed	1
	Sturnira giannae	3			open	0.25
	Sturnira tildae	4			open	0.125
Rhinophyllinae	*Rhinophylla pumilio*	1			open	1
Carolliinae	*Carollia brevicauda*	4			open	0.125
	Carollia perspicillata	6			open	< **0.05**
	Carollia castanea	8			open	< **0.01**
Glyphonycterinae	*Glyphonycteris daviesi*	1			open	1
	Trinycteris nicefori	2			open	0.5
Lonchophyllinae	*Hsunycteris thomasi*	4			open	0.125
Lonchorhininae	*Lonchorhina aurita*	4	2		open/closed	1
Glossophaginae	*Glossophaga soricina*	3			open	0.25
	Anoura caudifer	1			open	1
Phyllostominae	*Lophostoma silvicolum*	7			open	< **0.05**
	Phyllostomus hastatus		9		closed	< **0.01**
	Phyllostomus elongatus		3		closed	0.25
	Phyllostomus discolor		5		closed	0.063
	Gardnerycteris crenulatum		2	2	open/closed	0.5
	Tonatia saurophila	5			open	0.063
	Trachops cirrhosus	6			open	< **0.05**
	Chrotopterus auritus	3	1		open/closed	0.625
Desmodontinae	*Desmodus rotundus*	21			open	< **0.001**
Micronycterinae	*Micronycteris microtis*	1			open	1
	Micronycteris hirsuta	1			open	1
	Lampronycteris brachyotis	2			open	0.5
# of individuals	177	88	83	6		

Gape angle

Among the open-mouth species, gape angle varied from 19° in *Artibeus gnomus* to 54° in *Desmodus rotundus* (Figure 4, Table 2).

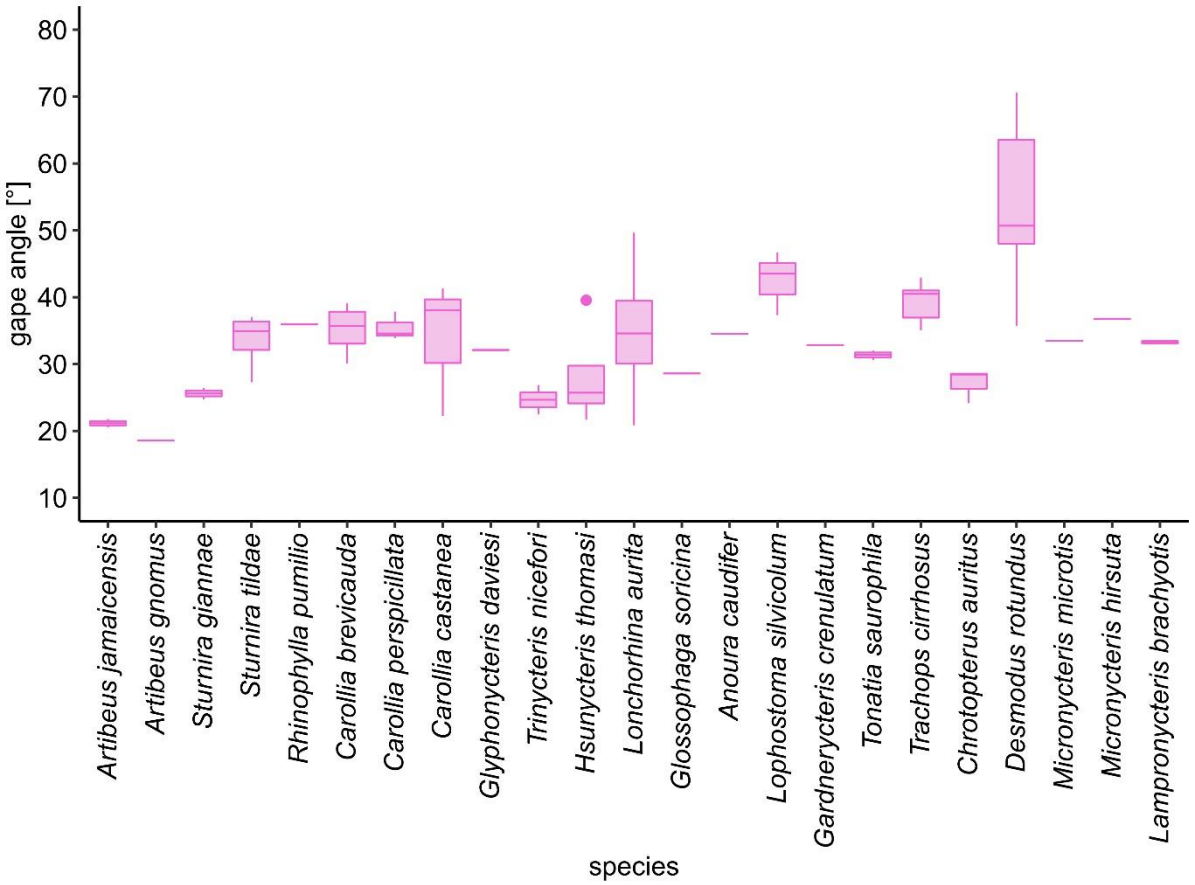


Figure 4: Gape angle of 23 phyllostomid species. Desmodus rotundus has the widest gape angle (54.3° ± 12.1°) while Artibeus gnomus (18.8°) opens the mouth only slightly during flight. The box incorporates the middle 50 % of the data and the line within the box the median value. Whiskers mark the 25th percentile and the 75th percentile of the data (range of the data). Outliers are plotted as dots (values that are more than 1.5 times the interquartile range beyond either end of the box). Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/

Table 2: Mean gape angle in degree [°] of 23 species and head position angle of 40 species of phyllostomid bats. Nasal emitters had their mouth consistently closed and no gape angle could be measured. Grey background indicates that individuals of this species were observed with open and closed mouth. For Glossophaga soricina no head position angle could be measured. n is the number of individuals. SD is the standard deviation. Based on Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/

subfamily	species	mouth	n	gape angle		SD	head angle		SD
Stenodermatinae	*Artibeus obscurus*	closed	3				148	±	6.4
	Artibeus jamaicensis	closed	6				140	±	9.1
		open	2	21	±	0.9	147	±	5.8
	Artibeus planirostris	closed	5				142	±	7.1
	Artibeus lituratus	closed	2				133	±	4.8
	Artibeus watsoni	closed	1				140		
	Artibeus ravus	closed	1				146		
	Artibeus anderseni	closed	1				141		
	Artibeus gnomus	open	1	19			147		
	Platyrrhinus infuscus	closed	2				147	±	6.0
	Platyrrhinus incarum	closed	2				142	±	8.5
	Platyrrhinus helleri	closed	1				151		
	Vampyrodes major	closed	1				147		
	Chiroderma villosum	closed	1				146		
	Vampyressa thyone	closed	1				157		
	Uroderma bilobatum	closed	2				144	±	7.9
	Uroderma magnirostrum	closed	1				151		
	Sturnira giannae	open	2	26	±	1.2	149	±	7.0
	Sturnira tildae	open	4	34	±	4.4	148	±	2.7
Rhinophyllinae	*Rhinophylla pumilio*	open	1	36			176		
Carolliinae	*Carollia brevicauda*	open	4	35	±	4.0	154	±	4.0
	Carollia perspicillata	open	3	35	±	2.1	157	±	5.5
	Carollia castanea	open	3	34	±	10.2	152	±	10.5
Glyphonycterinae	*Glyphonycteris daviesi*	open	1	32			159		
	Trinycteris nicefori	open	2	25	±	3.1	159	±	7.2
Lonchophyllinae	*Hsunycteris thomasi*	open	4	28	±	7.9	162	±	3.7
Lonchorhininae	*Lonchorhina aurita*	closed	2				152	±	0.5
		open	4	35	±	11.8	161	±	12.1
Glossophaginae	*Glossophaga soricina*	open	1	29					
	Anoura caudifer	open	1	35			180		
Phyllostominae	*Lophostoma silvicolum*	open	3	43	±	4.8	167	±	6.0
	Phyllostomus hastatus	closed	4				145	±	2.8
	Phyllostomus elongatus	closed	2				141	±	8.5
	Phyllostomus discolor	closed	3				140	±	3.8
	Gardnerycteris crenulatum	closed	3				143	±	5.8
		open	1	33			147		
	Tonatia saurophila	open	2	31	±	1.0	161	±	0.1
	Trachops cirrhosus	open	5	39	±	3.2	154	±	6.7
	Chrotopterus auritus	closed	1				137		
		open	3	27	±	2.5	138	±	2.5
Desmodontinae	*Desmodus rotundus*	open	7	54	±	12.1	183	±	8.9
Micronycterinae	*Micronycteris microtis*	open	1	33			178		
	Micronycteris hirsuta	open	1	37			159		
	Lampronycteris brachyotis	open	2	33	±	0.4	161	±	3.8
# of individuals			103						

Head position angle

Open-mouth species and closed-mouth species differed significantly in their head position angle (Wilcoxon rank sum test, W = 51, p < 0.001) (Figure 5). This difference in head angle according to mouth posture was confirmed by a phylogenetic ANOVA, including open, closed and "both" as mouth posture states (F=16.377, p <0.01). A post-hoc test shows that head position significantly differs both between species with open and closed mouths (t = 3.771, p < 0.05) and with open and ambiguous ("both") mouth posture (t = 5.272, p < 0.05), but not between closed and ambiguous states (t = 0.122, p = 0.916). Open-mouth species held their heads straighter, resulting in a larger angle (mean=159°± 11.8 SD). *Desmodus rotundus* even bent its head slightly upwards (mean=183° ± 8.9 SD). Closed-mouth species had a smaller head position angle, indicating they tilted their heads more downwards (mean=144°± 5.4 SD). We found the smallest angle of head position for *Artibeus lituratus* at 133°, followed by a closed-mouth individual of *Chrotopterus auritus* at 137°. Even within species, individuals flying with an open mouth held the head straighter than individuals flying with a closed mouth (Table 2).

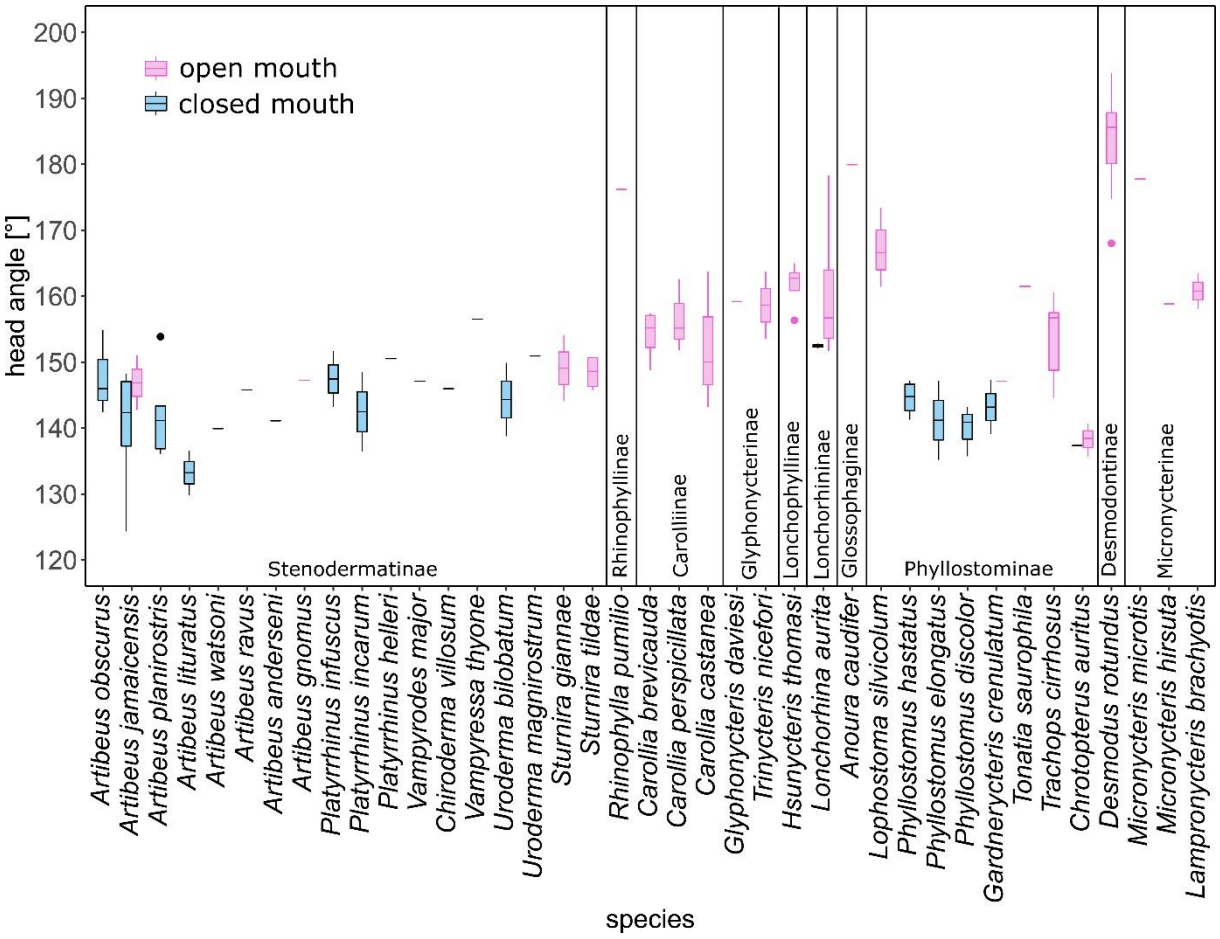


Figure 5: Boxplots of mean head position angle per species. Vertical lines separate subfamilies. Open-mouth species held the head straighter (mean=159° ± 12) than individuals with closed mouths (nasal emitters) (mean=183° ± 8). The box incorporates the middle 50 % of the data and the line within the box the median value. Whiskers mark the 25th percentile and the 75th percentile of the data (range of the data). Outliers (values more than 1.5 times the interquartile range beyond either end of the box) are plotted as dots. Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/

Roost emergence photographs

In addition to the flight cage observations of *Lonchorhina aurita* (n= 6 individuals), we scored 75 photographs of *L. aurita* emerging from their roost in the evening. In 41 photographs the mouth was closed (no teeth showing), while in 34 photographs the mouth was open (teeth visible, Supplementary Figure 3). There was no significant difference in number of observations between these two behaviors (exact binominal test, probability of closed mouth: 0.55, $p = 0.49$).

Discussion

Phyllostomidae is one of the ecologically most diverse families of bats. Yet, phyllostomid species show remarkably homogenous echolocation call structure, often assumed to be associated with the presence of a noseleaf, and, by extension, nasal emission of calls. In the context of recent growing interest in phyllostomid echolocation behavior (Yoh et al., 2020; Zamora-Gutierrez et al., 2020; Gessinger et al., 2019; Rodríguez-San Pedro and Allendes, 2017; Gonzalez-Terrazas et al., 2016), we explored mouth and head angle postures during echolocation within this family to make predictions about call emission modes. Following anecdotal and photographic evidence of phyllostomid bats flying with an open mouth, we investigated whether this behavior occurs occasionally or consistently across the family. We used high speed video recordings of a wide range of phyllostomid species in flight to show that the majority (88%) of the sampled species flew either consistently with the mouth opened or consistently with the mouth closed. Every bat in our study echolocated during the recordings. Supported by the consistent differences of the head position angle, we suggest that our observations of closed- and open-mouth species, reflect the emission mode of echolocation calls. Further, distribution of the closed- and open-mouth mode among the species was not random but showed distinct phylogenetic patterns supported by Pagel's lambda statistic. While bats flying with a closed mouth can emit echolocation calls only through the nostrils (nasal emission), open-mouth species may use: i) nasal emission, ii) oral emission, iii) alternating nasal and oral emission, iv) synchronous nasal and oral emission. The context in which plastic emitters shift between emission modes, and the possible ecological advantages of one emission mode over another in a given context, present future avenues of study.

With few exceptions, mouth posture (open/closed) and head angle were remarkably consistent among the individuals of a species. While the sample sizes are heterogeneously distributed among the species, our study across 40 phyllostomid species indicates a phylogenetic pattern in phyllostomid echolocation behavior. Below, we first show the distribution of the observed behaviors over the phylogenetic tree of the phyllostomid family (Figure 3) and then discuss their implications for echolocation.

Phylogenetic patterns

We hypothesized that phyllostomid bats with free-standing noseleaves would be exclusively nasal emitters and not, or only occasionally show an open mouth. However, we found that only 16 out of 40 species (40.0%) always flew with the mouth closed. In contrast, we found that 19 species (47.5%) consistently kept the mouth open during flight (Table 1, Figure 3). While we believe that this is representative behavior in most of our study species, we are less confident of this pattern in species with low sample sizes. The behavior of the single individual of *Artibeus gnomus*, for example, might have occurred by chance, because it contrasts with the main pattern found for the entire genus *Artibeus*. In only 5 species (12.5%), we found both behaviors to occur, which in some species might reflect natural variability that is better detected in larger sample sizes, such as in *Artibeus jamaicensis*, where 13 out of 16 animals (81%) always maintained a closed mouth.

Nasal emitters In support of our first hypothesis, 16 species with free-standing noseleaves used closed-mouth behavior, never showed an open mouth and thus were confirmed to be nasal emitters.

Within the Stenodermatinae subfamily, our sampling of the genera *Platyrrhinus, Vampyrodes, Chiroderma, Vampyressa,* and *Uroderma* revealed only nasal emitters. This group is the sister clade to the clade containing all species from the genus *Artibeus* (Figure 3), from which all individuals included in our study also maintained a closed mouth, confirming them to be nasal emitters. Only few individuals of *A. jamaicensis* and *A. planirostris* switched in consecutive flights from open-mouth to closed-mouth mode. This might indicate that they used both behaviors. Alternatively, the open mouth in these individuals could be unrelated to echolocation, but be an indicator, e.g., of stress related to handling. No individual of *A. jamaicensis* was observed flying exclusively with an open mouth and with 13 individuals flying exclusively with a closed mouth we reached the statistical power to confirm the original hypothesis of nasal emission (Table 1). In *A. planirostris* we observed also no individuals exclusively flying with an open mouth and a higher sampling effort would show whether nasal emission is indeed the most used behavior. The only member of the genus *Artibeus* we scored flying exclusively with an open mouth was *A. gnomus.* Unfortunately, we captured only a single individual of this species and obtained only a single recording that we could analyze. It would be important to explore whether the open mouth is really the consistent mode in this species or if this behavior changes between trials or individuals. In summary, for all eight *Artibeus* species tested, 40 out of 45 individuals or 89 % used nasal emission exclusively.

Within the Phyllostominae subfamily, all scored individuals from the three species of the genus *Phyllostomus* were exclusively observed with a closed mouth. While for *Phyllostomus hastatus* sampling effort was high enough for a significant result, we did not have the sufficient sample sizes to conclude the same for *P. discolor* and *P. elongatus* (Table 1).

Besides these, some individuals from *Gardnerycteris crenulatum, Chrotopterus auritus* and *Lonchorhina aurita* - had their mouths entirely closed at least occasionally, indicating exclusive nasal emission in these situations. In the case of *G. crenulatum*, two individuals started to fly with an open mouth but continued flying with a closed mouth in consecutive trials. An open mouth at the beginning of recordings in a nasal-emitting species could also be a sign, e.g., of stress as mentioned above. Two other individuals had the mouth consistently closed. Therefore, while nasal emission in this species is possible, our results are inconclusive. The same is true for *C. auritus*; three individuals flew with open mouth and teeth visible, another exclusively with a closed mouth. We cannot rule out that our experimental setup, adapted primarily for small to medium sized bats, affected the behavior of *C. auritus,* the largest species sampled in our data set. To clarify whether *G. crenulatum* and *C. auritus* use both open-mouth and closed-mouth mode in equal percentages, a larger sample size and a setup adjusted for large body size would be helpful. In contrast, *L. aurita* (Lonchorhininae) clearly used both behaviors; four individuals consistently flew with an open mouth (67%) and two with a closed mouth (33%), and neither behavior was significantly preferred (binomial test, $p = 1$). Additional roost emergence photographs further indicate equal use of both behaviors, with approximately half of the individuals flying with their mouth open (45%) and half with their moth closed (55%). *L. aurita* has the

longest noseleaf of the phyllostomidae, so an open mouth contradicts our original hypothesis. However, *L. aurita* stands out in this family by using long echolocation calls with constant frequency components (Gessinger et al., 2019). The inconsistent behavior of *L. aurita* might therefore actually reflect a sensory adaptation (see below).

Open mouth mode The common vampire bat *Desmodus rotundus* (Desmodontinae), the only species in our data set with a reduced noseleaf (Wilson and Mittermeier, 2019), is an open-mouth species. However, we also found that all sampled species from the subfamilies Micronycterinae, Glossophaginae, Rhinophyllinae, Carolliinae, Lonchophyllinae and Glyphonycterinae, all with free-standing noseleaves, are open-mouth species. This contradicts our first hypothesis that species with free-standing noseleaves are inherently nasal emitters. An interesting exception within the Stenodermatinae is the genus *Sturnira,* where both tested species (*S. giannae, S. tildae*) showed also only open-mouth individuals.

Summarizing our results, echolocation emission behaviors of phyllostomid bats are not randomly distributed over the phylogenetic tree (Fig. 3) and mouth posture shows a significant phylogenetic signal. Ancestral trait reconstruction showed that the open mouth mode is the ancestral state within the Phyllostomidae and that exclusive nasal emission (closed mouth) evolved at least twice in two independent clades (Stenodermatinae and the genus *Phyllostomus*). We suggest that exclusive nasal emission evolved within the large subfamily Stenodermatinae. While the genus *Sturnira*, which is the sister clade to all other Stenodermatinae, still uses the open-mouth mode, almost all other Stenodermatinae, comprising 45% of the sampled species, are exclusive nasal emitters. Within the subfamily Phyllostominae, exclusive nasal emission evolved at least once in the genus *Phyllostomus*. *Phyllostomus hastatus, P. discolor* and *P. elongatus,* representing three of the four extant species of the genus, showed exclusively nasal emission and were never observed with an open mouth. Additionally, exclusive nasal emission may have evolved at least twice more, in the genera *Gardnerycteris* and *Chrotopterus,* although this is unresolved as both behavior types were observed in these species.

In conclusion, as a whole phyllostomids are not characterized by a single behavior type. Rather, open-mouth or closed-mouth behavior is dominant or exclusively used *within* most phyllostomid subfamilies, which we posit has implications for the echolocation emission mode of this ecologically diverse group of bats. Higher behavioral variability is only found within the Phyllostominae, which happens to also be the ecologically most variable family, including on one hand animalivorous gleaners and “carnivores” but also omnivores feeding on insects, fruits and nectar (Gardner, 1977; Hoffmann et al., 2008). Open-mouth mode is reconstructed to be the ancestral behavior (Figure 3).

Echolocation call emission

Echolocation calls of laryngeally echolocating bats are either emitted orally, nasally, or in a combined manner through three sources, the two nostrils and the mouth (Jakobsen et al., 2018). In most families this trait seems to be remarkably constant (Metzner and Müller, 2016).

Closed-mouth species Anatomical features of the skull (Pedersen, 1998, 1995) and the presence of an often prominent noseleaf (Arita, 1990; Bogdanowicz et al., 1997) have been seen as evidence for nasal emission of echolocation calls in the Phyllostomidae (Brokaw and Smotherman, 2020; Hartley and Suthers, 1987; Jakobsen et al., 2018; Matsuta et al., 2013). Bats keeping their mouth completely closed must emit echolocation signals exclusively through the nostrils (e.g., Rübsamen, 1987; Suthers et al., 1988). We confirm that this is indeed the case for 16 phyllostomid species, all of which feed mainly on plant products (Giannini and Kalko, 2004).

Small sound-emitting structures such as the nostrils of a bat produce a much broader beam than larger structures, e.g., its mouth, for any given frequency (Kounitsky et al., 2015). Many phyllostomid bats forage in dense forest where a broader beam is unfavourable because it will result in more distracting echoes reflected by the background vegetation (Denzinger et al., 2018; Kalko et al., 2008). Generally, the beam can be narrowed either by enlarging the emitter size or by using higher frequencies (Jakobsen et al., 2013; Kounitsky et al., 2015). Bat nostrils as separate entities have a rather fixed size and - unlike mouth gapes - can probably not be enlarged enough to significantly narrow the beam width (Brokaw and Smotherman, 2020). However, a signal emitted by more than one emitter (e.g., two nostrils), may experience interference phenomena that can narrow the beam in the horizontal plane. Blocking one nostril in *Carollia perspicillata* resulted in horizontal widening of the beam pattern (Hartley and Suthers, 1987). Similar, an emission without the lancet of the noseleaf was shown to widen the emission pattern in the vertical plane in *Carollia perspicillata* (Hartley and Suthers, 1987), and in a model of *Phyllostomus discolor* and *Micronycteris microtis* (Vanderelst et al., 2010). Finally, in the nasally emitting rhinolophid *Rhinolophus ferrumequinum* beam width modeled without noseleaf was larger than the beam width actually measured, also suggesting that the noseleaf focuses the beam (Strother and Mogus, 1970).

Open-mouth species Nineteen species in our study consistently flew with an open mouth. Mouth opening could be involved in shaping the echolocation beam during flight. As mentioned above, open-mouth bats have four different options for the emission of echolocation calls: 1. Nasally, through the nostrils, 2. Through the mouth, 3. Alternately through mouth and nostrils, 4. Synchronously through mouth and nostrils.

1. Nasally. Emission of echolocation calls through nostrils integrated into a noseleaf has the advantage of a reduced vertical and horizontal beam width (Hartley and Suthers, 1987; Vanderelst et al., 2010). Phyllostomid bats usually filter out the fundamental harmonic and use several higher harmonics instead (Yoh et al., 2020). This is probably achieved through a filtering process in the nasal cavities, as demonstrated for *Rhinolophus hildebrandti* (Rhinolophidae). By sealing the nostrils bats were forced to echolocate through the mouth, which lead to a sudden increase of the energy in the fundamental harmonic, probably because

the filtering got disturbed (Suthers et al., 1988). Therefore, opening the mouth could even be disadvantageous for phyllostomids. Perching *Trachops cirrhosus* have been observed to occasionally echolocate with an open mouth, which resulted in some animals in an increase of intensity of the first harmonic (Surlykke et al., 2013). Anatomical evidence for an exclusively nasal sound emission can be found in the Hipposideridae and Rhinolophidae. In those bat families the lid of the larynx fits perfectly into the nasolaryngeal opening, thus creating a clear separation between the mouth- and the larynx-to-nostril air flow (Neuweiler, 1993). To our best knowledge, such a separation has not been discovered for phyllostomids, however, information on the anatomy of the vocal tract of phyllostomid species seems to be scarce.

2. Orally. Bats emitting echolocation calls orally can change the beam width by adjusting the mouth gape. Mouth-emitting Bodenheimer's pipistrelle bats *Hypsugo bodenheimeri* (Vespertilionidae) achieve a narrower beam width by increasing their mouth gape when flying into cluttered environments (Kounitsky et al., 2015). This easy way to adjust beam width could be used by open-mouth phyllostomids in their narrow space forest habitats. In *Desmodus rotundus*, with its almost entirely reduced noseleaf, we actually scored a large variability in mouth gape, suggesting that this species might adjust the mouth gape and therefore also beam width during flight. All other open- mouth phyllostomids in our study had free-standing noseleaves and we consider it unlikely that these structures would have evolved if echolocation calls were emitted exclusively orally.

3. Alternating oral and nasal emission. A bidirectional echolocation mode involving both nasal and oral emission has been suggested in *Barbastella barbastellus* (Vespertilionidae). This aerial hunting bat emits two alternating echolocation call types, one of which is presumably emitted through the nostrils and the other through the mouth. In a single channel recording this alternation can be detected by distinct shifts in intensity, as the two sound beams differ in their orientation towards the microphone (Seibert et al., 2015). It is suggested that the nasally emitted calls serve mainly for detection and localization of prey, while the orally emitted call supports spatial orientation.

4. Simultaneous nasal and oral emission. Emitting sound through a nostril causes a large beam width due to the small emitter size. Beam width can be horizontally decreased through interference of the two nostril sound beams and vertically controlled by the noseleaf (Hartley and Suthers, 1987; Vanderelst et al., 2010). Also, vertical beam could be narrowed by adding a third point source, i.e., oral sound emission. The measured sound beam of the vespertilionid *Plecotus auritus* was best explained by a triple-emitter model involving both nostrils and the mouth. This model was the only one consistent with the observed narrowing of the beam in the vertical plane (Jakobsen et al., 2018). So far, directionality of echolocation calls of open-mouth phyllostomids has only been studied for *Carollia perspicillata* and *Trachops cirrhosus* (Brinkløv et al., 2011; Surlykke et al., 2013). Both species showed narrow sonar beam widths. Interestingly, beam width of *Carollia perspicillata* was narrower than in previous studies where oral emission was prevented by sealing the mouth (Hartley and Suthers, 1987), supporting the idea of synchronous mouth and nostril emission.

Since open-mouth mode is the ancestral behavior in the phyllostomid tree the question arises, why stenodermatines and the species of the genus *Phyllostomus* do not use the open-mouth mode? Perhaps their

generally rather broad noseleaves are specially adapted to focusing the beam in the vertical plane, thus making additional oral emission unnecessary. On the other hand, the advantages of being able to carry relatively large fruits might outweigh the advantages of a more narrow echolocation beam, especially as they may also use olfactory cues during foraging (Ripperger et al., 2019; Hodgkison et al., 2013; Korine and Kalko, 2005). A narrow beam could be more important for animalivorous gleaning bats (e.g., *Trachops cirrhosus, Lophostoma silvicolum*), which listen to prey- generated sounds, have to capture potentially mobile prey and face a dilemma between calling and listening (Jones et al., 2016). A narrow beam produces less irrelevant echoes potentially interfering with the sounds produced by the prey. In this context, a future comparison of beam widths between nasal emitters and open-mouth species might be extremely interesting.

Several species pointed their nostrils in the direction of flight, confirming our second hypothesis that head position enables the emitters to point in the main direction of flight. Open mouth individuals showed a larger head position angle, pointing both mouth gape and nostrils in the flight direction. Even within a species, individuals flying with an open mouth held their head straighter than individuals flying with closed mouths. However, due to the small sample size we could not obtain more conclusive data. Nevertheless, the difference in head position between the two behavioral groups further supports the idea that call emission through the mouth is much more prominent than previously thought in phyllostomid echolocation. The combined evidence of mouth posture and head angle therefore suggests that open-mouth phyllostomids use a synchronous emission of echolocation calls through mouth and nostrils.

Open-mouth mode/nasal emissions: While everything mentioned above for open-mouth species and nasal emitters applies for species using both open-mouth mode and nasal emission as well, the question arises why a few species sometimes fly with an open mouth and sometimes keep their mouth closed. The clearest case here is *Lonchorhina aurita*, where a solid sample size underlines an almost equal use of nasal emission and open-mouth mode. *Lonchorhina aurita* shows an echolocation call design strikingly different from all other phyllostomid genera, composed of a constant frequency (CF) and a frequency modulated (FM) component (Gessinger et al., 2019). One explanation would be that the two components could be emitted separately through nostrils and mouth, respectively. Synchronised high-speed video and audio recordings would be necessary to test this hypothesis and, combined with recent advances in bat tagging technology, might shed light on the functional significance of this pattern (Stidsholt et al., 2021).

Conclusion

With the 40 species included in our study we assembled a phylogenetically broad sample that covers almost all subfamilies and close to 20% of extant species of the ecologically highly diverse phyllostomid family. While sample size in some species is admittedly limited, our study reveals for the first time an intriguing phylogenetic pattern within the family. Within almost all phyllostomid subfamilies we found a remarkably uniform mouth posture and matching head position. An interesting exception were the Phyllostominae, where the genus *Phyllostomus* showed the same closed mouth posture (indicating nasal emission) as did the vast majority of the exclusively plant-visiting species from the Stenodermatinae subfamily. This coincides with the fact that the genus *Phyllostomus* is ecologically distinct from other, exclusively animalivorous species in the phyllostomines, by being highly omnivorous, consuming many insects and even small vertebrates like the other phyllostomines, but utilizing also a high proportion of plant resources (fruit, nectar). These results suggest that diet and the corresponding foraging behavior might influence the actual emission type. In conclusion, the considerable proportion of open-mouth species and the significant difference of head position angles between open-mouth species and nasal emitters highly suggests that the phyllostomid family contains not just exclusively nasal-emitting species. Future investigation may reveal additional fully or partly oral-emitting species to be mapped on the phylogenetic phyllostomid tree. Experiments, such as selectively obstructing sound emission through nostrils or mouth, respectively, will be necessary to clarify to what extent open-mouth phyllostomids are emitting sound through the mouth and/or nostrils, respectively, and the potential consequences for echolocation beam width and niche adaptation in the ecologically highly diverse phyllostomid family.

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Statements

Data availability statement

The datasets presented in this study can be found in the article/Supplementary Material under the link: https://www.frontiersin.org/articles/10.3389/fevo.2021.630481/full#supplementary-material.

Ethics statement

The animal study was reviewed and approved by STRI's animal ethics committee (IACUC protocols 2014-0101-2017 and 2017-0102-2020). We followed the guidelines for the use of wild mammal species in research as recommended by the American Society of Mammalogists.

Author contributions

GG, AS and MT had the idea for the study. Flight cage recordings in Panamá and Perú were made by GG and MT. GG did the video analysis and prepared figures and tables. LW did the phylogenetic analysis. GG and LW did the statistics. GG drafted the manuscript and RP, SB, LW, and MT, contributed to finishing the paper. All authors gave final approval for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal ethics statement

We followed the guidelines for the use of wild mammal species in research as recommended by the American Society of Mammalogists. The work was approved by STRI's animal ethics committee (IACUC protocols 2014-0101-2017 and 2017-0102-2020).

Permission to carry out fieldwork statement

Field work in Panama was conducted under permits no. SE/A 69-15, SE/AH-2-16 and SE/AP-13-18 from Panama's Ministerio de Ambiente (MiAmbiente). Field work in Peru was conducted under permit no. 0648-2017-SERFOR/DGGSPFFS-DGSPFS from Peru's Servicio Nacional Forestal y de Fauna Silvestre – SERFOR.

Supplementary material

Supplementary Data 1: Raw data of i. mouth posture, ii. gape and head position angle, iii. mouth posture of L. aurita during roost emergence. Based on Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/
https://www.frontiersin.org/journals/ecology-and-evolution/articles/10.3389/fevo.2021.630481/full#supplementary-material

i. Mouth posture.

date	country	bat_id	species	subfamily	sex	mouth_posture
27.03.2015	Panama	4	*Artibeus literatus*	Stenodermatinae	F	closed
27.03.2015	Panama	5	*Artibeus jamaicensis*	Stenodermatinae	F	closed
27.03.2015	Panama	6	*Artibeus jamaicensis*	Stenodermatinae	M	closed
29.03.2015	Panama	7	*Vampyrodes major*	Stenodermatinae	F	closed
29.03.2015	Panama	8	*Carollia castanea*	Carolliinae	M	open
29.03.2015	Panama	9	*Carollia castanea*	Carolliinae	M	open
29.03.2015	Panama	10	*Artibeus literatus*	Stenodermatinae	F	closed
03.04.2015	Panama	11	*Artibeus jamaicensis*	Stenodermatinae	M	closed
03.04.2015	Panama	12	*Chiroderma villosum*	Stenodermatinae	M	closed
03.04.2015	Panama	13	*Chiroderma villosum*	Stenodermatinae	M	closed
06.04.2015	Panama	14	*Carollia castanea*	Carolliinae	F	open
06.04.2015	Panama	16	*Vampyrodes major*	Stenodermatinae	M	closed
06.04.2015	Panama	17	*Carollia perspicillata*	Carolliinae	M	open
06.04.2015	Panama	18	*Phyllostomus hastatus*	Phyllostominae	F	closed
08.04.2015	Panama	25	*Desmodus rotundus*	Desmodontinae	M	open
11.04.2015	Panama	26	*Platyrrhinus helleri*	Stenodermatinae	M	closed
20.04.2015	Panama	44	*Tonatia saurophila*	Phyllostominae	F	open
20.04.2015	Panama	45	*Artibeus literatus*	Stenodermatinae	M	closed
22.04.2015	Panama	52	*Artibeus jamaicensis*	Stenodermatinae	F	open/closed
22.04.2015	Panama	53	*Carollia castanea*	Carolliinae	F	open
22.04.2015	Panama	54	*Artibeus literatus*	Stenodermatinae	F	closed
22.04.2015	Panama	55	*Lophostoma silvicolum*	Phyllostominae	M	open
24.04.2015	Panama	63	*Carollia castanea*	Carolliinae	M	open
24.04.2015	Panama	64	*Artibeus ravus*	Stenodermatinae	F	closed
24.04.2015	Panama	65	*Artibeus ravus*	Stenodermatinae	F	closed
24.04.2015	Panama	66	*Artibeus ravus*	Stenodermatinae	F	closed
24.04.2015	Panama	70	*Gardnerycteris crenulatum*	Phyllostominae	M	closed
01.05.2015	Panama	71	*Lampronycteris brachyotis*	Micronycterinae	F	open
01.05.2015	Panama	72	*Carollia castanea*	Carolliinae	M	open
01.05.2015	Panama	73	*Artibeus jamaicensis*	Stenodermatinae	F	closed
01.05.2015	Panama	74	*Artibeus jamaicensis*	Stenodermatinae	F	closed
01.05.2015	Panama	75	*Artibeus jamaicensis*	Stenodermatinae	F	closed
01.05.2015	Panama	78	*Artibeus jamaicensis*	Stenodermatinae	M	closed
01.05.2015	Panama	80	*Artibeus jamaicensis*	Stenodermatinae	M	closed
01.05.2015	Panama	81	*Artibeus jamaicensis*	Stenodermatinae	F	closed
04.05.2015	Panama	82	*Artibeus watsoni*	Stenodermatinae	F	closed
04.05.2015	Panama	84	*Lophostoma silvicolum*	Phyllostominae	M	open
04.05.2015	Panama	85	*Artibeus literatus*	Stenodermatinae	F	closed
06.05.2015	Panama	88	*Tonatia saurophila*	Phyllostominae	M	open
06.05.2015	Panama	89	*Tonatia saurophila*	Phyllostominae	M	open

date	country	bat_id	species	subfamily	sex	mouth_posture
06.05.2015	Panama	90	*Phyllostomus discolor*	Phyllostominae	M	closed
06.05.2015	Panama	91	*Phyllostomus discolor*	Phyllostominae	F	closed
06.05.2015	Panama	92	*Phyllostomus discolor*	Phyllostominae	F	closed
06.05.2015	Panama	93	*Phyllostomus discolor*	Phyllostominae	F	closed
06.05.2015	Panama	94	*Phyllostomus discolor*	Phyllostominae	M	closed
09.05.2015	Panama	95	*Artibeus watsoni*	Stenodermatinae	F	closed
09.05.2015	Panama	96	*Platyrrhinus helleri*	Stenodermatinae	F	closed
09.05.2015	Panama	98	*Carollia castanea*	Carolliinae	M	open
13.05.2015	Panama	101	*Artibeus jamaicensis*	Stenodermatinae	M	closed
13.05.2015	Panama	102	*Lophostoma silvicolum*	Phyllostominae	F	open
13.05.2015	Panama	103	*Tonatia saurophila*	Phyllostominae	M	open
13.05.2015	Panama	104	*Vampyrodes major*	Stenodermatinae	M	closed
13.05.2015	Panama	114	*Artibeus literatus*	Stenodermatinae	F	closed
13.05.2015	Panama	115	*Artibeus literatus*	Stenodermatinae	M	closed
23.05.2015	Panama	117	*Carollia perspicillata*	Carolliinae	F	open
23.05.2015	Panama	123	*Artibeus literatus*	Stenodermatinae	M	closed
03.06.2015	Panama	130	*Artibeus watsoni*	Stenodermatinae	M	closed
03.06.2015	Panama	131	*Artibeus watsoni*	Stenodermatinae	F	closed
03.06.2015	Panama	132	*Uroderma bilobatum*	Stenodermatinae	M	closed
05.06.2015	Panama	133	*Uroderma bilobatum*	Stenodermatinae	F	closed
05.06.2015	Panama	134	*Artibeus ravus*	Stenodermatinae	M	closed
06.06.2015	Panama	135	*Lophostoma silvicolum*	Phyllostominae	F	open
06.06.2015	Panama	136	*Artibeus watsoni*	Stenodermatinae	M	closed
11.06.2015	Panama	137	*Uroderma bilobatum*	Stenodermatinae	M	closed
11.06.2015	Panama	139	*Uroderma bilobatum*	Stenodermatinae	M	closed
11.06.2015	Panama	140	*Glossophaga soricina*	Glossophaginae	F	open
17.06.2015	Panama	141	*Micronycteris hirsuta*	Micronycterinae	F	open
22.06.2015	Panama	142	*Carollia castanea*	Carolliinae	M	open
22.06.2015	Panama	143	*Carollia perspicillata*	Carolliinae	F	open
22.06.2015	Panama	144	*Vampyressa thyone*	Stenodermatinae	F	closed
22.06.2015	Panama	145	*Carollia perspicillata*	Carolliinae	M	open
22.06.2015	Panama	146	*Carollia perspicillata*	Carolliinae	M	open
25.06.2015	Panama	147	*Artibeus ravus*	Stenodermatinae	F	closed
25.06.2015	Panama	148	*Glossophaga soricina*	Glossophaginae	M	open
30.06.2015	Panama	149	*Phyllostomus hastatus*	Phyllostominae	F	closed
30.06.2015	Panama	150	*Phyllostomus hastatus*	Phyllostominae	M	closed
30.06.2015	Panama	151	*Phyllostomus hastatus*	Phyllostominae	F	closed
01.07.2015	Panama	152	*Glossophaga soricina*	Glossophaginae	F	open
01.07.2015	Panama	153	*Artibeus watsoni*	Stenodermatinae	F	closed
03.07.2015	Panama	154	*Micronycteris microtis*	Micronycterinae	F	open
03.07.2015	Panama	155	*Gardnerycteris crenulatum*	Phyllostominae	M	open/closed
03.07.2015	Panama	156	*Trinycteris nicefori*	Phyllostominae	F	open
06.07.2015	Panama	157	*Tonatia saurophila*	Phyllostominae	F	open
06.07.2015	Panama	158	*Trachops cirrhosus*	Phyllostominae	M	open
12.07.2015	Panama	159	*Desmodus rotundus*	Desmodontinae	F	open
12.07.2015	Panama	160	*Desmodus rotundus*	Desmodontinae	F	open
12.07.2015	Panama	161	*Desmodus rotundus*	Desmodontinae	F	open

date	country	bat_id	species	subfamily	sex	mouth_posture
12.07.2015	Panama	162	*Desmodus rotundus*	Desmodontinae	M	open
12.07.2015	Panama	163	*Desmodus rotundus*	Desmodontinae	M	open
12.07.2015	Panama	164	*Desmodus rotundus*	Desmodontinae	F	open
18.09.2017	Peru	232	*Gardnerycteris crenulatum*	Phyllostominae	M	closed
18.09.2017	Peru	236	*Phyllostomus elongatus*	Phyllostominae	M	closed
18.09.2017	Peru	240	*Trachops cirrhosus*	Phyllostominae	M	open
20.09.2017	Peru	253	*Artibeus anderseni*	Stenodermatinae	M	closed
20.09.2017	Peru	254	*Uroderma bilobatum*	Stenodermatinae	M	closed
20.09.2017	Peru	256	*Trachops cirrhosus*	Phyllostominae	M	open
20.09.2017	Peru	257	*Artibeus obscurus*	Stenodermatinae	M	closed
20.09.2017	Peru	258	*Artibeus planirostris*	Stenodermatinae	M	open/closed
20.09.2017	Peru	259	*Artibeus obscurus*	Stenodermatinae	M	closed
21.09.2017	Peru	267	*Carollia brevicauda*	Carolliinae	M	open
21.09.2017	Peru	268	*Platyrrhinus infuscus*	Stenodermatinae	F	closed
21.09.2017	Peru	269	*Trachops cirrhosus*	Phyllostominae	F	open
21.09.2017	Peru	271	*Trachops cirrhosus*	Phyllostominae	M	open
21.09.2017	Peru	272	*Trachops cirrhosus*	Phyllostominae	M	open
24.09.2017	Peru	285	*Uroderma magnirostrum*	Stenodermatinae	M	closed
24.09.2017	Peru	289	*Sturnira tildae*	Stenodermatinae	F	open
24.09.2017	Peru	290	*Sturnira tildae*	Stenodermatinae	M	open
24.09.2017	Peru	291	*Sturnira tildae*	Stenodermatinae	F	open
25.09.2017	Peru	292	*Platyrrhinus infuscus*	Stenodermatinae	M	closed
25.09.2017	Peru	294	*Hsunycteris thomasi*	Lonchophyllinae	F	open
25.09.2017	Peru	295	*Artibeus planirostris*	Stenodermatinae	F	closed
25.09.2017	Peru	296	*Phyllostomus elongatus*	Phyllostominae	M	closed
25.09.2017	Peru	297	*Lophostoma silvicolum*	Phyllostominae	M	open
25.09.2017	Peru	298	*Lophostoma silvicolum*	Phyllostominae	F	open
25.09.2017	Peru	299	*Artibeus planirostris*	Stenodermatinae	M	closed
27.09.2017	Peru	300	*Artibeus planirostris*	Stenodermatinae	F	closed
28.09.2017	Peru	301	*Platyrrhinus infuscus*	Stenodermatinae	F	closed
28.09.2017	Peru	302	*Sturnira tildae*	Stenodermatinae	M	open
28.09.2017	Peru	303	*Carollia brevicauda*	Carolliinae	F	open
28.09.2017	Peru	304	*Carollia brevicauda*	Carolliinae	M	open
28.09.2017	Peru	305	*Carollia brevicauda*	Carolliinae	M	open
28.09.2017	Peru	306	*Artibeus planirostris*	Stenodermatinae	M	closed
29.09.2017	Peru	309	*Platyrrhinus incarum*	Stenodermatinae	M	closed
29.09.2017	Peru	310	*Platyrrhinus incarum*	Stenodermatinae	M	closed
29.09.2017	Peru	311	*Artibeus obscurus*	Stenodermatinae	M	closed
29.09.2017	Peru	314	*Platyrrhinus incarum*	Stenodermatinae	M	closed
15.06.2016	Panama	169	*Artibeus jamaicensis*	Stenodermatinae	M	open/closed
15.06.2016	Panama	170	*Artibeus jamaicensis*	Stenodermatinae	M	open/closed
15.06.2016	Panama	171	*Artibeus jamaicensis*	Stenodermatinae	M	closed
24.06.2016	Panama	172	*Lampronycteris brachyotis*	Micronycterinae	M	open
24.06.2016	Panama	173	*Chrotopterus auritus*	Phyllostominae	M	open
29.06.2016	Panama	176	*Lophostoma silvicolum*	Phyllostominae	M	open
29.06.2016	Panama	177	*Carollia perspicillata*	Carolliinae	M	open
29.06.2016	Panama	178	*Artibeus jamaicensis*	Stenodermatinae	M	closed

date	country	bat_id	species	subfamily	sex	mouth_posture
29.06.2016	Panama	179	*Artibeus jamaicensis*	Stenodermatinae	M	closed
05.06.2016	Panama	180	*Desmodus rotundus*	Desmodontinae	M	open
06.06.2016	Panama	181	*Desmodus rotundus*	Desmodontinae	M	open
06.06.2016	Panama	182	*Desmodus rotundus*	Desmodontinae	M	open
06.06.2016	Panama	183	*Desmodus rotundus*	Desmodontinae	M	open
06.06.2016	Panama	184	*Desmodus rotundus*	Desmodontinae	M	open
06.06.2016	Panama	186	*Desmodus rotundus*	Desmodontinae	M	open
06.06.2016	Panama	187	*Desmodus rotundus*	Desmodontinae	M	open
07.07.2016	Panama	189	*Lonchorhina aurita*	Lonchorhininae	F	open
07.07.2016	Panama	190	*Lonchorhina aurita*	Lonchorhininae	M	closed
07.07.2016	Panama	191	*Lonchorhina aurita*	Lonchorhininae	M	closed
07.07.2016	Panama	192	*Lonchorhina aurita*	Lonchorhininae	M	open
07.07.2016	Panama	194	*Lonchorhina aurita*	Lonchorhininae	M	open
08.06.2016	Panama	196	*Desmodus rotundus*	Desmodontinae	M	open
08.06.2016	Panama	197	*Desmodus rotundus*	Desmodontinae	M	open
08.06.2016	Panama	198	*Desmodus rotundus*	Desmodontinae	M	open
08.06.2016	Panama	199	*Desmodus rotundus*	Desmodontinae	M	open
08.06.2016	Panama	200	*Desmodus rotundus*	Desmodontinae	M	open
08.06.2016	Panama	201	*Desmodus rotundus*	Desmodontinae	M	open
08.06.2016	Panama	202	*Desmodus rotundus*	Desmodontinae	M	open
17.07.2016	Panama	206	*Phyllostomus hastatus*	Phyllostominae	F	closed
17.07.2016	Panama	207	*Phyllostomus hastatus*	Phyllostominae	M	closed
17.07.2016	Panama	208	*Phyllostomus hastatus*	Phyllostominae	F	closed
17.07.2016	Panama	209	*Phyllostomus hastatus*	Phyllostominae	F	closed
17.07.2016	Panama	210	*Phyllostomus hastatus*	Phyllostominae	F	closed
21.08.2018	Peru	G3031	*Gardnerycteris crenulatum*	Phyllostominae	F	open/closed
21.08.2018	Peru	G2034	*Sturnira giannae*	Stenodermatinae	M	open
22.08.2018	Peru	G4026	*Phyllostomus elongatus*	Phyllostominae	M	closed
22.08.2018	Peru	G4036	*Chrotopterus auritus*	Phyllostominae	M	closed
22.08.2018	Peru	G4038	*Rhinophylla pumilio*	Carolliinae		open
22.08.2018	Peru	G4035	*Glyphonycteris daviesi*	Phyllostominae		open
23.08.2018	Peru	G4055	*Trinycteris nicefori*	Phyllostominae	M	open
24.08.2018	Peru	G4069	*Hsunycteris thomasi*	Lonchophyllinae	M	open
24.08.2018	Peru	G4074	*Sturnira giannae*	Stenodermatinae	F	open
25.08.2018	Peru	G4089	*Anoura caudifer*	Glossophaginae	M	open
25.08.2018	Peru	G4110	*Hsunycteris thomasi*	Lonchophyllinae	M	open
26.08.2018	Peru	G4135	*Platyrrhinus infuscus*	Stenodermatinae		closed
27.08.2018	Peru	G6032	*Sturnira giannae*	Stenodermatinae	M	open
27.08.2018	Peru	G6035	*Artibeus gnomus*	Stenodermatinae	F	open
27.08.2018	Peru	G6033	*Chrotopterus auritus*	Phyllostominae	M	open
27.08.2018	Peru	G4168	*Chrotopterus auritus*	Phyllostominae		open
27.08.2018	Peru	G6021	*Hsunycteris thomasi*	Lonchophyllinae		open
12.04.2019	Panama	704	*Lonchorhina aurita*	Lonchorhininae	M	open

ii.Gape and head position angle.

mouth posture	species individuals	id	gape angle	head position angle
closed	*Artibeus anderseni*	253	0.0	141
closed	*Artibeus jamaicensis*	75	0.0	146
closed	*Artibeus jamaicensis*	167	0.0	139
closed	*Artibeus jamaicensis*	169	0.0	148
closed	*Artibeus jamaicensis*	171	0.0	147
closed	*Artibeus jamaicensis*	178	0.0	124
closed	*Artibeus jamaicensis*	179	0.0	137
closed	*Artibeus lituratus*	114	0.0	130
closed	*Artibeus lituratus*	115	0.0	137
closed	*Artibeus obscurus*	257	0.0	142
closed	*Artibeus obscurus*	259	0.0	146
closed	*Artibeus obscurus*	311	0.0	155
closed	*Artibeus planirostris*	258	0.0	136
closed	*Artibeus planirostris*	295	0.0	137
closed	*Artibeus planirostris*	299	0.0	143
closed	*Artibeus planirostris*	300	0.0	154
closed	*Artibeus planirostris*	306	0.0	141
closed	*Chiroderma villosum*	13	0.0	146
closed	*Chrotopterus auritus*	G4036	0.0	137
closed	*Dermanura watsoni*	95	0.0	140
closed	*Dermanura watsoni*	147	0.0	146
closed	*Gardnerycteris crenulatum*	70	0.0	139
closed	*Gardnerycteris crenulatum*	232	0.0	135
closed	*Gardnerycteris crenulatum*	G3031	0.0	147
closed	*Lonchorhina aurita*	190	0.0	152
closed	*Lonchorhina aurita*	191	0.0	153
closed	*Phyllostomus discolor*	91	0.0	143
closed	*Phyllostomus discolor*	92	0.0	141
closed	*Phyllostomus discolor*	93	0.0	136
closed	*Phyllostomus elongatus*	236	0.0	135
closed	*Phyllostomus elongatus*	296	0.0	147
closed	*Phyllostomus hastatus*	149	0.0	147
closed	*Phyllostomus hastatus*	206	0.0	143
closed	*Phyllostomus hastatus*	208	0.0	146
closed	*Phyllostomus hastatus*	210	0.0	141
closed	*Platyrrhinus helleri*	96	0.0	151
closed	*Platyrrhinus incarum*	309	0.0	136
closed	*Platyrrhinus incarum*	310	0.0	149
closed	*Platyrrhinus infuscus*	268	0.0	143
closed	*Platyrrhinus infuscus*	292	0.0	152
closed	*Uroderma bilobatum*	132	0.0	150
closed	*Uroderma bilobatum*	254	0.0	139
closed	*Uroderma magnirostrum*	285	0.0	151
closed	*Vampyressa thyone*	144	0.0	157
closed	*Vampyrodes major*	104	0.0	147
open	*Anoura caudifer*	G4089	34.5	180
open	*Artibeus gnomus*	G6035	18.6	147
open	*Artibeus jamaicensis*	169	20.5	143
open	*Artibeus jamaicensis*	170	21.8	151
open	*Carollia brevicauda*	267	30.1	157
open	*Carollia brevicauda*	303	34.0	149
open	*Carollia brevicauda*	304	39.1	153
open	*Carollia brevicauda*	305	37.4	157
open	*Carollia castanea*	14	41.3	150

mouth posture	species individuals	id	gape angle	head position angle
open	*Carollia castanea*	9	22.3	143
open	*Carollia castanea*	98	38.1	164
open	*Carollia perspicillata*	17	37.9	152
open	*Carollia perspicillata*	143	34.6	163
open	*Carollia perspicillata*	177	33.9	155
open	*Chrotopterus auritus*	173	28.4	141
open	*Chrotopterus auritus*	G4168	28.7	136
open	*Chrotopterus auritus*	G6033	24.2	138
open	*Desmodus rotundus*	161	46.7	175
open	*Desmodus rotundus*	181	35.7	185
open	*Desmodus rotundus*	182	63.9	194
open	*Desmodus rotundus*	183	50.7	187
open	*Desmodus rotundus*	199	63.2	189
open	*Desmodus rotundus*	202	70.6	186
open	*Desmodus rotundus*	184b	49.3	168
open	*Gardnerycteris crenulatum*	155	32.9	147
open	*Glossophaga soricina*	148	28.6	
open	*Glyphonycteris daviesi*	G4035	32.1	159
open	*Hsunycteris thomasi*	294	39.6	165
open	*Hsunycteris thomasi*	G4069	24.9	163
open	*Hsunycteris thomasi*	G4110	26.5	156
open	*Hsunycteris thomasi*	G6021	21.7	162
open	*Lampronycteris brachyotis*	71	33.6	158
open	*Lampronycteris brachyotis*	172	33.0	163
open	*Lonchorhina aurita*	189	49.6	159
open	*Lonchorhina aurita*	192	36.1	154
open	*Lonchorhina aurita*	194	33.1	152
open	*Lonchorhina aurita*	704	20.9	178
open	*Lophostoma silvicolum*	135	37.3	173
open	*Lophostoma silvicolum*	176	43.5	167
open	*Lophostoma silvicolum*	298	46.7	161
open	*Micronycteris hirsuta*	141	36.8	159
open	*Micronycteris microtis*	154	33.5	178
open	*Rhinophylla pumilio*	G4038	35.9	176
open	*Sturnira giannae*	G4074	26.5	154
open	*Sturnira giannae*	G6032	24.8	144
open	*Sturnira tildae*	289	33.7	151
open	*Sturnira tildae*	290	37.0	146
open	*Sturnira tildae*	291	36.1	147
open	*Sturnira tildae*	302	27.3	151
open	*Tonatia saurophila*	88	30.6	161
open	*Tonatia saurophila*	103	32.1	162
open	*Trachops cirrhosus*	158	35.1	157
open	*Trachops cirrhosus*	256	37.0	149
open	*Trachops cirrhosus*	269	40.5	145
open	*Trachops cirrhosus*	271	41.0	161
open	*Trachops cirrhosus*	272	42.9	157
open	*Trinycteris nicefori*	156	22.5	164
open	*Trinycteris nicefori*	G4055	26.9	154

iii. mouth posture of L. aurita during roost emergence.

continuation of table

photograph #	mouth posture	photograph #	mouth posture	photograph #	mouth posture
9510	closed	9577	closed	9628	open
9516	open	9579	open	9629	closed
9517	open	9581	open	9631	closed
9521	open	9582	open	9633	closed
9523	open	9583	open	9635	closed
9525	closed	9586	open	9639	closed
9529	closed	9587	open	9641	closed
9532	open	9589	closed	9642	closed
9539	open	9591	closed	9644	closed
9540	closed	9592	closed	9645	closed
9541	closed	9594	open	9646	closed
9544	open	9595	open	9647	closed
9546	closed	9597	open	9651	closed
9548	open	9599	closed	9656	closed
9551	open	9601	closed	9657	closed
9554	closed	9603	open	9658	closed
9555	open	9605	closed	9659	open
9560	closed	9608	open	9660	closed
9564	open	9609	open	9662	open
9565	closed	9610	closed	9663	open
9569	open	9615	open	9664	closed
9570	open	9620	closed	9666	open
9571	closed	9622	closed	9668	open
9573	open	9626	closed	9670	open
9574	closed	9627	closed	9671	closed

Supplementary Data 2: Example of angle measurements and their digitalization errors (Glyphonycteris daviesi). Based on Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/, https://www.frontiersin.org/journals/ecology-and-evolution/articles/10.3389/fevo.2021.630481/full#supplementary-material



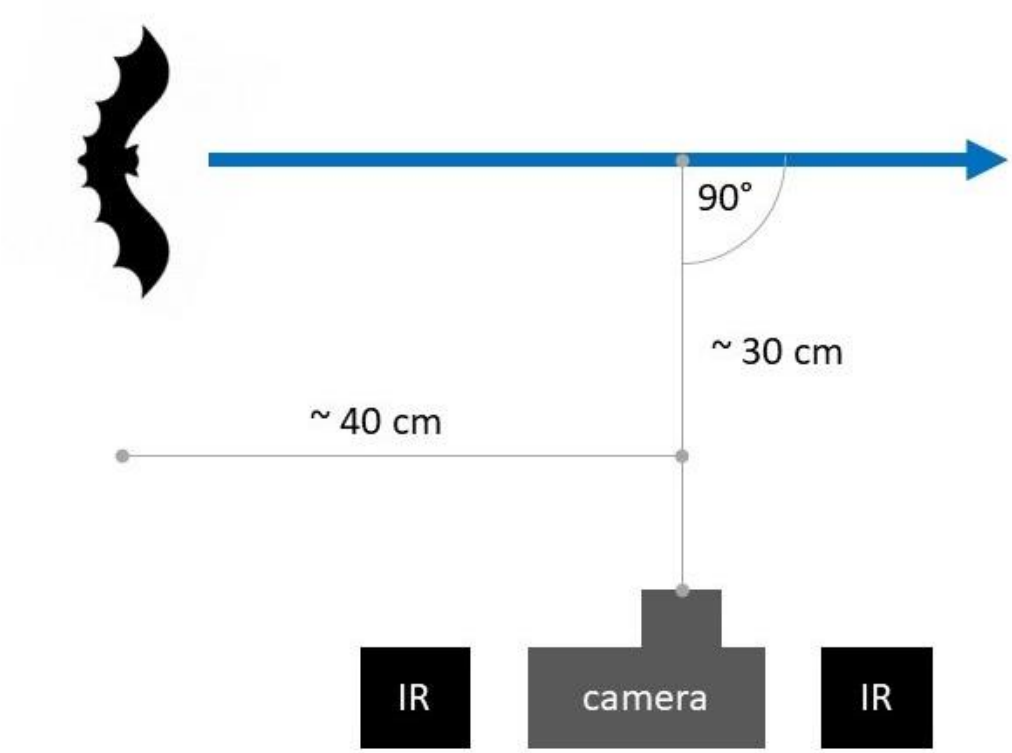
Mouth gape		Head angle	
15 measurements taken		15 measurements taken	
1	31.8	1	160.1
2	30.6	2	159.0
3	30.3	3	158.3
4	28.7	4	157.8
5	33.7	5	157.4
6	34.2	6	156.8
7	33.7	7	161.4
8	30.4	8	158.2
9	32.4	9	159.5
10	31.2	10	163.2
11	35.0	11	158.4
12	33.8	12	160.2
13	32.8	13	162.5
14	32.0	14	159.6
15	34.5	15	157.4
mean	32.4	mean	159.3
standard deviation	1.85	standard deviation	1.91
3 measurements taken		3 measurements taken	
1	33.4	1	157.4
2	32.7	2	157.7
3	29.3	3	161.4
mean	31.8	mean	158.8
standard deviation	2.21	standard deviation	2.24

Supplementary Data 3: Genbank sequence IDs of the sequences used for reconstructing the phylogenetic tree, modified from Rojas et al. (2016) and pers comm. Paul Velazco. Based on Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0:

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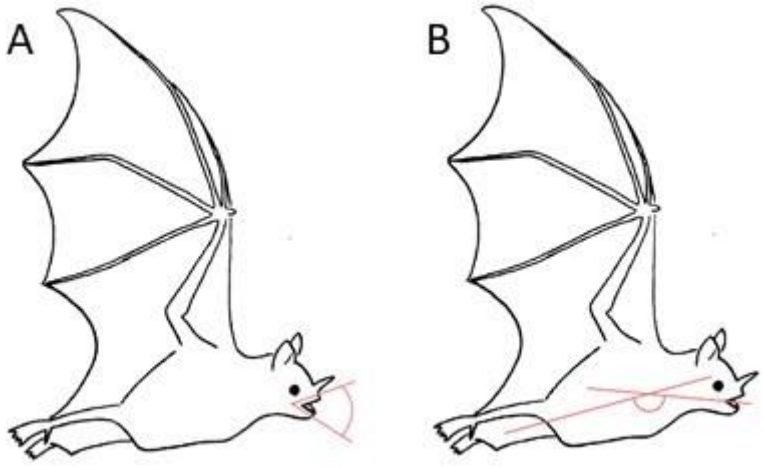
Species	mouth posture	*atp7a*	*bdnf*	*plcb4*	*rag2*	*stat5A*	*thy*	*ttn6*	*coxI*	*cyt-b*	*12S, tRNA-val, 16S*
Artibeus watsoni	x	KF569416	KF569465	KF569312	KF569341	KF569364	KF569441	KF569385	JF459377	U66516	FJ179205
Rhinophylla pumilio	o	KC783047	KC782995	KC783104	AF316484	KC783207	EU371960	KC783167	EF080598	AF187031	AY395827
Carollia perspicillata	o	KC783012	KC782959	KC783065	KC783114	KC783177	KC783218	KC783130	EF080211	FJ589715	AY395836
Glyphonycteris daviesi	o	KC783019	KC782966	KC783073	AF316464	KC783183	KC783224	KC783137	EF080364	AY380747	AY395812
Hsunycteris thomasi	o	KC783021	KC782969	KC783076	AF316456	KC783186	KC783227	KC783140	EF080377	AF423086	AY395842
Lonchorhina aurita	b	KC783022	KC782970	KC783077	AF316457	KM362087	KC783228	KC783141	JF435491	FJ155494	AY395843
Anoura caudifer	o	KC783005	KC782952	KC783058	KC783112	KF569360	KC783214	KC783123	EF079981	L19506	AY395835
Gardnerycteris crenulatum	b	KC783035	KF569475	KC783091	AF316472	KY129856	KF569458	KC783155	EU096781	FJ155478	AF411543
Tonatia saurophila	o	KC783049	KC782998	KC783107	AF442086	KC783210	KC783250	KC783169	EF080734	FJ155488	AF411530
Chrotopterus auritus	b	KC783014	KC782961	KC783067	DQ903851	KC783178	KC783220	KC783132	EF080303	FJ155481	AF411538
Micronycteris hirsuta	o	KC783029	KC782978	KC783084	AF316466	KC783192	KC783235	KC783149	EF080447	AY380769	AY395819
Lampronycteris brachyotis	o	KC783020	KC782967	KC783074	AF316463	KC783184	KC783225	KC783138	EF080370	AY380748	AF411536
Artibeus jamaicensis	b	-	KC782954	KC783060	AY834663	KC783174	KC783215	KC783125	AF061340	DQ869518	AF061340
Artibeus anderseni	x	KF569411	KF569460	KF569311	-	KF569363	KF569439	KF569384	JF448543	U66509	FJ179197
Platyrrhinus helleri	x	KC783043	KC782991	KC783100	FJ154339	KC783204	KC783244	KC783163	EF080579	FJ154140	-
Vampyressa thyone	x	KF569437	KF569483	KF569337	KF569358	KF569379	KF569448	KF569408	JF449316	DQ312429	-
Uroderma magnirostrum	x	KF569435	KF569481	KF569335	KF569356	KF569377	KF569449	KF569407	JF456032	AY169957	-
Sturnira tildae	o	KF569434	KF569480	KF569331	DQ903847	KF569375	KF569452	KF569404	EF080704	DQ903816	-
Carollia castanea	o	KF569417	KF569466	KF569314	FN641676	KF569366	KF569450	KF569388	JF447989	AF512006	-
Trinycteris nicefori	o	KC783051	KC783000	KC783108	-	KC783211	KC783252	KC783170	EF080784	AY380749	AY395830

Species	mouth posture	*atp7a*	*bdnf*	*plcb4*	*rag2*	*stat5A*	*thy*	*ttn6*	*coxI*	*cyt-b*	*12S, tRNA-val, 16S*
Lophostoma silvicolum laephotis	o	KC783026	KC782975	KF569323	AF442083	-	KC783232	KF569397	HQ919730	FJ155492	AF263230
Phyllostomus hastatus	x	KC783042	-	KC783099	AF316479	KM362096	KC783243	KC783162	EF080556	FJ155479	AF411541
Desmodus rotundus	o	KC783015	KC782962	KC783068	AF316444	KC783179	KC783221	KC783133	JF435307	-	AF263228
Micronycteris microtis	o	KC783032	KC782981	KC783087	-	KC783194	KC783237	KC783152	HQ625008	AY380756	AY395822
Artibeus obscurus	x	KC783009	KC782956	KC783062	-	-	KC783217	KC783127	EF080106	AY684768	AY395805
Artibeus lituratus	x	KC783008	KC782955	KC783061	DQ985530	-	KC783216	KC783126	EF080083	AY684740	-
Artibeus gnomus	o	KC783006	KC782953	KC783059	-	KC783173	-	KC783124	JF452461	EU160957	FJ179209
Uroderma bilobatum	x	KC783052	KC783001	KC783109	AF316491	-	-	KC783171	EF080788	AY169955	AY395831
Sturnira giannae	o	KC783048	KC782996	KC783105	KC754300	KC783208	KC783248	-	EF080684	KC753832	-
Glossophaga soricina	o	KC783018	-	KC783072	AF316452	KC783182	-	KC783136	EF080360	AF423081	AY395840
Trachops cirrhosus	o	KC783050	KC782999	-	AF316490	AJ865422	KC783251	-	EF080747	FJ155483	AF411539
Platyrrhinus incarum	x	KM361957	-	KM362033	FJ154344	KM362099	KM362133	KM362163	-	FJ154147	-
Carollia brevicauda	o	KC783011	KC782958	KC783064	-	KC783176	-	KC783129	JF453684	AF511951	-
Phyllostomus discolor	x	KF569427	KF569476	KF569325	KM362066	KF569373	-	KF569400	EF080546	-	-
Platyrrhinus infuscus	x	-	-	KF569326	KF569350	-	-	KF569401	JF444938	FJ154148	-
Phyllostomus elongatus	x	KC783041	-	KC783098	-	KC783203	-	KC783161	EF080551	-	-
Chiroderma villosum	x	-	-	-	AF316439	-	EU371975	-	JF454581	L28943	-
Artibeus planirostris	b	-	-	-	-	-	-	-	JF459102	AY642920	U26287
Lophostoma silvicolum centralis	o	-	-	-	AF442082	-	-	-	-	FJ155493	AF442092
Vampyrodes major	x	-	-	-	-	-	-	-	-	HQ637422	-



Supplementary Figure 1: Setup for high speed video recordings: bat illustration indicating the point of release, blue error indicating the anticipated, ideal flight path of the bat, infrared lights (IR) and high-speed camera (camera) located at ca. 30 cm at a 90° angle on a parallel line at ca. 40 cm distance to point of release. Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0:

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Supplementary Figure 2: Schematic drawings illustrating the measured angles: A) two axes were drawn along the upper (palate) and lower (dentary) jawbone and the gape angle measured in between; B) a line along the main body axis was drawn and crossed with a second line drawn between the upper and lower jawbone (angle dissector of the gape angle) and the head position angle was measured. Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0:
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Supplementary Figure 3: Examples of Lonchorhina aurita photographed during roost emergence. In 41 individuals no teeth were visible and their mouth posture was scored as closed (left). 34 individuals were scored with open mouths because teeth were clearly visible (right). (Photos by MT). Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0:https://creativecommons.org/licenses/by/4.0/, https://www.frontiersin.org/journals/ecology-and-evolution/articles/10.3389/fevo.2021.630481/full#supplementary-material

Supplementary Video 1: High speed videos of flying Phyllostomid bat, slowed down by factor 10. Species: Artibeus jamaicensis, Plathyrrinus incarum, Vampyrodes major, Uroderma magnirostrum, Carollia brevicauda, Carollia perspicillata, Carollia castanea, Hsunycteris thomasi, Phyllostomus hastatus, Tonatia saurophila, Trachops cirrhosus and Lampronycteris brachyotis. Published in Gessinger et al. (2021). Creative Commons Attribution License (CC BY) 4.0: https://creativecommons.org/licenses/by/4.0/, https://www.frontiersin.org/journals/ecology-and-evolution/articles/10.3389/fevo.2021.630481/full#supplementary-material

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Chapter 2

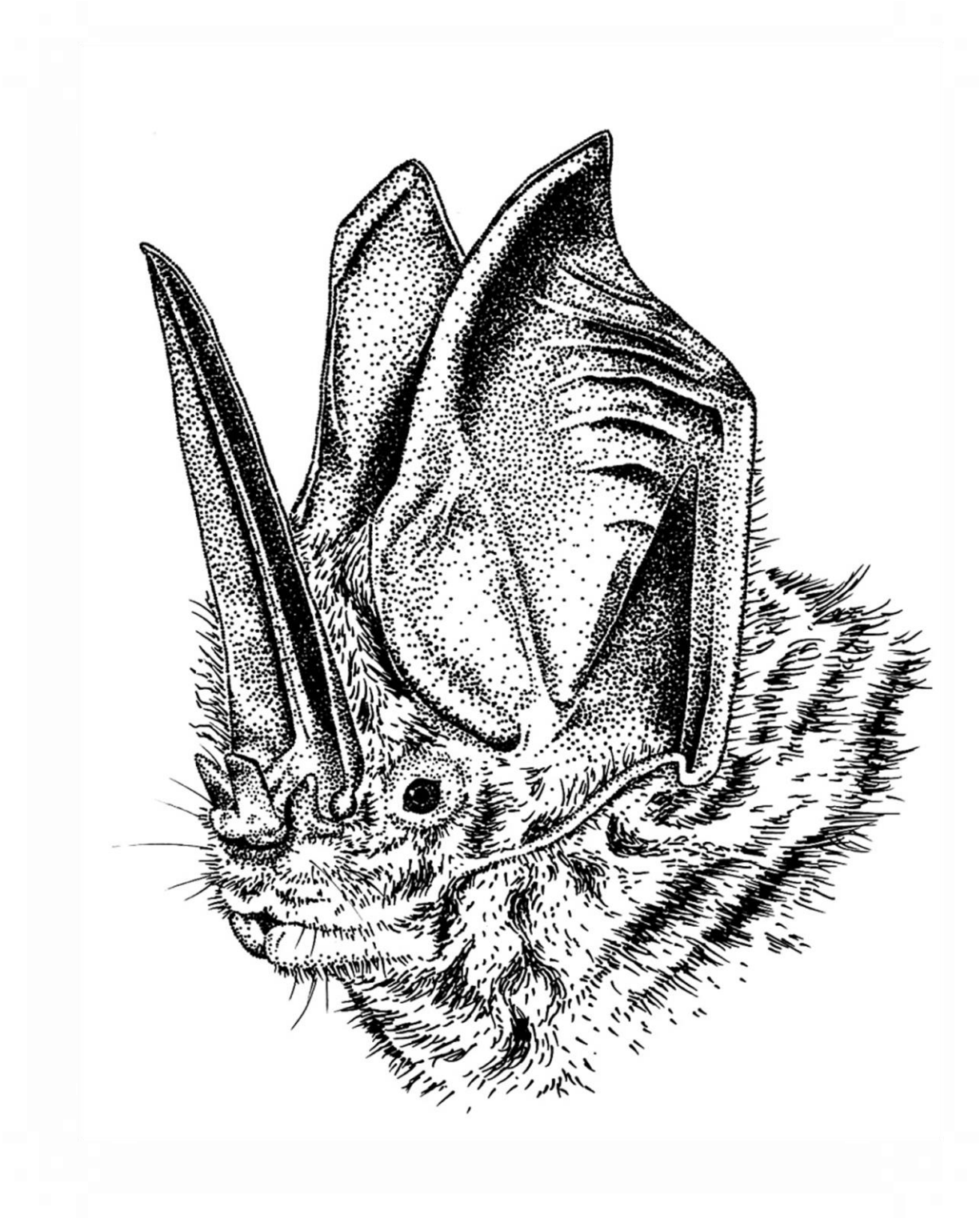


Illustration by Gloria Gessinger

Chapter 2: Aerial insectivory in a phyllostomid bat: *Lonchorhina aurita* uses unique multi pulse echolocation as well as feeding buzzes to catch airborne prey.

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Abstract

The sensory system of predatory bats is highly adapted to their respective hunting style. Gleaning bats usually possess large ears to detect prey-generated cues, while bats hunting airborne prey often use feeding buzzes, a behavioral adaptation during echolocation for successfully locating fast-moving prey in the air. *Lonchorhina aurita* belongs to the neotropical family Phyllostomidae, which comprises many gleaning species hunting in dense forest. However, its long, partially constant frequency search calls deviate distinctly from the standard phyllostomid echolocation call structure. We therefore hypothesized that *L. aurita* is no typical gleaning bat but an edge space aerial insectivore. We tested *L. aurita's* ability to catch aerial prey and predicted that its echolocation behavior during approach is adapted to foraging on aerial prey. We demonstrate that *L. aurita* readily catches aerial prey on the wing. Our analysis revealed decreases in both pulse interval and pulse duration during the approach to aerial prey. This is achieved initially by the use of unique multi pulses, a combination of CF-FM (constant frequency – frequency modulated) search calls and consecutive FM (frequency modulated) approach calls. Finally, *L. aurita* emitted classic feeding buzzes with call durations as short as 1.0 ms and pulse intervals as brief as 4.0 ms. These feeding buzzes exhibit many similarities to the feeding buzzes of edge space aerial insectivores from different bat families. Therefore, we conclude that *L. aurita* is the first phyllostomid bat reported to occupy the niche of an edge space aerial insectivore.

Introduction

Localizing and catching prey imposes many challenges on sensory and motor systems of predators because prey species generally do their best to avoid being captured. Therefore, predators show distinct adaptations to the respective prey and their hunting style. Feather morphology of owls (predator) allows for a silent flight preventing the mice (prey) from hearing the approaching danger (e.g., Wagner et al., 2017). Wolf packs (predators) show a district group hunting behaviour, allowing them to kill even prey larger then themselves (e.g., Escobedo et al., 2014).

Most species of bats (Mammalia: Chiroptera) are highly efficient predators of insects (Simmons, 2005). Bats either glean insects from the substrate or capture them in flight, using aerial hawking (Schnitzler and Kalko, 2001). These hunting strategies differ widely in their demands on the motor and sensory systems. Most gleaning bats rely mainly on prey-generated cues for prey detection and localization (Arlettaz, 1996; Bell, 1982; Fenton, 1990; Page and Ryan, 2008) and use echolocation mainly to guide the approach to the prey (Schnitzler et al., 2003). During the approach, call repetition rate is increased and calls are grouped (Barclay et al., 1981; Denzinger and Schnitzler, 2013; Schnitzler and Kalko, 2001). In contrast, aerial insectivorous bats mainly use echolocation for the detection and localization of moving airborne prey. The approach ends with a feeding buzz consisting of numerous short calls emitted at high repetition rates. The echoes from these buzzes provide rapid spatial updates, which help to track even erratically moving prey (Griffin, 1958; Griffin et al., 1960; Schnitzler and Kalko, 2001).

Phyllostomid bats (Chiroptera: Phyllostomidae) are considered the ecologically most diverse bat family, with feeding habits ranging from frugivory, over nectarivory to animalivory and sanguivory in the vampire bats (Giannini and Kalko, 2004). Most animalivorous phyllostomids are considered to be passive gleaners (Denzinger et al., 2018; Giannini and Kalko, 2004; Kalko et al., 2008). They usually have large ears that are typical for gleaning bats (Coles et al., 1989; Fleming et al., 2020; Wetterer et al., 2000), as they rely on prey-generated acoustic cues to detect prey. They forage mostly in rather dense vegetation (Denzinger et al., 2016; Giannini and Kalko, 2004). Echolocation is mainly used for obstacle avoidance and for coordinating the approach to a landing site (Kalko and Condon, 1998; Page and Ryan, 2008; Schnitzler and Kalko, 2001; Thies et al., 1998) and sounds are in many species at least partially emitted also through the mouth (Gessinger et al., 2021). Due to largely similar echolocation tasks and the close phylogenetic relationship, echolocation calls are relatively similar in structure among the species. They are short (< 2ms), multiharmonic, frequency modulated calls with broad bandwidth (Kalko, 2004; Yoh et al., 2020). Even *Macrophyllum macrophyllum*, the only edge space trawling phyllostomid species emits these call types, but shows a temporal call pattern similar to trawling or aerial hawking species from other families (Weinbeer and Kalko, 2007).

An interesting outlier among the animalivorous phyllostomids is the Sword-nosed bat *Lonchorhina aurita* (Tomes 1863). Together with its congeners it has the tallest nose leaf in the family and its echolocation call design deviates drastically from the standard phyllostomid pattern (Gessinger et al.,

2019). In search phase they use two different call types: constant frequency – frequency modulated (CF-FM) calls and pure constant frequency (CF) calls, that are unlike any other phyllostomid calls (Gessinger et al., 2019). *Lonchorhina aurita* has been reported to feed on insects (Howell and Burch, 1973; Lassieur and Wilson, 1989). Further, *Lonchorhina aurita* is a forest bat roosting in caves and artificial human structures (Bloedel, 1955; Deleva and Chaverri, 2018, Gessinger personal observation).

Lonchorhina aurita was previously assumed to be a gleaning bat, as this seems to be the main hunting style among the animalivorous phyllostomids (Denzinger and Schnitzler 2013). However, due to the unique search call structure, Gessinger et al. (2019) suggested that the echolocation system of *L. aurita* is adapted to aerial hawking.

Aim of this study was to characterize the potential foraging niche used by *Lonchorhina aurita*. The previously observed hunting habitat (smaller open areas within the forest, e.g., above streams) suggests an edge space hunting strategy, where flying prey is caught in the aerial mode. We therefore tested *L. aurita's* ability to catch aerial prey. We hypothesized that the echolocation behavior of *L. aurita* during approach is adapted to this foraging on aerial prey. Specifically, we predicted that *Lonchorhina aurita* should reduce call interval and duration when closing in to the prey target, resulting in a final feeding buzz immediately before an aerial capture attempt, as commonly seen in aerial hawking species from other families (Griffin, 1958; Griffin et al., 1960; Schnitzler and Kalko, 2001).

Therefore, we observed *Lonchorhina aurita* hunting exposed to free-flying insect prey in the controlled situation of a flight cage at the Smithsonian Tropical Research Institute in Panama. We recorded the echolocation behavior of the animals during hunting. Additionally, the echolocation behavior during landing on a stationary target was compared to prey capture echolocation.

Material and methods

Studied bats

Between January and April 2019, we caught *Lonchorhina aurita* (Chiroptera: Phyllostomidae: Lonchorhininae; Lassieur and Wilson, 1989; Rojas et al., 2016) in Soberania National Park, Gamboa, Panama and measured weight with a Pesola balance and length of forearm, tail as well as 5th and 3rd finger with calipers and a ruler. Aspect ratio index was calculated following Findley et al. (1972).

Bat behavior was observed in a 5 m x 5 m x 2.5 m outdoor flight cage located at the forest edge near the Smithsonian Tropical Research Institute in Gamboa, Panama. In order to habituate the animals to the captive situation, up to three bats at a time were kept prior to experiments in a small pop-up mosquito tent (1.8 m x 2 m, maximum height 1.5 m) inside the flight cage over one night and were hand-fed in at least three feeding sessions. As it was not possible to get sufficient natural food for the bats, we developed an artificial diet consisting of a baby food mash (Hero Baby Natur, Arroz con pollo) enriched with protein powder (Nutricia, Protifar) and vitamin paste (Trixie, TX-2578 Multivitamin paste), and either mealworms (*Tenebrio* sp.) or freshwater fish (*Tilapia* sp., modified after Barnard, 2009). This food paste was fed to the bat, using a plastic pipette. To ensure the wellbeing of the animals, bats were weighed before and after feeding sessions. Flight cage observations and different experimental trials of up to two hours per bat were conducted over up to five consecutive nights. Bats were fed at the beginning of the night and in between trials, at least three times per night. Finally, prior to release bats were fed and returned to the capture site at least two hours before sunrise.

In all experiments we followed the guidelines for the use of wild mammal species in research as recommended by the American Society of Mammalogists (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists, 2016). The work was conducted under STRI IACUC protocol no. 2017-0102-2020. Fieldwork in Panama was conducted under permits no. SE/AP-13-18 from Panama's Ministerio de Ambiente (MiAmbiente), issued to R.A.P.

Further we also recorded *Lonchorhina aurita* foraging in the field at 15 different recording sites in the Soberanía National Park on 28 nights in total, from 18:00-21:00 hrs. in July 2016, in March 2017 and between January and May 2018. We selected gaps close to known roosts or flight paths that had been previously identified through mistnetting.

Experimental setup

We observed and recorded the flight and echolocation behavior of 16 individuals hunting free-flying prey in a flight cage. Only four bats did not start to hunt in the flight cage. These animals either focused predominantly on trying to escape, or were just sitting on the walls and did not respond at all to prey released in the flight cage. Therefore, they were excluded from any further analysis and quickly released. We obtained high quality capture or capture attempt sequences from 9 bats (24 sequences). In the forest we recorded an additional 4 approach phases.

Bats were able to roam freely inside of the flight cage for up to two hours per trial. Live moths were released near a red light bulb (60 W, dimmed down to ca. 30 %) hanging from the ceiling in the center of the flight cage 150 cm above ground. The moths commonly approached and circled the light for different durations before landing on the ceiling, walls or ground. Sitting moths were encouraged to fly again. Persistently non-flying individuals were excluded from the experiment. When a bat caught a moth, we waited until the animal had finished eating. A new moth was only released as soon as the bat resumed search flight. Only occasionally several moths were flying around the light at the same time, when formerly sitting moths had started to fly again.

A preliminary diet analysis showed that in the study area *Lonchorhina aurita* is mainly foraging on moths (Kalbantner, 2018). Medium-sized moths (Lepidoptera, wingchord range 15 – 35 mm) were either caught using a light trap (superactinic neon tube, 12 V, 15 W; white cloth) in Soberanía National Park or were collected around illuminated buildings close to the forest edge in Gamboa from 19:00 to 23:00, just before the start of the experiments. Most of the moths belonged to four families: Notodontidae, Noctuidae, Erebidae, Geometridae and Saturnidae (Janzen and Hallwachs, 2009).

Recordings

Video recordings. We filmed the foraging behavior of *Lonchorhina aurita* in the flight cage, using a Sony Handycam DCR-SR72 in nightshot™ mode with an additional wide-angle lens (Opteka, 37mm, 0.45x Wide Angle Lens) positioned in a corner of the flight cage. Additional light was provided by several infrared spotlights. Landing behavior was filmed without wide-angle-lens. Before starting an experiment, we manually synchronized audio and video recordings with a custom-built trigger, which synchronously started the audio recording while an LED lit up that was also recorded in the video.

Audio recordings. The echolocation behavior was recorded with ultrasound microphones (Avisoft-Bioacoustics, CM16). In the foraging experiments we used four microphones, with three being attached to the ceiling and pointing down at the red light and one attached to the flight cage wall pointing horizontally at the red light. A single microphone was used for recording landing sequences and when recording foraging *Lonchorhina aurita* at 15 different gaps in the forest. Here, audio recordings were manually triggered at the instant of landing or when passes were observed on the computer screen in a real time spectrogram with a 3-5 second pre-trigger and a hold time of 5 seconds, respectively. Signals were digitized using a four channel Avisoft Bioacoustics UltraSoundGate (USG) 416H (foraging experiment) and a single channel USG 116Hm (landing behavior, forest recordings) (sampling rate 300 Hz, sampling rate forest recordings 500 Hz, 16 bit) and stored as wave-files (Panasonic Toughbook CF-F9 or Microsoft SurfacePro Tablet PC). Audio recordings during foraging experiments were automatically started when echolocation call intensity on any channel exceeded a threshold of 3 % above the background noise within a frequency range between 43 – 52 kHz. We selected this range as it encompasses the dominant third harmonic of the constant frequency component of *L. aurita* search calls

(Gessinger et al. 2019). Recordings were restricted to a maximum length of 60 seconds and pre-trigger was set to 3 or 5 seconds, respectively.

Landing buzzes were recorded in a small popup tent (2.0 m x 1.8 m, height 1.5 m). Bats would either fly a small circle or a straight line before landing on one of the mesh walls. We obtained the best recordings if the bats landed right in front of the handheld microphone. This way we collected 7 landing buzzes from 7 individuals.

Data analysis

Video analysis. Using the software Pinnacle Studio 23 (version 23.1.1.242 ultimate) we scored unsuccessful and successful captures. Attacks were considered unsuccessful when the bat attacked the moth but missed to catch it. Successful captures involved the bat catching the moth and generally carrying it away. Occasionally, a bat dropped a moth after a successful capture, however, this was still counted as successful, as the bat had initially successfully captured the prey.

Audio analysis. We analyzed entire capture sequences, search sequences, aborted approach sequences and one landing sequence per individual. Due to the directionality of the microphone and the bat's sound emission, the quality of the recordings varied and we selected the sequences with fewest missing calls and good signal-to-noise-ratio. From the originally recorded four channels we chose for analysis in each case the channel with the best amplitude and signal-to-noise-ratio.

Signals were displayed as color spectrograms (FFT 512, Blackman, dynamic range 60 dB) with the custom-made sound analysis software Selena (University of Tübingen, Germany). Spectrograms were plotted with a temporal resolution of 0.08 ms and a spectral resolution of 196 Hz. Signal parameters were always measured in the harmonic with the highest amplitude. Peak frequency was detected automatically and start and end of a call were defined at – 10 dB below peak amplitude (Figure 1, Table 1). We measured start and end frequency, and calculated bandwidth, pulse duration and repetition rate (calls per second). We determined the pulse interval to the previous call during terminal groups and landing buzzes, as well as within multi pulses (MPs). Multi pulses are groups of an initial CF-FM call followed by several FM calls. For FM calls duty cycle was calculated as the percentage of pulse duration divided by the pulse interval to the previous call. Additionally, we determined the pulse interval to the previous CF-FM (constant frequency – frequency modulated) call for search calls and multi pulse CF-FM calls. Consecutively, we calculated the duty cycle of these CF-FM calls by dividing the pulse duration by the pulse interval to the previous CF-FM call multiplied by 100 %.

We calculated group duration for multi pulses (MPs), feeding buzzes, and landing buzzes as follows: I. we determined the duration of the group from the beginning of the first call to the end of the last call. II. If first or last call of a group were to faint to measure start and end, we used the time of the peak frequency of the call to determine beginning and end of the group. We calculated MP group interval as the time difference between the first call of the group to the first call of next group. Spectrograms for

illustration purposes were exported from Avisoft-SASLab Pro (Version 5.2.13, 2019 by Raimund Specht) with an FFT 512 in Hamming Window with different overlap, as indicated in each figure's caption.

Statistical analysis

We used R v. 3.6.1 for all statistical analysis (R Core Team, 2019). To compare approach group (feeding buzz, landing buzz, multi pulse) call parameters we used a Kruskal-Wallis rank sum test with Dunn post-hoc test. To compare CF-FM calls of search phase and multi pulses we used a Wilcoxon rank-sum test.

We used the R package "dplyr" (Wickham et al., 2019) to prepare data for analysis and illustrated the data using "ggraptR" (Dubossarsky and Song, 2020) and "ggplot" (Wickham, 2016).

Table 1: Overview of the measured and calculated temporal call and group parameters. Illustrations in Fig. 1.

Parameter ID	Parameter name	Parameter measurement/calculation
1	CF-FM pulse interval	Start of call to start of previous CF-F/CF call
2	CF-FM pulse duration	End of call minus start of call at – 10 dB below peak amplitude
	CF-FM duty cycle	(CF-FM pulse duration / CF-FM pulse interval) *100 %
	CF-FM repetition rate	1000 / CF-FM pulse interval.
MP	Multi pulses	Groups of CF-FM calls and consecutive FM calls
3	FM pulse interval	Start of call to start of previous call
4	FM pulse duration	End of call minus start of call at – 10 dB below peak amplitude
	FM duty cycle	(FM pulse duration / FM pulse interval) *100 %
	FM repetition rate	1000 / FM pulse interval.
5	Start of group	
6	Start of next group	
7	Group interval	Start of next group minus start of group
8	Group duration	I. end of the last call minus the start of first call or II. for faint calls peak frequency time was used instead of end and start time.
	Within group duty cycle	((Sum of CF-FM call duration + Sum of FM call Duration) / (Group duration)) *100 %

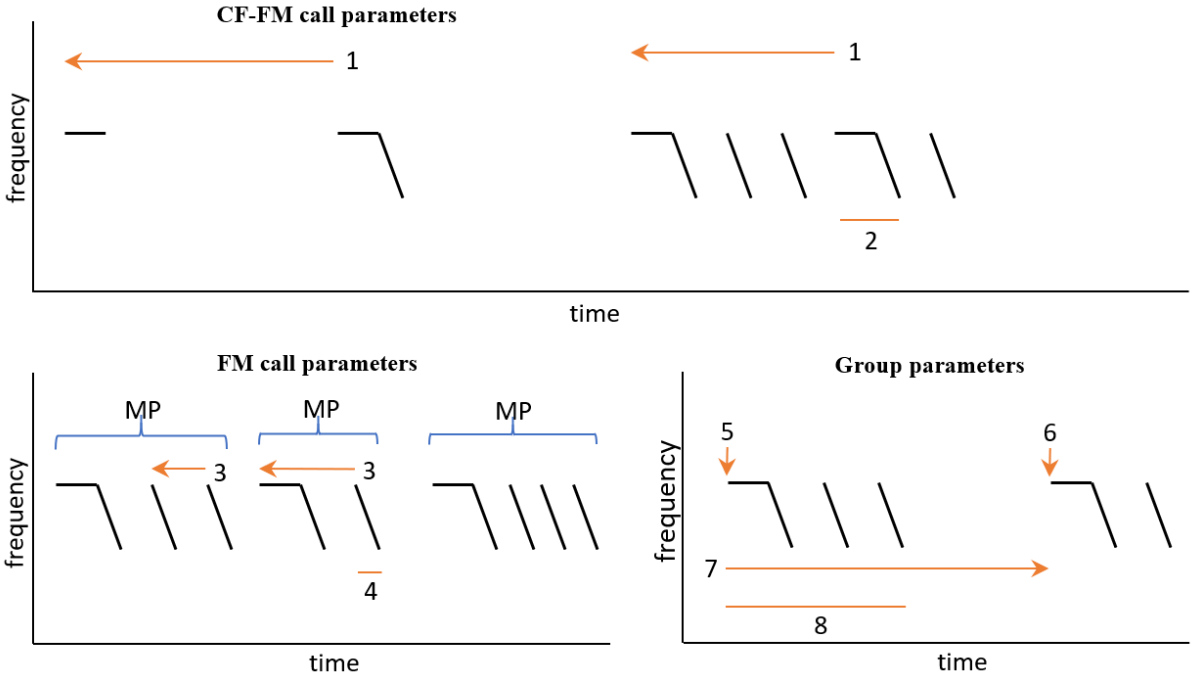


Figure 1: Schematic call sequence to illustrate measured and calculated temporal call and group parameters. For legend see Tab. 1.

Results

Morphology. *Lonchorhina aurita* individuals showed a 51 ± 1.9 mm SD long tail that is completely integrated into the uropatagium (n = 14). Mean forearm length was 51 ± 1.1 mm SD (n= 178). The wing aspect ratio index was 2.18 ± 0.071 SD (n = 61, Supplementary Table 1).

Capture behaviour. Bats captured free flying moths in the air by intercepting their flight path and following them. When closing in on the moths, bats used their wings to first guide the flying insects into their uropatagium to secure it and then seized it with the mouth. Most bats ate their prey during flight, except for a single individual who landed for eating in all trials. Moths resting on the walls or ceiling were never approached.

Hunting success in the flight cage varied widely between the individuals. Mean hunting success of all bats who caught more than two moths (6 individuals) was 48 %. Some individuals were able to immediately catch any offered moth with a hunting success of up to 71 %. Others showed many unsuccessful capture attempts with a minimum hunting success of 30 %.

Search phase echolocation. Bats were mostly flying in large circles within the flight cage, staying close to the walls, ceiling or floor while searching for prey. The most common search calls (92 % of all analysed search calls) were CF-FM (constant frequency – frequency modulated) calls (Figure 2, A). Additionally, 6 bats used CF calls that often were emitted before a series of CF-FM calls (Figure 2, B). Occasionally, the two call components, CF and FM, were clearly separated by a distinct gap, visible not only in the spectrogram but also in the oscillograms (Figure 2, C and D).

Search calls were emitted at median pulse intervals of 59.2 ms and median call duration of 4.9 ms, resulting in a median duty cycle of 6.1 % (Figure 3, Table 2, Supplementary Figure 1).

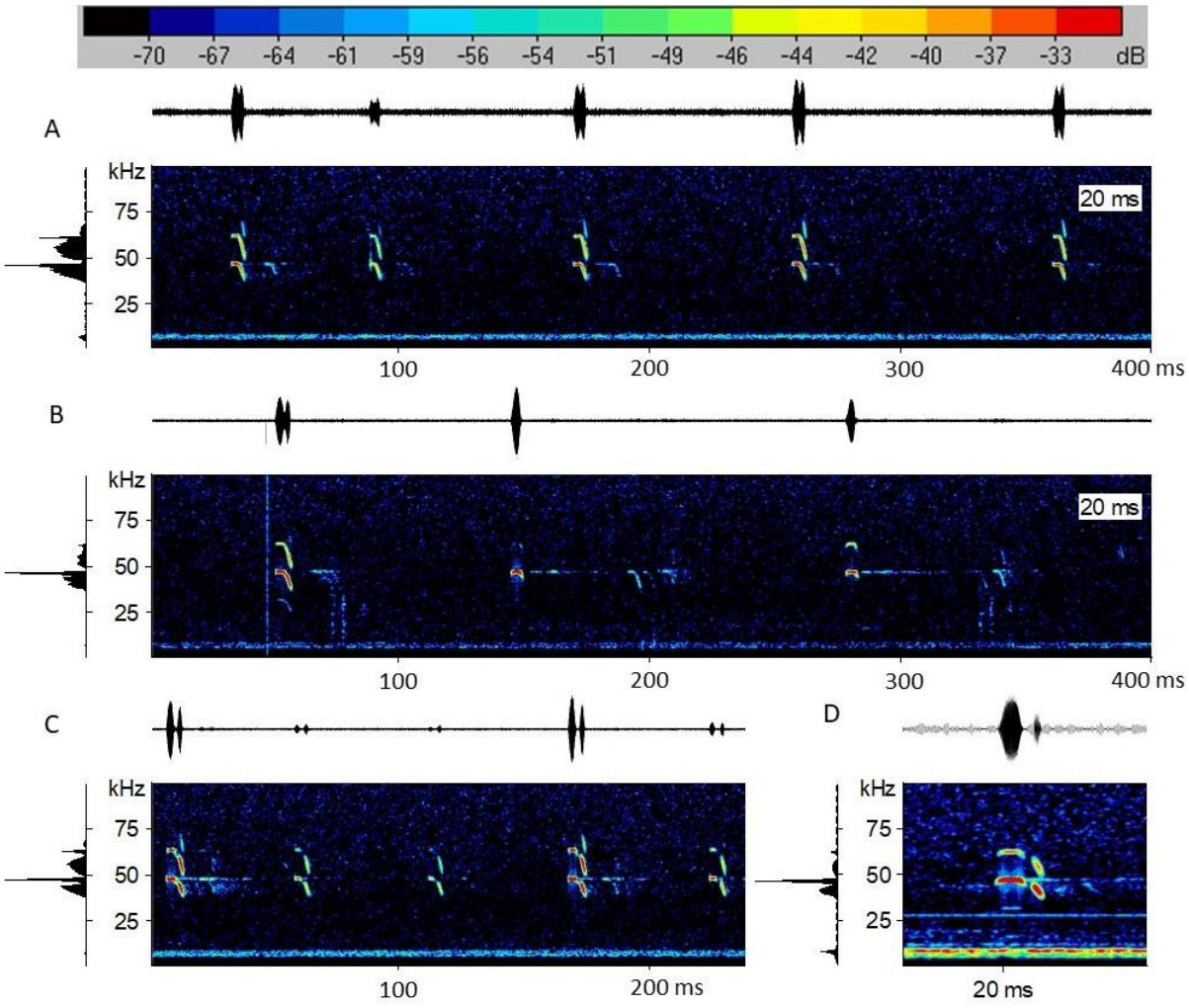


Figure 2: Spectrogram (FFT 512, Hamming Window) of search calls emitted in the flight cage. A) CF-FM search calls, B) alternating call types CF-FM and CF with alternating call amplitudes, C) CF-FM search calls with a gap between CF and FM component as well as alternating call amplitudes, overlap 75 %. D) Detail of search call with separation between CF and FM component clearly visible in both spectrogram (overlap 87.5%) and oscillogram.

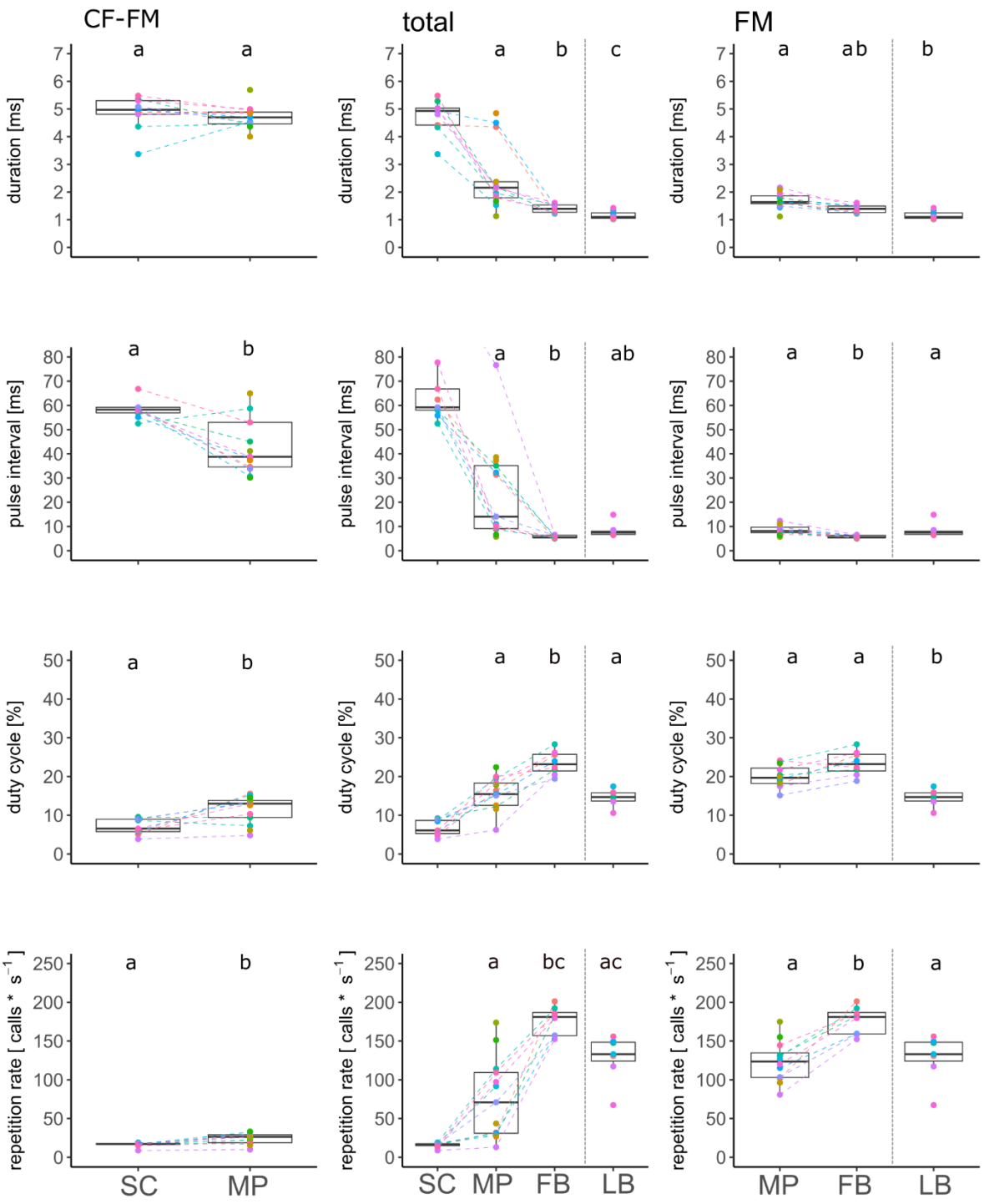


Figure 3: Temporal call parameters (duration, pulse interval, duty cycle and repetition rate) of CF-FM calls, both call types together (total) and FM calls in the different phases of hunting and landing: SC search phase, MP multi pulses, FB feeding buzz, LB landing buzz. Black bars indicate the median of each parameter. Dots represent the median of each individual. Coloured dashed lines connect the same individual in different hunting phases. Dashed grey line separates hunting and landing sequence. Letters a, b, c indicate statistical differences of the parameters between phases (same letter: no significant difference, different letter: significant difference, Wilcoxon-Mann-Whitney-Test for CF-FM calls, Kruskal Wallis rank sum test with Dunn's post hoc test for total and FM-calls).

Table 2: Temporal call parameters. For each bat median was calculated and the median taken from this median. n are the number of calls measured for each parameter.

	call type	call duration [ms]	range	ind.	# of calls	pulse interval [ms]	range	ind.	# of calls	duty cycle [%]	range	ind.	# of calls	repetition rate [calls-1]	range	ind.	# of calls
search calls	total	4.9	2.0-6.4	9	321	59.2	29.5-797.8	9	274	6.1	0.5-15.9	9	272	16.9	1.3-33.9	9	274
	CF	3.0	2.0-3.6	6	25	103.7	52.2-502	6	16	3.0	0.5-4.1	6	16	10.1	2.0-19.1	6	16
	CF-FM	5.0	2.2-6.4	9	296	58.3	29.5-797.8	9	258	6.6	0.6-15.9	9	256	17.2	1.3-33.9	9	258
multi pulses	CF-FM	4.7	2.0-6.1	13	158	38.8	25.0-259.4	12	102	13.0	1.5-18.5	12	101	26.3	3.9-40.0	12	101
	FM	1.6	1.0-3.9	12	225	8.1	4.1-30.0	12	215	19.7	5.7-40.2	12	204	123.5	33.4-241.0	12	215
feeding buzzes	CF-FM	4.1	2.7-4.6	5	6												
	FM	1.4	1.3-1.0	8	311	5.5	4.0-35.5	8	277	23.2	3.8-42.7	8	254	181.0	28.2-248.2	8	277
landing approach	FM	1.8	1.0-2.7	7	166	21.8	10.6-77.7	7	159	8.6	2.3-16.0	7	158	46.0	12.9-94.4	7	159
landing buzzes	FM	1.1	1.0-1.8	7	183	7.5	5.4-38.5	7	184	14.7	3.8-42.7	7	176	133.0	26-186.1	7	184
					1204				1098				1030				1098

The main energy of most search calls was in the third harmonic at a peak frequency of 47 kHz with the fourth harmonic often at similar amplitude (Figure 2, Supplementary Table 2). CF-FM search calls had a mean bandwidth of 6 kHz, while CF search calls showed a bandwidth of only 0.5 kHz in the third harmonic (Table 3). Whenever harmonics were present, they did not overlap in frequency (Supplementary Table 3). The amplitude of consecutive calls often varied a lot. We observed several different rhythmic patterns of single alternatively loud and silent calls as well as groups of a loud call in the beginning followed by several, more silent calls (Figure 2, B and C) which may indicate head movements of the calling bat.

Table 3: Bandwidth. For each bat means were calculated and the mean taken from these means. n are the number of calls measured.

		bandwidth [kHz]											
type	call type	2nd harmonic	SD	# of ind.	# of calls	3rd harmonic	SD	# of ind.	# of calls	4th harmonic	SD	# of ind.	# of calls
search calls	CF					0.5	0.21	6	24				
	CF-FM					6.1	1.39	9	294				
multi pulses	CF-FM					8.2	0.81	13	156				
	FM					6.2	1.05	11	112	7.4	0.85	7	102
feeding buzzes	CF-FM					8.0	1.11	4	5				
	FM					5.4	0.89	7	144	6.3	1.64	5	167
landing buzzes	FM	3.0	1.28	2	40	3.4	1.14	6	143				
total # of calls					40				878				269

Approach phase echolocation. Upon detection of a prey target, bats stopped flying circles and closed in on the free-flying moths, intercepting and/or following the targets' flight path. In this situation bats stopped emitting pure CF-calls and used now only CF-FM calls. In some cases, bats continuously reduced pulse intervals between CF-FM calls and eventually emitted a terminal buzz consisting of pure FM calls (Figure 4, Figure 6). However, more often they emitted during approach or just before the terminal buzz conspicuous groups of calls (hereafter referred to as multi pulses, MPs). These multi pulses were emitted in 84 % of all capture sequences (21 out of 25).

In the forest habitat we recorded a total of four approach phases, also consisting of CF-FM calls and MPs. Parameters of MPs from the forest were within the variation found for this call type in the flight cage (Figure 5, Figure 7), therefore we pooled the data for further analysis.

Multi pulses (MP) are groups of at least 2 and up to 18 calls at high repetition rates, with pulse intervals decreasing to a minimum of 4.1 ms (Table 2). MP groups usually consisted of an initial CF-FM call with a median duration of 4.7 ms (Figure 3, Table 2), directly followed by 1 - 17 short FM calls with a

median duration of 1.6 ms (median # of calls per MP group = 3, Table 4). The MP groups were emitted at median group intervals of 41.5 ms, and were distinctly shorter than the pulse interval of search calls (59.2 ms, Wilcoxon rank-sum test: W = 18, p-value < 0.01, Table 4, Supplementary Figure 1). Compared to the search phase, the median duty cycle of the CF-FM calls increased to 13.0 % (Wilcoxon rank-sum test: W = 99, p-value < 0.01, Figure 3, Table 2,). MPs were emitted by all bat individuals during at least some approaches. Only very rarely the initial CF-FM call was missing. After a MP group, bats could either abort the attack or proceed with the approach and end with a terminal group consisting of a feeding buzz (FB) (Figure 4).

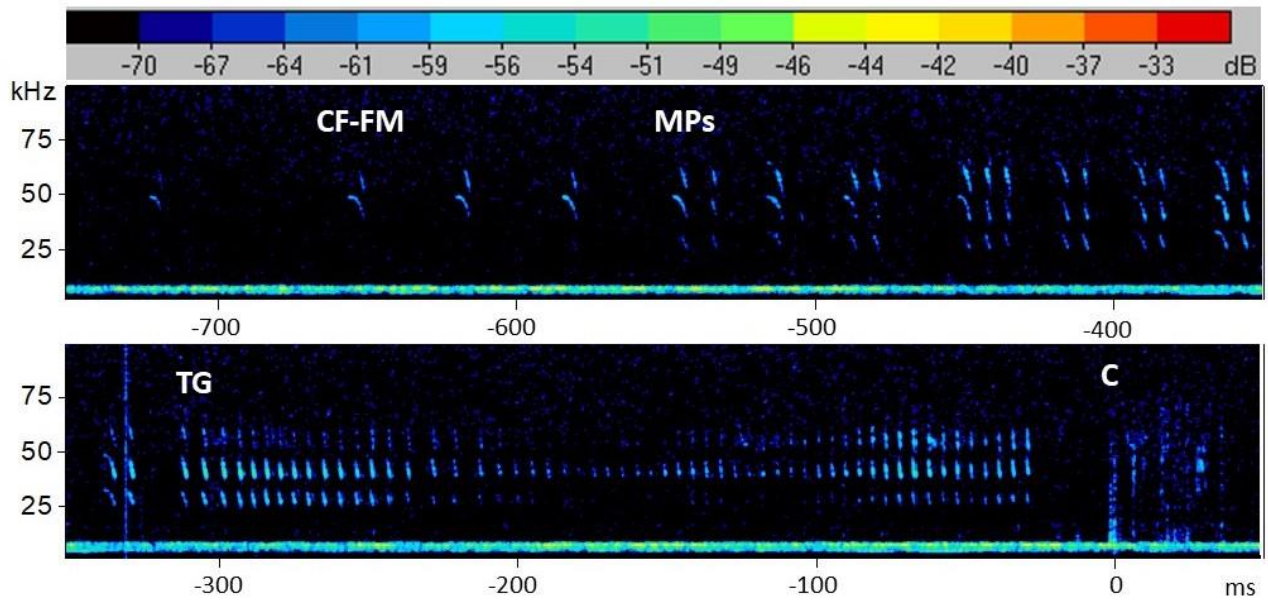


Figure 4: Spectrogram of a capture sequence with a terminal group (TG) emitted prior capture (C). Prior to the TG the bat emitted CF-FM calls and multi pulses (MPs) (FFT 512, Hamming Window, overlap 75%).

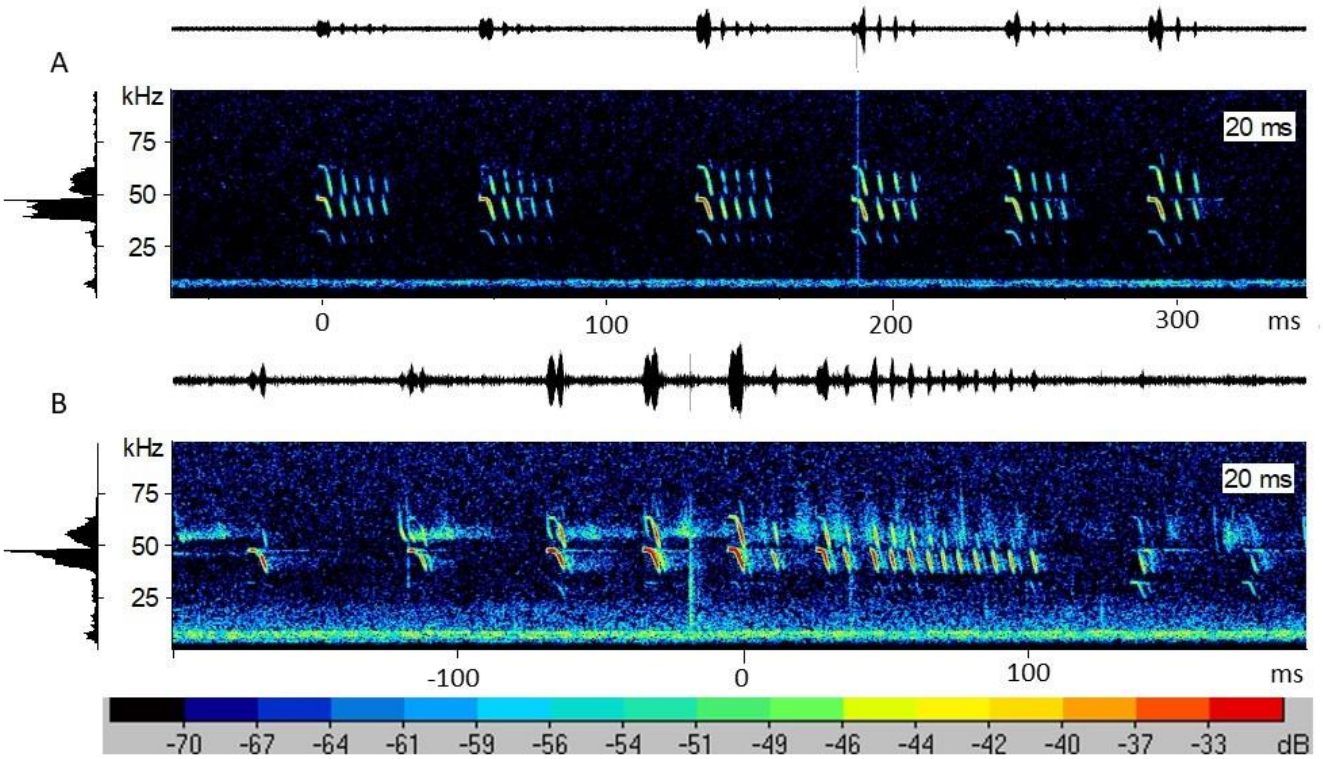


Figure 5: Spectrograms of multi pulses (MPs) emitted during approach. A) in the flight cage, B) in the forest. Pulse interval between groups is larger than within a group. CF-FM calls had the longest pulse durations and FM calls were usually very short (FFT 512, Hamming Window, overlap 75%).

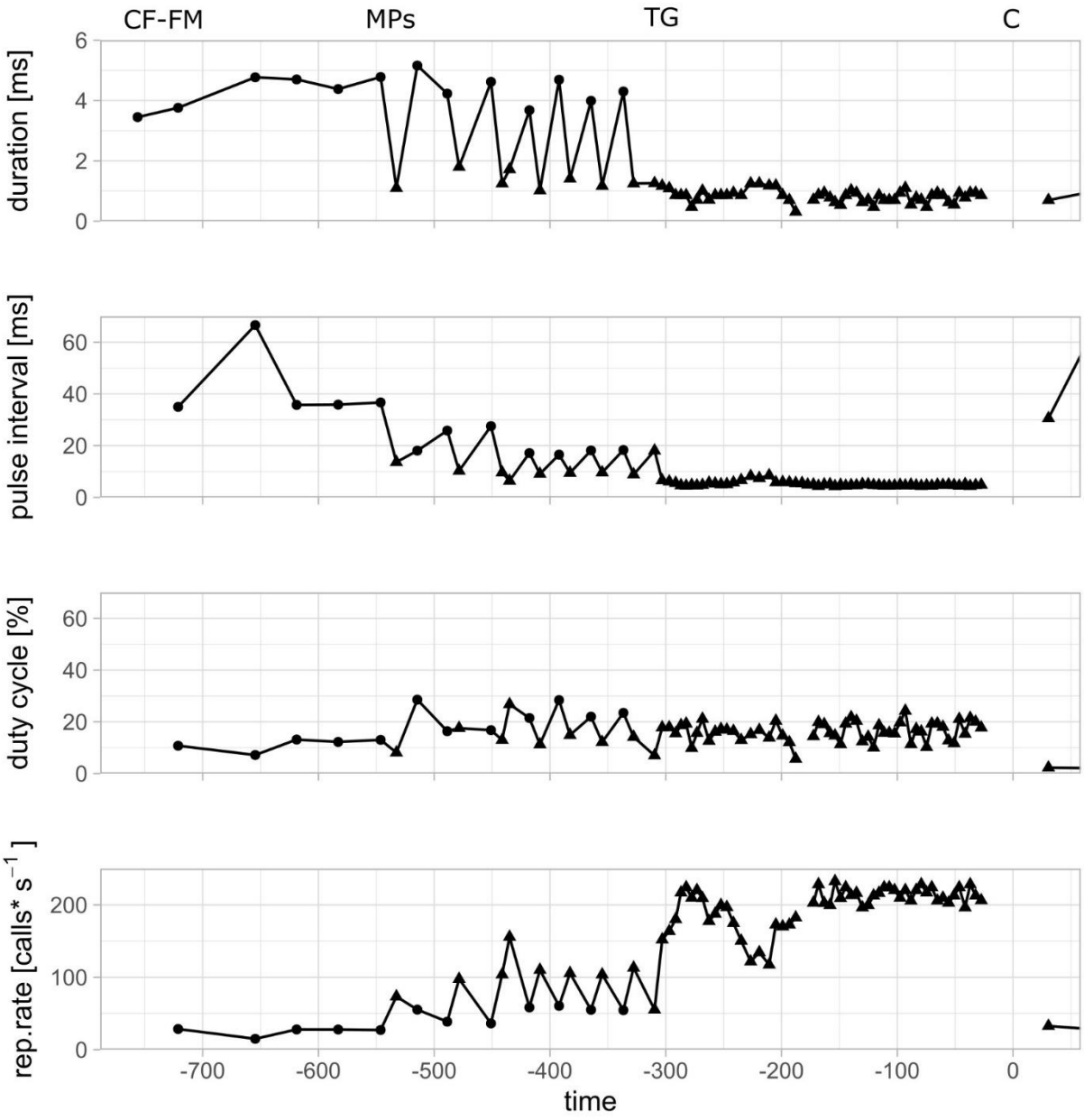


Figure 6: Derived parameters from the capture sequence in Fig. 4. Circles represent CF-FM calls, triangles represent FM calls. Duration was measured at -10 dB (for illustration purposes faint calls were occasionally measured at -6 dB). Pulse interval was measured as start of the call to start of the previous call. Duty cycle was calculated as pulse duration divided by pulse interval times 100 %. Repetition rate (rep.rate) is the calculated number of calls per second.

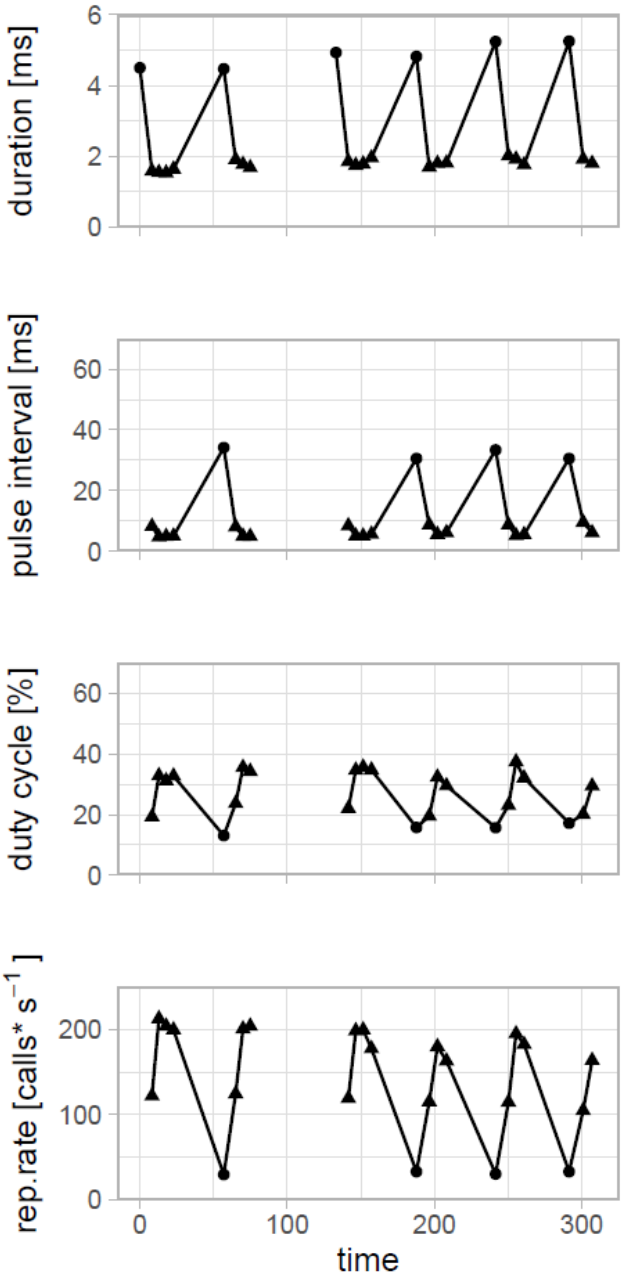


Figure 7: Derived parameters of the multi pulse sequence from Fig. 5 A. Circles represent CF-FM calls, triangles represent FM calls. Duration was measured at -10 dB. Pulse interval was measured as start of the call to start of the previous call. Duty cycle was calculated as pulse duration divided by pulse interval times 100 %. Repetition rate (rep.rate) is the calculated number of calls per second

Generally, each MP has the potential to develop into a feeding buzz. The feeding buzz comprised all coherent FM call groups prior to an (attempted) capture of the prey target and the last emitted CF-FM call, if present as part of a group (Figure 4). We considered MP groups of more than 18 FM calls right before capture as part of the feeding buzz. Buzz duration varied between 28 ms and 1886 ms (median = 288.7 ms, n_{buzzes}=18, $N_{individuals}$=8) with a minimum of 4 calls and a maximum of 233 calls (median = 36 calls, Table 4). Further, feeding buzzes were characterized by a continuous reduction of the pulse interval within a few calls down to 4.0 ms (median = 5.5 ms) and pulse duration down to 1.0 ms (median = 1.4, Figure 6, Table 2). Bats kept both pulse interval and pulse duration relatively constant. This resulted in a median duty cycle of 23.2 % and a repetition rate of 181.0 calls * s^{-1}. Main energy in these calls was generally in the third harmonic with a mean FM-call bandwidth of 5.5 kHz (Table 3).

Table 4: Temporal group parameters. For each bat a median group duration of multi pulses and feeding buzzes was calculated and the median taken from these medians. One landing buzz per individual was measured. ind. is the number of individuals. Minimum (min) and maximum (max) values were lowest and highest values, respectively.

parameter	median	min	max	calls analyzed	groups analyzed	# of ind.
multi pulses						
duration [ms]	15.1	5.6	153.5	813	232	13
group interval [ms]	41.5	11.8	191.0	732	214	12
within group duty cycle [%]	48.9	11.8	67.6	193	64	12
# of calls per group	3	2	18	820	235	13

parameter	median	min	max	calls analyzed	buzzes analyzed	# of ind.
feeding buzz						
duration [ms]	288.7	28.3	1885.9	946	18	8
# calls per buzz	36	4	233	946	18	8
landing buzz duration [ms]	252.4	183.8	367.4	223	7	7
# of calls per buzz	31	25	44	223	7	7

Landing echolocation. When landing, bats emitted groups of calls with a large median pulse interval of 21.8 ms prior to emitting a final group, the so-called landing buzz (Figure 8, Figure 9, Table 2). As the initial CF-FM-call was always missing, with a large pulse interval between FM calls, these groups were no MPs. We defined the landing buzz as the last group of FM calls before landing (Figure 8). When the bat initiated the buzz, pulse interval was reduced to 5.4 ms (median = 7.5 ms) and often got longer again towards the end of the buzz right before landing (Figure 9, Table 2). Median landing pulse duration was 1.1 ms. The median landing buzz duration was 252 ms (Table 4). Most energy of the landing buzzes was in the 3rd harmonic (Supplementary Table 2). Mean bandwidth of the landing buzz was 3.4 kHz (Table 3).

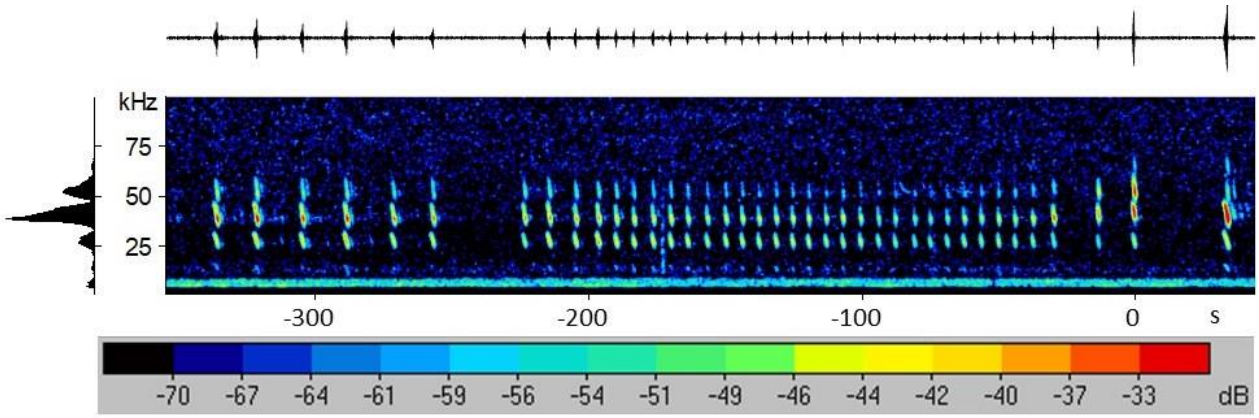


Figure 8: Spectrogram of a landing sequence (FFT 512, Hamming Window, overlap 75%). Landing event at t = 0s.

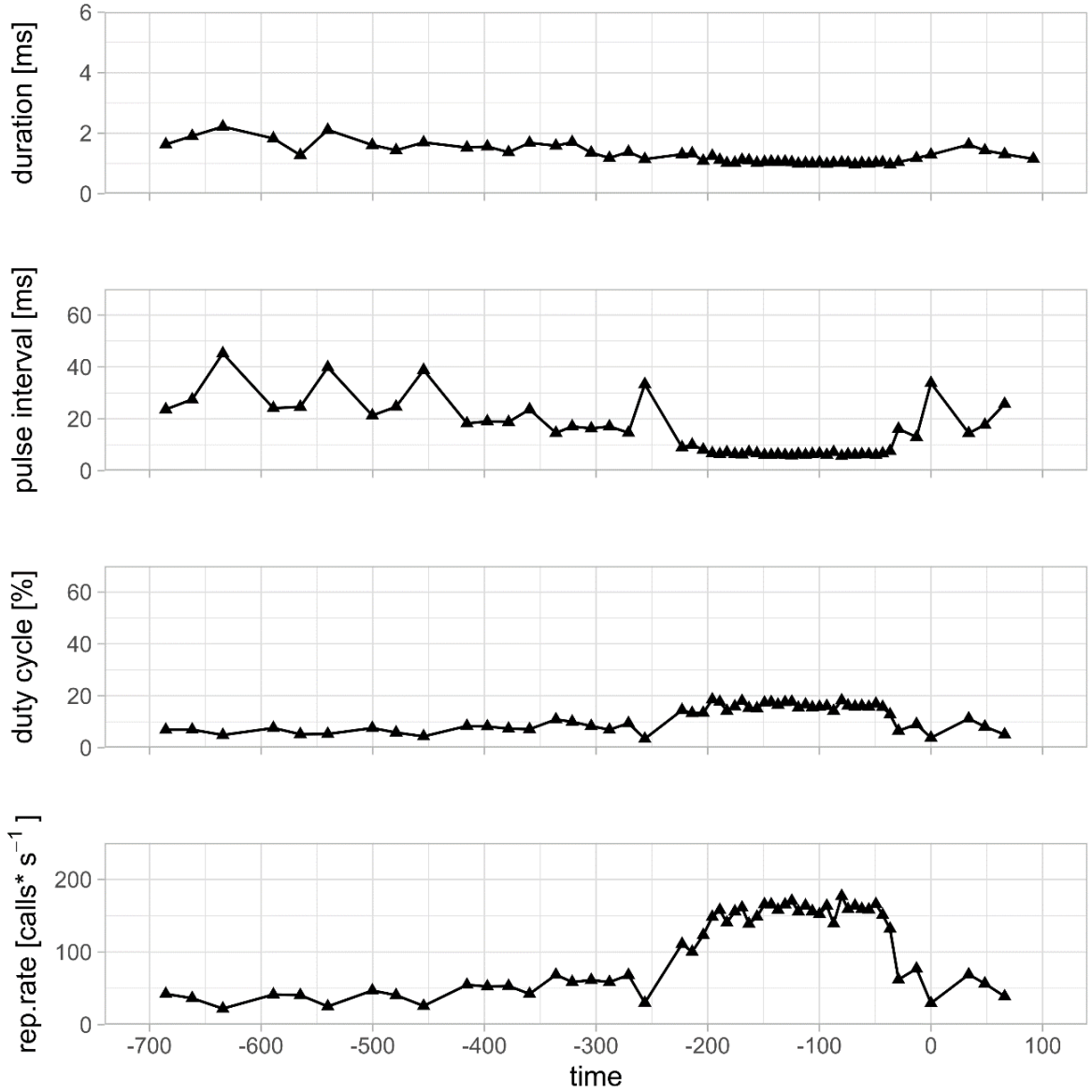


Figure 9: Derived parameters of the landing sequence from Fig.8. Circles represent CF-FM calls, triangles represent FM calls. Duration was measured at -10 dB. Pulse interval was measured as start of the call to start of the next call. Duty cycle was calculated as pulse duration divided by pulse interval times 100 %. Repetition rate (rep.rate) is the number of calls per second.

Comparisons between call types. We used a Wilcoxon rank-sum test to compare CF-FM calls emitted during search phase and during multi pulses. Additionally, we used a Kruskal Wallis rank sum test followed by a Dunn's post hoc test to compare all temporal parameters between the three phases multi pulses, feeding buzzes and landing buzzes (Figure 2, Table 2).

The CF-FM calls emitted in search phase did not differ in duration to those emitted at the beginning of multi pulses (Wilcoxon rank sum test: $W=40$, $p =0.23$). However, during multi pulses the pulse intervals between CF-FM calls were significantly shorter than during search (Wilcoxon rank sum test: $W=22$, $p < 0.05$). Consequently, duty cycle (Wilcoxon rank sum test: $W=99$, $p < 0.01$) and repetition rate were also significantly higher (Wilcoxon rank sum test: $W=95$, $p < 0.05$, Figure 3, Table 2).

FM call duration was significantly shorter in landing buzzes compared to FM calls in the multi pulses during approach (Kruskal-Wallis chi-squared = 17.024, df = 2, $p < 0.001$, Dunn Kruskal-Wallis multiple comparison, $Z=-4.11$, $p < 0.001$). However, there was no difference between multi pulse FM calls and feeding buzz FM calls (Dunn Kruskal-Wallis multiple comparison, $Z= 1.92$, $p = 0.05$) nor between feeding buzz FM calls and landing buzz FM calls (Dunn Kruskal-Wallis multiple comparison, $Z= -2.08$, $p = 0.05$, Figure 3). Feeding buzz sequences have both the shortest pulse interval (Kruskal-Wallis chi-squared = 13.98, df = 2, $p < 0.001$) compared to multi pulse FM calls (Dunn Kruskal-Wallis multiple comparison, $Z= 3.67$, $p < 0.001$) and landing buzz FM calls (Dunn Kruskal-Wallis multiple comparison, $Z= 2.55$, $p < 0.05$, Figure 3), as well as the highest repetition rate (Kruskal-Wallis chi-squared = 14.40, df = 2, $p < 0.001$, Dunn Kruskal-Wallis multiple comparison with multi pulse FM calls, $Z= -3.72$, $p < 0.001$, Dunn Kruskal-Wallis multiple comparison with landing buzz FM calls, $Z= -2.58$, $p < 0.05$). The duty cycle of FM calls did not differ between multi pulses and feeding buzzes (Kruskal-Wallis chi-squared = 14.288, df = 2, $p < 0.001$, Dunn Kruskal-Wallis multiple comparison, $Z= -1.70$, $p = 0.09$, Figure 3).

Discussion

Most of the animalivorous phyllostomid bat species glean insects and small vertebrates from substrates (Schnitzler and Kalko, 2001) and use rather uniform echolocation calls (Kalko, 2004; Yoh et al., 2020). In this study we analyzed the hunting behavior of *Lonchorhina aurita*, the Common sword-nosed bat, a phyllostomid bat with extraordinarily big noseleaf and ears that shows an echolocation behavior distinctly different from all other phyllostomid genera and that was suggested to be a specialized aerial insectivore (Gessinger et al., 2019). Our results confirm clearly that *L. aurita* hunts aerial prey and that the capture behavior corresponds to the typical patterns reported from other aerial insectivorous families, including a feeding buzz sequence immediately before prey capture.

Behavior and morphology. Confirming our initial hypothesis, we found that *Lonchorhina aurita* readily caught free-flying moths on the wing. We observed that *L. aurita* first secured the prey in its uropatagium and then took it out with the mouth, a behaviour typically found in aerial and trawling foraging bat families (e.g., Vespertilionidae, *Eptesisus fuscus* (Surlykke and Moss, 2000), *Myotis daubentonii* (Kalko and Schnitzler, 1989) and *Myotis nattereri* (Melcón et al., 2007); Noctilionidae, *Noctilio albiventris* (Kalko et al., 1998)). The genus *Lonchorhina* shows the longest tail within the phyllostomid family. It is entirely enclosed into the tail membrane and may aid in stabilizing it during aerial captures (Gardner, 2008; Wilson and Mittermeier, 2019). Actually, the phyllostomid bat genus with the second largest tail, *Macrophyllum*, contains the only other species in this family that has been reported to use a feeding buzz in aerial captures and trawling approaches over water (Gardner, 2008; Weinbeer et al., 2013). Tail membranes in other animalivorous phyllostomid genera are smaller and tails do not reach the edge of the membrane (e.g., *Tonatia, Lophostoma, Trachops, Micronycteris*). In plant visiting phyllostomid genera tails are often entirely missing (e.g., *Sturnira, Artibeus, Leptonycteris, Anoura*, Wilson and Mittermeier, 2019). At a value of 2.18, the Aspect Ratio Index (ARI) of *L. aurita* is rather high compared to members of the Phyllostominae (= 1.99 ± 0.04, Findley et al., 1972) - mostly gleaning species - indicating a narrower wing compared to the average species of this animalivorous subfamily. This suggests for *L. aurita* a faster flight style similar to other edge space aerial insectivores such as in the Mormoopid family (mean ARI = 2.22 ± 0.4, Findley et al., 1972; Tschapka, 2004).

Echolocation in search phase. Deviations from general phyllostomid patterns occur particularly in the echolocation behavior. Search calls of *Lonchorhina aurita* are composed of a CF- and an FM-component merged together, which, within the phyllostomid family, is known only from the genus *Lonchorhina* (Barataud et al., 2013; Gessinger et al., 2019). Interestingly, we observed in many call sequences calls with a clear temporal separation between the components (Figure 2, D). To our knowledge, species with similar call structure from other families, e.g., *Noctilio leporinus*, do not show this temporal separation (Schnitzler et al., 1994; Übernickel et al., 2013). Perhaps, in *Lonchorhina aurita*, these two call components are emitted separately through mouth and nostrils and precise vocal coordination is required for merging them into a single call. In contrast to most other phyllostomid species that fly consistently

either with a closed or an open mouth, *L. aurita* was observed both flying with an open and with a closed mouth (Gessinger et al., 2021), however, more detailed observations will be necessary to determine whether one mouth posture is preferred in a specific situation over the other and whether mouth posture is related to the respectively emitted CF or FM component. An alternating emission of echolocation signals through nostrils or mouth has already been shown for the aerial insectivore *Barbastella barbastellus* (Vespertilionidae), although at a larger temporal scale (Seibert et al., 2015).

The structure of search calls emitted by *Lonchorhina aurita* is very similar to the call structure of edge space foragers (Gessinger et al., 2019). They typically emit combined calls consisting of a shallowly modulated more or less CF component and a steeply modulated component at intermediate frequency with pulse intervals of 90-100 ms (Denzinger and Schnitzler, 2013; Schnitzler et al., 2003). While the CF component is suited to detect low intensity target echoes, FM components allow for a more precise measurement of distances and direction of targets (Jones, 1999; Schnitzler and Denzinger, 2011; Siemers and Schnitzler, 2004; Simmons and Stein, 1980). With a peak frequency of 47 kHz search calls of *L. aurita* are within the expected frequency range of search calls of edge space foragers (Denzinger and Schnitzler, 2013, e.g., most emballonurids (Jung et al., 2007) or most mormoopids (Mora et al., 2013)). While the median search call pulse interval in our flight cage recordings was well below the 90-100 ms (median=59 ms) typical for edge space insectivores, previously published field recordings suggest larger pulse intervals (> 100 ms) when sequences of only CF search calls are emitted (Gessinger et al., 2019).

Compared to that of other phyllostomid species of similar size, peak frequency in *L. aurita* is rather low. Based on the allometric relations for phyllostomid bats, at a mean forearm of 51 mm *L. aurita* should have a peak frequency of ca. 78 kHz (Yoh et al., 2020). A similar downward deviation in peak frequency is observed for the smaller *Macrophyllum macrophyllum* (forearm 33-40 mm, Wilson and Mittermeier, 2019) which emits search calls with a peak frequency of 55 kHz (Weinbeer and Kalko, 2007) instead of the predicted 90 kHz. This species is found outside the forest, hunting insects above large water bodies (Brinkløv et al., 2010; Weinbeer et al., 2006). Lower frequencies are less attenuated in air and therefore allow for an increased target detection range in more open habitats (Stilz and Schnitzler, 2012).

Lonchorhina aurita has recently been reported to emit shorter search calls when flying in a small flight cage (ca. 9 m^2), compared to an open habitat in the forest (Gessinger et al., 2019). The search calls recorded inside our flight cage (25 m^2) are about 1.5 ms shorter than the calls recorded in the field (pulse duration = 6.6 ms, Gessinger et al., 2019), indicating that the space inside the flight cage is more confined than the space in which *L. aurita* forage in their natural habitat. However, it was clearly not too small for the bats to successfully hunt moths.

Echolocation during approach. When reacting to the presence of a moth, bats decreased the use of CF calls and employed mostly FM calls that are more suitable to obtaining exact distance information during the feeding buzz (Denzinger et al., 2018; Schnitzler and Kalko, 2001; Yovel et al., 2011). As predicted,

bats reduced the pulse interval during the approach. Additionally, they emitted so called multi pulses (MPs), a behavior previously not described in any other species. These groups of calls are only used during approach and are characterized by an initial, longer CF-FM call, followed by one to many shorter FM calls with very low pulse intervals. Maintaining a CF-FM call at the beginning of an MP suggests that the CF component provides additional information that is useful at this early point in time of the approach to a prey. Pulse interval within the MPs was extremely shortened down to 4.1 ms, just like in the feeding buzz (4.0 ms), resulting in a highly increased repetition rate. During our flight cage observations MPs were often emitted prior to feeding buzzes, and the duty cycle drastically increased to almost 49 % within the group (Table 4), compared to search phases with a duty cycle of about 6 % (Table 2). MPs of foraging bats were also recorded in the wild. However, we never recorded MPs during landing sequences, instead we observed here typical phyllostomid call groups without the initial CF-FM call (Kalko and Condon, 1998; Schnitzler and Kalko, 2001; Thies et al., 1998). Therefore, we assume that MPs are used by *Lonchorhina aurita* to collect more information about a prey. There are at least two possible ways how *L. aurita* could benefit from using MPs during the approach.

The first component of the MPs is typically a CF-FM call. Through the combined use of CF-FM and FM calls, the duty cycle within the multi pulse group is tremendously increased (48.9 %, Table 4) compared to search phase duty cycle (6.1 % , Table 2) which increases the probability of perceiving a glint (Denzinger et al., 2018). Acoustic glints occur, when the impinging sound wave emitted by the bat is perpendicular to the moving wing of an insect. A glint consists of a short and prominent amplitude peak (Schnitzler et al., 2003). The information conveyed in single glints may allow a rough classification of the prey object. *Lonchorhina aurita* is a moth specialist (Kalbantner, 2018) and might use its echolocation to differentiate moths from other flying insects such as potentially harmful or unpalatable species.

Secondly, higher repetition rate and lower pulse interval will result in an increased information flow, just as during feeding buzzes. This will increase precision in localization of an erratically moving target and seems to be a common phenomenon in echolocating bats (Griffin, 1958; Griffin et al., 1960; Schnitzler and Kalko, 2001). We suppose, that multi pulses are also used to pre-assess the current position and flight direction of a moth before initiating the final intercept of the moths' flight path with a feedingg buzz. This does however not explain the presence of the initial CF-FM component. Further research is needed to finally evaluate the advantages of multi pulse use.

Feeding buzz. Finally, when approaching the target, *Lonchorhina aurita* used a feeding buzz with call durations as short as 1.0 ms and minimum pulse intervals of 4.0 ms. As predicted, both call duration and pulse interval were drastically reduced. Per our definition the feeding buzz comprised the last emitted CF-FM call and all coherent FM call groups prior to an (attempted) contact with the prey target. However, the transition between multi pulses and feeding buzzes is fluid. The actual use of a feeding buzz in *L. aurita* differs distinctly from the behavior of most other phyllostomid bats. The majority of

these are gleaning bats, which have not been reported to use feeding buzzes. For example, while the frog-eating bat *Trachops cirrhosus* emits echolocation calls throughout the attack, the pulse interval in the final phase is still about 12 ms, which is longer than the pulse intervals found in common feeding buzzes of aerial insectivores (Barclay et al., 1981; Simmons et al., 1979). Similarly, *Tonatia saurophila* reduces the pulse interval, during a gleaning approach, but the pulse intervals stay well above 10 ms (Schnitzler and Kalko, 2001). However, emission of feeding buzzes is common in bats with aerial hunting strategies from other families (Griffin 1958; Griffin, Webster, and Michael 1960; Schnitzler and Kalko 2001). Buzzes provide a fast update of the acoustically available information and allow for precise localization of even fast-moving prey (Schnitzler and Kalko, 2001). The feeding buzzes of the phyllostomid *L. aurita* - especially with regard to pulse interval - are most similar to the buzzes of edge space insectivores from other families, for example the feeding buzzes of *Saccopteryx bilineata* and *S. leptura* (Jakobsen et al., 2015; Schnitzler and Kalko, 2001). However, unlike in pipistrelles and some vespertilionids, we did not observe anything like a distinct buzz I and buzz II (Griffin et al., 1960; Melcón et al., 2007; Schnitzler and Kalko, 2001). Feeding buzzes of *L. aurita* had a median duration of 288.7 ms ± 228.53 SD. This is both quite long and highly variable. We found *L. aurita* to be very maneuverable and observed it closely following moths, while emitting terminal buzzes. Longer feeding buzzes could be a result of the bat chasing an erratically moving or evasive behavior showing moth, rather than just intercepting the moths flight path. Feeding buzzes of the phyllostomid *M. macrophyllum* hunting stationary prey above water in a flight cage were much shorter (median duration 179.6 ± 32.0 ms; range 98–301 ms) and less variable (Weinbeer and Kalko, 2007).

Another interesting variability can be seen in bandwidth. Most phyllostomids use 2 harmonics during the search phase, which overlap in frequency (Kalko, 2004; Thies et al., 1998; Yoh et al., 2020). In contrast, *Lonchorhina aurita* uses single 3rd harmonic search calls (Gessinger et al., 2019). Bandwidth of one single harmonic of *L. aurita* during a feeding buzz is rather low (6.4 kHz in the third harmonic) compared to, e.g., *Molossus rufus* with its bandwidth > 25 kHz (Grinnell et al., 2016) or pipistrelles (Kalko and Schnitzler, 1993) but higher than during landing (3.4 kHz). However, when initializing the approach phase, *L. aurita* maximizes call bandwidth by spreading the main energy over the third and fourth harmonics, while avoiding harmonic overlap. This behavior is maintained throughout the feeding buzz, thus reaching an overall bandwidth of ca. 13 kHz. The observed dynamic harmonic hopping, as well as the lack of a frequency overlap between the harmonics make *L. aurita* a good candidate to test whether a heteroharmonic computation strategy is used. The response of delay-tuned neurons to a lower frequency harmonic in the call and to a higher frequency harmonic in the echo was hypothesized to be advantageous for categorizing prey-size, for hunting insects with ultrasound hearing capabilities and for living in large colonies (Mora et al., 2013). For the only phyllostomid bat tested so far, *C. perspicillata*, a homoharmonic computation strategy has been shown (Hagemann et al., 2011). In this species a clear harmonic overlap is observed during search flight, which gets reduced towards the end of the approach (Thies et al., 1998), whereas in *L. aurita* harmonic overlap was never observed.

Landing buzz. Many phyllostomid bats use short groups of calls for the coordination of landing (Kalko and Condon, 1998; Schnitzler and Kalko, 2001; Thies et al., 1998). The flower visiting bat *Leptonycteris yerbabuenae* uses in addition to these groups a longer, buzz-like terminal group (mean: 12 calls) with low pulse intervals (mean: 9.8 ms) when visiting cactus flowers (Gonzalez-Terrazas et al., 2016). We showed that *Lonchorhina aurita* also used both, first groups with pulse intervals of 21.8 ms, followed by long landing buzzes (median: 31 calls) with median pulse intervals of 7.5 ms. The FM-calls of these buzzes had larger pulse intervals compared to the feeding buzzes, just as expected for a stationary target. The use of buzzes outside of prey context strongly strengthens the hypothesis that aerial captures are indeed the main hunting strategy of *L. aurita*, as aerial insectivore species from other families also use landing buzzes (Griffin et al., 1960; Melcón et al., 2007). While echolocation behavior during landing and hunting resembles, the lack of a CF-FM call during landing further strengthens its role in the prey context.

Conclusion

In conclusion, *Lonchorhina aurita* is an extremely interesting phyllostomid species with a specialized hunting behavior, whose morphology and behavior differs distinctly from almost all other animalivorous family members. We were able to verify our hypothesis and could show that *L. aurita* is an aerial insectivore with the typical adaptations in behavior and echolocation. As such, it represents an interesting outlier within the Phyllostomidae, together with *M. macrophyllum*, which, however, seems to be more specialized on trawling over water surfaces (Weinbeer et al 2013). The fact that *L. aurita* forages in gaps within forests, strongly suggests that it belongs to the guild of edge space aerial insectivores, which represents a new niche for phyllostomid predators. Unlike other edge space foragers *L. aurita* emits multi pulses during approach which may deliver additional information on the nature of prey and its movement in space. Further investigations in *L. aurita* should focus on the prey cues used to detect, localize and classify prey and the role of multi pulses for prey classification.

Acknowledgements

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Supplementary material

Supplementary Table 1: Forearm and tail length, 3rd finger and 5th finger length and aspect ratio of Lonchorhina aurita.

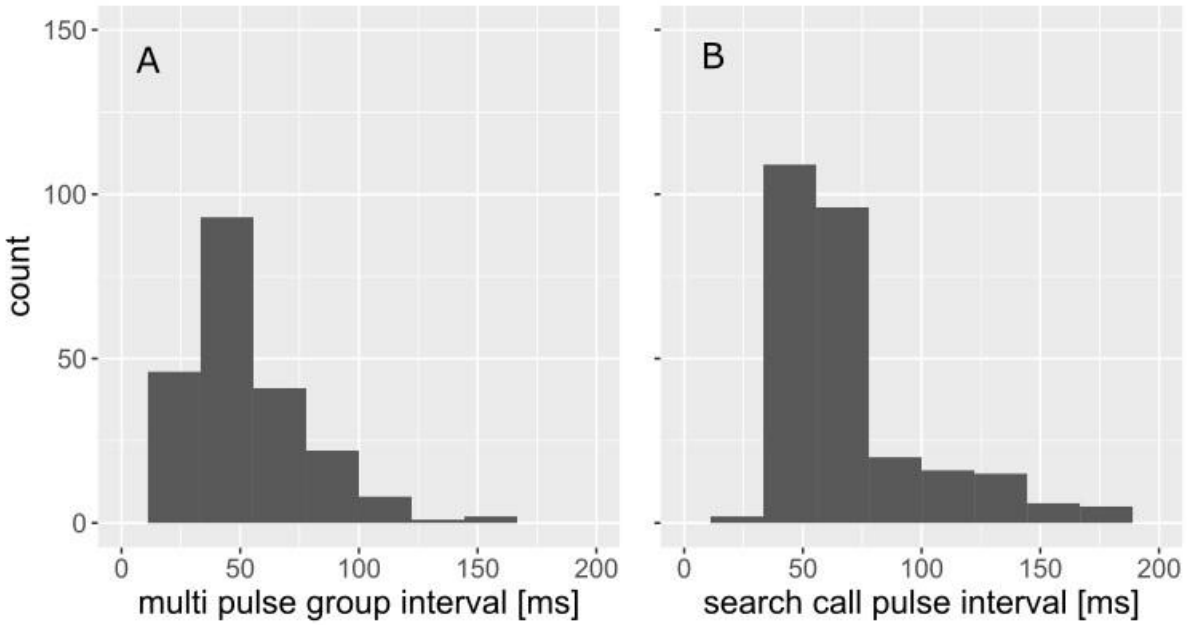
forearm [mm]									tail [mm]
51.9	52.5	51.2	51.0	51.2	50.8	52.7	50.3	51.1	53
50.5	51.2	50.5	49.8	50.2	51.5	49.3	50.6	50.9	53
50.8	50.3	49.4	51.8	50.4	49.0	51.1	50.6	51.1	51
50.6	50.7	50.0	51.3	49.7	51.0	50.7	49.5	58.8	52
49.5	50.9	50.2	51.0	50.0	50.3	51.2	48.8	53.2	52
49.7	50.5	50.9	49.8	49.8	50.1	51.3	50.1	51.8	50
48.9	52.2	51.0	50.2	50.4	50.5	49.6	52.0	51.9	48
51.9	50.4	50.0	51.2	51.5	49.7	50.2	49.8	51.1	51
51.6	50.0	49.8	51.0	51.3	48.5	50.8	50.9	51.6	50
49.9	50.1	49.7	50.5	53.5	49.5	51.1	51.2	50.8	46
50.2	52.1	50.8	48.6	49.5	50.1	51.4	51.4	51.2	50
51.0	51.4	49.1	51.0	51.3	51.2	51.4	51.8	51.8	52
52.0	50.1	49.0	49.6	49.9	51.5	50.6	51.2	48.5	53
50.4	51.6	51.5	50.0	49.9	50.5	49.8	51.6	50.9	50
48.9	51.1	52.0	49.4	50.2	50.8	50.4	51.8	50.8	
52.1	50.8	51.1	51.7	51.4	50.0	50.3	51.4	50.4	
51.9	50.3	51.5	49.7	51.1	51.5	51.4	50.7	48.5	
50.2	52.2	48.0	49.1	52.1	51.0	49.4	51.1	50.7	
51.5	50.0	50.3	51.4	49.8	51.9	48.1	50.7		
50.1	50.5	50.0	48.5	50.3	51.9	51.8	51.1		

continuation of table

5. finger [mm]	3. finger [mm]	Aspect Ratio (Findley 1972)	5. finger [mm]	3. finger [mm]	Aspect Ratio (Findley 1972)	5. finger [mm]	3. finger [mm]	Aspect Ratio (Findley 1972)	5. finger [mm]	3. finger [mm]	Aspect Ratio (Findley 1972)
64	90	2.20	62	80	1.29	66	90	1.36	64	87	1.36
62	90	2.27	68	89	1.31	61	85	1.39	70	89	1.27
64	90	2.20	68	92	1.35	60	87	1.45	65	90	1.38
65	93	2.34	68	93	1.37	68	92	1.35	64	90	1.41
60	90	2.39	68	93	1.37	62	88	1.42	67	93	1.39
60	85	2.28	64	88	1.38	63	90	1.43	65	90	1.38
64	95	2.30	61	84	1.38	64	89	1.39	65	88	1.35
65	92	2.20	63	91	1.44	63	88	1.40	65	90	2.16
64	90	2.23	70	90	1.29	63	90	1.43	65	90	2.13
63	93	2.28	63	91	1.44	63	88	1.40	69	88	2.01
60	90	2.35	65	95	1.46	63	88	1.40	64	88	1.38
68	95	2.16	63	86	1.37	64	88	1.38	62	89	1.44
68	95	2.11	65	90	1.38	67	90	1.34	60	86	1.43
66	95	2.21	65	90	1.38	66	95	1.44			
67	93	2.15	66	88	1.33	68	90	1.32			
64	90	1.41	65	88	1.35	67	90	1.34			

Supplementary Table 2: Peak frequencies. For each bat means were calculated and the mean taken from these means. n are the number of calls measured for each harmonic.

		peak frequency											
g_type	calltype	2nd harmonic	SD	ind.	# of calls	3rd harmonic	SD	ind.	# of calls	4th harmonic	SD	ind.	# of calls
search calls	total					46.9	0.78	9	353				
	CF					46.6	0.68	6	26				
	CF-FM					47.1	0.82	9	327				
multi pulses	total					46.5	1.46	13	539	58.9	0.52	8	279
	CF-FM					47.6	0.63	13	188				
	FM					45.3	1.13	12	351	58.9	0.52	8	279
feeding buzzes	total					44.8	2.34	9	473	57.4	0.64	7	594
	CF-FM					47.8	0.59	4	5				
	FM					43.4	1.15	9	468	57.4	0.64	7	594
landing buzzes	FM	32.0	3.20	2	49	43.1	1.06	6	174				
total # of calls					49				1539				873



Supplementary Figure 1: Histograms of multi pulse group intervals (A, n groups=214) and search call pulse intervals below 200 ms (B, n pulse intervals=269).

*Supplementary Table 3: Start (top) and End (bottom) frequency. For each bat means were calculated and the mean taken from these means. n are the number of calls measured for each harmonic. * marks that all calls were from one single individual. * indicates calls of only one individual were available for analysis.*

start frequency

g_type	calltype	2nd harmonic	SD	ind.	# of calls	3rd harmonic	SD	ind.	# of calls	4th harmonic	SD	ind.	# of calls
search calls	total					46.7	0.77	9	324				
	CF					46.5	0.67	6	25				
	CF-FM					46.9	0.83	9	299				
multi pulses	total					46.5	1.26	13	290	59.3	1.13	8	116
	CF-FM					47.4	0.76	13	160				
	FM					45.4	0.72	11	130	59.3	1.13	8	116
feeding buzzes	total					45.7	1.60	8	174	58.4	1.30	5	188
	CF-FM					47.5	0.18	4	5				
	FM					44.7	0.98	7	169	58.4	1.30	5	188
landing buzzes	FM	32.1	3.24	2	41	41.7	1.48	6	153				
total # of calls					41				941				304

end frequency

g_type	calltype	2nd harmonic	SD	ind.	# of calls	3rd harmonic	SD	ind.	# of calls	4th harmonic	SD	ind.	# of calls
search calls	total					42.9	2.91	9	324				
	CF					46.0	0.59	6	25				
	CF-FM					40.8	1.57	9	299				
multi pulses	total					39.2	0.80	13	295	51.9	0.66	8	119
	CF-FM					39.2	0.91	13	162				
	FM					39.2	0.71	11	133	51.9	0.66	8	119
feeding buzzes	total					39.3	0.74	8	164	52.1	0.72	5	187
	CF-FM					39.4	1.26	4	5				
	FM					39.3	0.33	7	159	52.1	0.72	5	187
landing buzzes	FM	29.1	4.53	2	40	38.2	0.50	6	147				
total # of calls					40				930				306

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Chapter 3



Illustration by Gloria Gessinger

Chapter 3: Prey movement and wing beat as cues in prey captures of the phyllostomid aerial hawking bat *Lonchorhina aurita*.

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Abstract

Lonchorhina aurita (Phyllostomidae) hunts moths with an aerial hunting strategy in the edge space of tropical rain forests. We investigated the prey cues needed by the species to capture moths in a multiple-choice flight cage experiment. Bats could choose between different prey cues, flutter or spatial movement, and a control. We hypothesized that an 'acoustic image' (present for all targets) is sufficient for prey detection and would motivate bats to approach, but flutter is necessary for prey capture. Our results show that bats were able to differentiate between fluttering and non-fluttering prey types. While the bats occasionally investigated other targets, they clearly preferred the fluttering target and caught it significantly more often. Therefore, this cue is especially important for the prey classification by *Lonchorhina aurita.* Flutter could be perceived by echolocation and/or through passive listening. Independent of the target, feeding buzzes were emitted during prey capture. We suggest that *Lonchorhina* may flexibly use several sensory channels to classify and localize their prey.

Introduction

Insects are not only very nutritious but also highly abundant in many ecosystems. Particularly in tropical rainforests, insects can be found everywhere: On the ground, in the canopy, on water surfaces, in the leaf litter, and flying through the air (McCaig et al., 2020). Further, most insect groups in tropical rainforests are rather aseasonal and thus represent a year-round available prey (Kishimoto-Yamada and Itioka, 2015). Especially for the flying bats with their high energy demands, it is crucial to have a reliable food resource, and indeed, most bats have specialized on insect prey (Schnitzler and Kalko, 2001).

The sensory system of bats is adapted to the available resources within their respective hunting habitat. In loud and cluttered hunting habitats the cues available to bats are different from those used by bats in wide open habitats (Denzinger et al., 2016). In open space, bats use an aerial hunting strategy and predominantly perceive prey cues through echolocation. Since the open space is free of obstacles, most, if not all, returning echoes come from potential prey or other flying animals. Background echoes arrive much later and thus can easily be separated from prey echoes (Denzinger and Schnitzler, 2013). In narrow space, echolocation signals are not only reflected from prey but also from the rather close background (Arlettaz et al., 2001). Here, many insectivorous bats are gleaners, for example many of the New World phyllostomid bats that use other detection strategies such as passive listening for sounds produced by the prey (Arlettaz, 1996; Bell, 1982; Page and Ryan, 2008). In edge space, a combination of both methods is conceivable. Sitting insects might be easier found by passive listening to rustling or courtship sounds, whereas quietly flying insects, such as moths, might be easier detected by echolocation. Once detected, bats may classify an item as potential prey and finally localize it precisely for successful capture (Kalko and Schnitzler, 1998).

Flying moths are erratically moving targets with moving wings. Bats using an aerial hunting strategy when hunting for moths may theoretically use several prey cues. The presence of a moth could be perceived as an ‘acoustic image’ produced by echolocation (Simmons and Stein, 1980). Echoes are simply returned by the relatively large moth wings and body. Additionally, the wing beat could be either detected by echolocation through rhythmical shifts in amplitude and frequency of the returning echo (Baier and Wiegrebe, 2018; Bell and Fenton, 1984; Emde and Schnitzler, 1990), or by passive listening to the low frequency sound produced by the wing beat (Anderson and Racey, 1993).

Unlike other phyllostomid bats, *Lonchorhina aurita* is an edge space aerial insectivore and hunts moths with an aerial hunting strategy at more open sites inside the forest (Chapter 2, Gessinger et al., 2019; Kalbantner, 2018). Relatively open hunting habitats within forests are found in the proximity of small streams with little to no understory (Gessinger et al., 2019). For prey localization *L. aurita* uses a feeding buzz similar to that of edge space hunting species from other families (Chapter 2, Denzinger et al., 2016). Echolocation call design during search flight differs distinctly from the standard phyllostomid echolocation pattern (Kalko, 2004; Yoh et al., 2020). The calls are longer, up to 7 ms, and consist of a constant frequency onset followed by a downward frequency modulated call component (Gessinger et

al., 2019). For the current study we were interested in the cues that *L. aurita* uses for prey detection, as well as those used for prey selection. To test this, we performed a forced choice experiment with different prey types. We offered moth targets with a wing beat, as well as targets without a wing beat, which were either moved or motionless.

We hypothesize that an 'acoustic image' of prey (Simmons and Stein, 1980) produced by echolocation is sufficient for prey detection in an edge space situation and therefore all targets can be perceived by *L. aurita* in a flight cage setup. If this is the case, we expect *L. aurita* to investigate all targets with approach flights. However, for prey classification, and the final decision to attack a target we expect further necessary cues. We hypothesize that only a wing beat cue would lead to a positive classification of the target as prey and a final capture by the bat. Therefore, we scored both, approach flights as a measure of successful detection and captures as a measure of prey classification.

Material and methods

Studied bats

Study area was the canal region close to Gamboa in Panama. We caught *Lonchorhina aurita* (Chiroptera: Phyllostomidae, Lassieur and Wilson, 1989) in Soberania National Park from January to April 2019 during emergence from their roost or along flyways above streams. We used six-meter nylon mist nets for capture (ECOTONE®, Warszawa, Poland). We determined sex and age and used only adult animals of both sexes. Lactating and pregnant females as well as clearly juvenile bats were immediately released and not used in experiments.

Up to three bats at a time were marked by cutting small patches of dorsal fur and habituated during one night in a small tent (2 m x 1.8 m, height 1.5 m) to an outdoor flight cage environment (N_{total}=12). Animals were hand fed in at least three feeding sessions over the night. We fed a custom developed mixture of baby food (Hero Baby Natur, type arroz con pollo) enriched with protein powder (Protifar, Nutricia) and vitamin paste (Trixie, TX-2578 Multivitamin paste), and either mealworms (*Tenebrio* sp. larvae) or freshwater fish (*Tilapia* sp.) all finely pureed with a blender and offered to the bats with a plastic pipette. We monitored weight of the bats throughout the night and made sure they ate at least 6 g of our nutritious mixture (bat body mass: 13 g). Bats participated in experimental trials for up to five consecutive nights. Each trial lasted up to 2 hours. After conclusion of the experiments, bats were fed again and released at the capture site at least two hours prior to sunrise. All captures and experimental procedures were carried out following IACUC regulations of STRI (IACUC protocol 2017-0102-2020) under permit no. SE/AP-13-18 from the Panama's Ministerio de Ambiente (Miambiente). All research complied with the laws of Panamá.

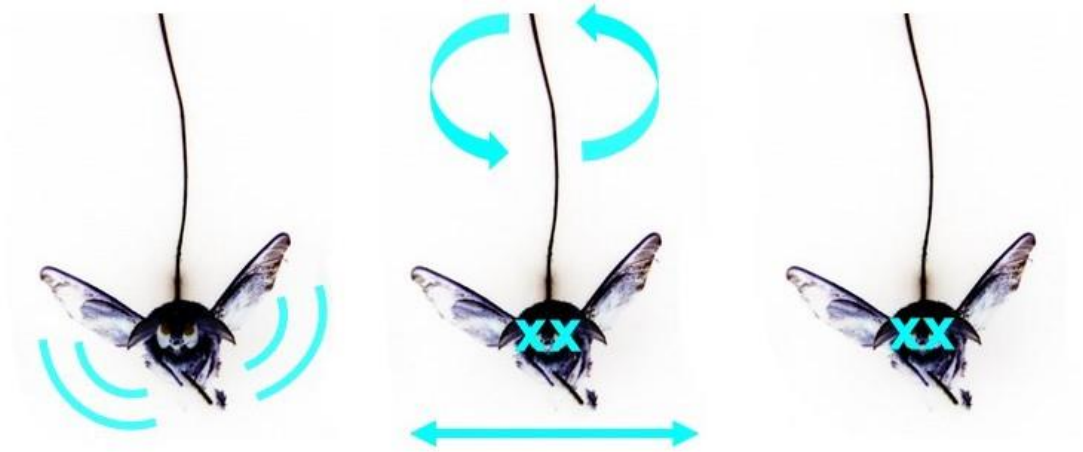
Prey: Moths

We used light traps (superactinic neon tube, 12 V, 15 W; white cloth) to capture live medium-sized moths of various species (Lepidoptera; wingchord range 15 – 35 mm) in Soberanía National Park between 19:00 and 23:00. Additionally, we collected moths around lit buildings in Gamboa. Moths were individually photographed on graph paper and their body sizes and wingchord measured for classification. Five moth families were most present in the area: Notodontidae, Noctuidae, Erebidae, Geometridae, and Saturnidae (Janzen and Hallwachs, 2009). In our experiments moths were offered to the bats hanging from the ceiling, tethered with a thin wire (0.2 mm diameter) which was poked through the thorax of the moths. This way the moths could still move their wings. We had to pause our experiments prior and on full moon nights due to low moth capture success.

Choice experiment

We studied the prey choice behavior of single individuals of *Lonchorhina aurita.* We tested the bats preferences for two different moth prey cues, movement and wing beat. All experiments were conducted in a 5 m x 5 m x 2.5 m flight cage located on the forest edge at the Smithsonian Tropical Research Institute in Gamboa, Panama. Each bat was able to roam freely inside of the flight cage.

We offered three targets for the bats to choose from: a stationary moth with wing beat (WB), a manually moved moth without wing beat (M) and a control moth with neither wing beat nor movement (C)(Figure 1). The stationary live moth targets with wing beat (WB) were attached to the end of thin 60 cm long wooden poles hanging from the ceiling (Figure 2). These were held in place by styrofoam stoppers but could also be moved from the distance by the observer via a nylon string. When a moth stopped flying, a quick pull usually re-elicited flight behavior with beating wings. Most moths flew spontaneously and for periods of up to several minutes. If not, we had to exchange the moth. Analogously, the control moth (C) was offered on a wooden pole, but remained motionless. Both the control and the moved target were moths killed in a freezer. Their wings were fully extended to provide a maximum echo-reflecting target (Kober and Schnitzler, 1990). To imitate the erratic flight behavior of moths the manually moved target was not directly attached to the stiff wooden poles but to a flexible 60 cm long wire hanging right next to the pole (Figure 2). This wire could also be moved from the distance via a nylon string. In the experiment all three targets were offered at the same time and were presented in a triangle, equally spaced at a distance of 120 cm from each other in the center of the flight cage.



	WB	M	C
Movement	No	Yes	No
Wingbeat	Yes	No	No
Alive	Yes	No	No

Figure 1: Prey targets and their corresponding prey cues. (WB) stationary moth target with wing beat. (M) manually moved moth target without wing beat. (C) stationary moth target without wing beat as control.

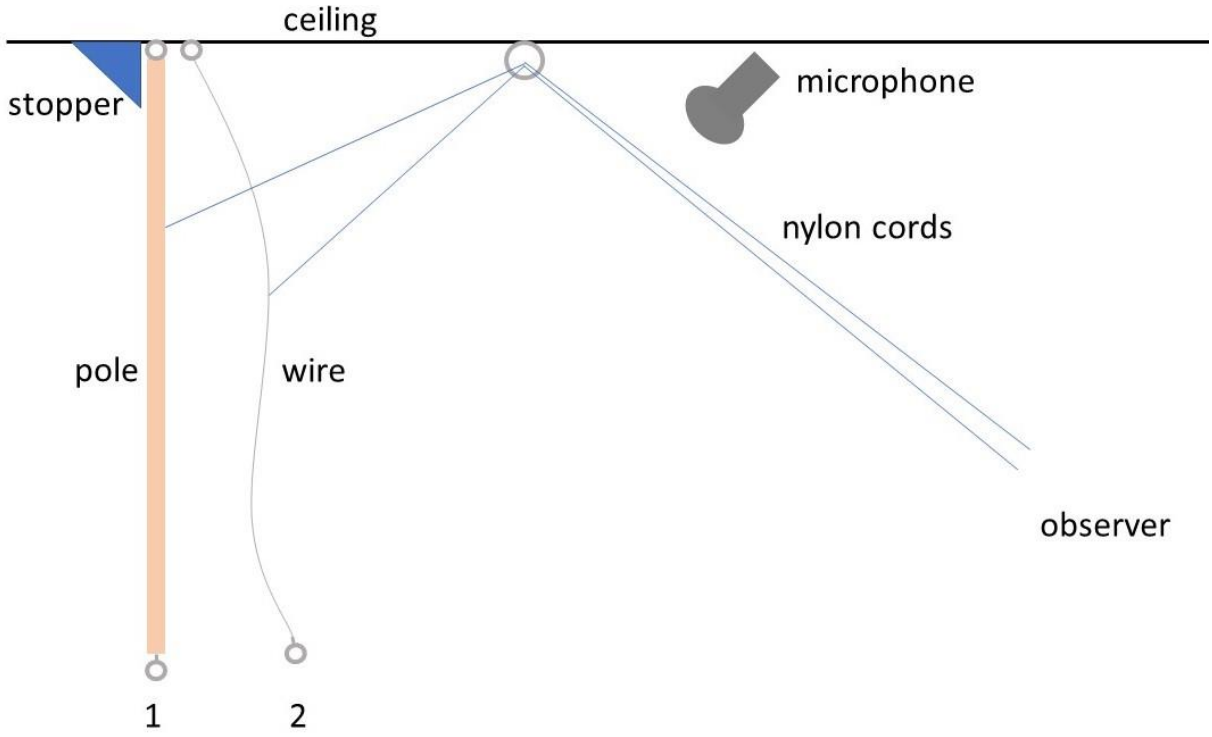


Figure 2: Setup of one representative prey offering position. Prey was either attached at point 1 (WB, C) to a 60 cm long wooden pole or point 2 (M) to a 60 cm long wire. Both pole and wire could be moved by the observer via nylon strings. While prey attached to the wire would always flexibly move, the stopper next to the pole kept it in its position unless intentionally moved by the observer.

Video and audio recordings

We recorded the performance of the bat using a Sony camcorder (Handycam DCR-SR72) in night shot mode with an additional wide-angle lens (Opteka 37mm 0.45x Wide Angle Lens for Camcorders) positioned in a corner of the flight cage. At the same time, we observed the bat on the display of the camcorder. To monitor wing motion the moth with the flapping wings (WB) was additionally filmed up close, with a camera positioned close to the flight cage wall (Sony Handycam DCR-SR45, night shot mode). We used up to six infrared spotlights for illumination (12 V, 4 LED bulbs). Additionally, we monitored the bats' echolocation behavior with four ultrasound microphones (Avisoft-Bioacoustics, CM16). Three microphones were attached to the ceiling, pointing down at a 45° angle directed at each of the three tethered moth targets, while the fourth microphone was positioned as an overview microphone close to the wall. Microphones were connected to an Avisoft Bioacoustics UltraSoundGate 416H (USG) interface controlled by a Panasonic Toughbook CF-F9. We used a sampling rate of 300 kHz. Audio recordings started automatically when echolocation call intensity on any channel exceeded a threshold of 3 % above noise. Pre-trigger recording duration was set to at least 3 s. We were able to synchronize audio and video recordings using a custom-built trigger that started the audio recording, while also switching on a LED light that was visible in the video recordings.

Data analysis

In the video recordings of the triple choice experiments we monitored the movement of the moth with flapping wings and scored the behavior of the bat. Only trials with all three target types present at the same time were scored for the choice experiment. We scored the number of attacks and approaches towards the respective prey targets (WB, M, C). An approach was scored when the bat clearly oriented itself towards a target. An attack was scored when the bat got into contact with the prey target. By definition, prior to every attack the bat had to approach, so for each attack an additional approach was scored. Sometimes the fluttering moth would stop flying when the bat approached. Therefore, we additionally scored the number of aborted approaches. Further, we scored the presence and absence of feeding buzzes (Chapter 2).

Statistical analysis

After scoring the approaches and attacks, we conducted a Kruskal-Wallis test followed by Dunn's test with Benjamini-Hochberg stepwise adjustment (Dinno, 2015) to identify the preferred prey type. The statistical analyses were performed with R v. 3.6.1 (R Core Team, 2019). Data was prepared for analysis using the R package "dplyr" (Wickham et al., 2019) and illustrated with the R packages "ggraptR" (Dubossarsky and Song, 2020) and "ggplot" (Wickham, 2016).

Results

Choice experiment

Five bats spontaneously started hunting in the flight cage and approached and attacked at least one of the offered targets several times. Prior to prey interaction bats flew circles in the flight cage, while continuously emitting search calls. The bats were mostly successful in removing prey from the wire using their wings to guide the prey into their uropatagium and consumed it either directly in flight or after perching on the flight cage walls (Supplementary Video 1). The bats ate the moths headfirst and discarded the wings.

Approaches All five hunting bats approached the wing beat (WB) target several times (Figure 3 A, range WB target: 22-50 times). Four bats approached the manually moved (M) target (range: 1-18 times). Three bats also approached the control up to three times. Despite the large differences among the individuals in the total number of approaches (range: 23-64 approaches), there was a significant difference between the number of approaches to the three treatments (Kruskal-Wallis $x^2 = 9.9819$, df = 2, p=0.007). Bats approached WB targets significantly more often than M targets (Dunn Benjamini-Hochberg, p = 0.031) and the control (Dunn Benjamini-Hochberg, p = 0.008). There was no significant difference in the number of approaches between M targets and the control (Dunn Benjamini-Hochberg, p = 0.477).

Attacks While all five individuals recognized WB targets as potential prey items and collected them from the poles, the M target was never collected and consumed. The M target was only attacked by two individuals, while the control was never attacked, resulting in a significant difference between the number of attacks to the targets (Kruskal-Wallis $x^2 = 12.091$, df = 2, p = 0.002). Three bats attacked exclusively WB targets (Figure 3 B). The WB target was clearly preferred over M targets (Dunn Benjamini-Hochberg, p = 0.008). Further, WB targets were attacked much more than the control (Dunn Benjamini-Hochberg, p = 0.004), while M targets were not attacked significantly more often than the control (Dunn Benjamini-Hochberg, p = 0.690).

Occasionally, we observed moths stopping to flap their wings while a bat approached. As a result, all bats observed in such a situation (N_{bats} = 3) consistently aborted the attack ($n_{aborted\ attacks}$ = 9) (Supplementary Video 2). At least one moths started to flap its wings again and as a result the bat resumed approaching.

Additional observations Sporadically, bats would hover in front of an offered prey (n_{hovers}=16, N_{bats}=4). The bats hovered both at fluttering moth targets (n=11) as well as at manually moved moth targets (n=5). Five hover flights resulted in an attack of the target, with the manually moved moth being attacked only once after a hover. Hovering bats emitted feeding buzz-like pulsed calls (Supplementary Figure 1). Three bats unsuccessfully attacked a fluttering moth which thereby was detached from the wire and dropped to the floor. Within seconds the bats returned and reattacked the now empty pole. Another bat

repeatedly approached empty poles in between trials, while the observer was preparing the next experiment switching target position or renewing targets (N_{bats}=1, $n_{approaches}$=5). One bat approached a moth which had fallen to the ground and was still fluttering.

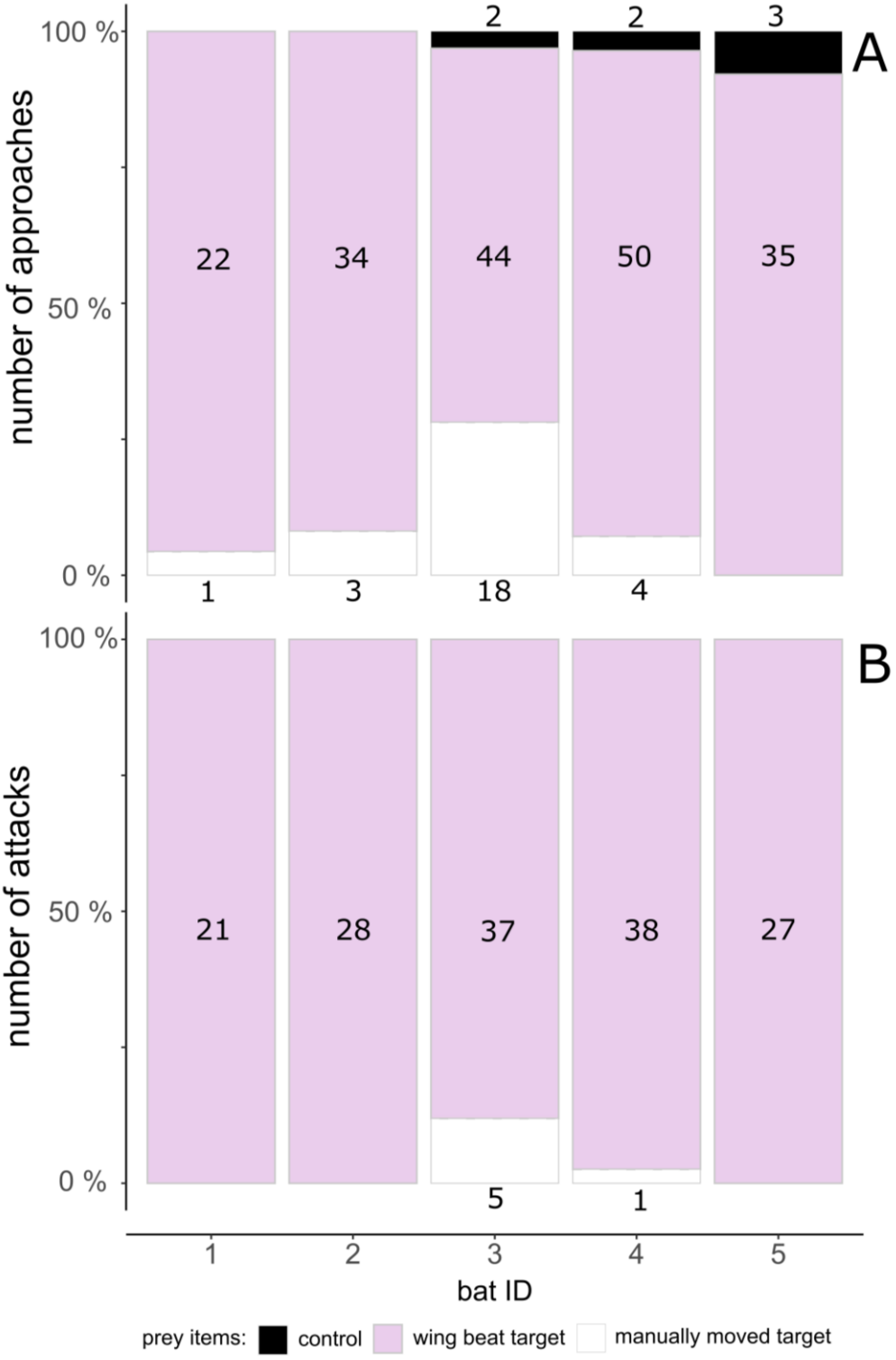


Figure 3: A approaches and B attacks of bats at different prey items (control in black/top, wing beat target in pink/middle and manually moved target in white/bottom). Bars represent the percentage of the approaches in relation to the total number of approaches per bat (bat 1-5). Numbers indicate the absolute number of approaches or attacks to each target.

Echolocation behavior

We recorded the echolocation behaviour of the bats during approaches and attacks and obtained several feeding buzzes irrespective of the prey target (Figure 4). However, not all audio recordings of approaches/attacks contained echolocation calls and therefore not every recording contained a feeding buzz.

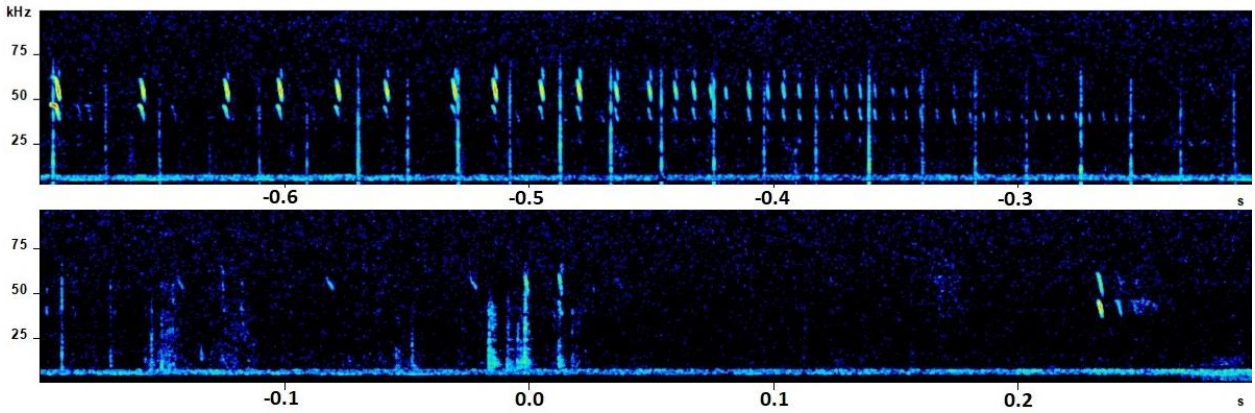


Figure 4: Feeding buzz sequence of L. aurita attacking a wing beat target with a wing beat frequency of approximately 50 Hz (moth wing beat visible as vertical lines in spectrogram). At time 0.0s the bat removes target from the wire. Spectrogram parameters: FFT 512, Hamming window, 75 % overlap.

Discussion

Insects are a valuable and, in most habitats, abundantly available source of nutrients for bats. Depending on their species-specific life style they emit different cues. Some insect species emit audible sounds when calling for mates, walking through leave litter or flying through the air (Goerlitz et al., 2008; Neil and Holderied, 2021). Others are colorful and visually very conspicuous (Lotts and Naberhaus, 2023). Further, insects can be smelly (Allison and CardŽ, 2016; Ayasse et al., 2001). In addition to those cues, predators equipped with an echolocation system, may perceive from a moth an 'acoustic image' that contains additional information, e.g., for relative flight speed, size and distance of the insect (Simmons and Stein, 1980).

We closely examined the cues *Lonchorhina aurita* can use while hunting for flying moths in aerial hunting mode in a confined space. We tested which prey cues are used to successfully capture prey. Since olfactory cues are assumed to be of rather limited relevance for insectivorous bats (Faure and Barclay, 1992; Geipel et al., 2013) we focused on audible cues, namely target movement and wing beat. We reduced visual cues using infra-red illumination (Winter et al., 2003).

In a choice experiment we presented three moth targets simultaneously: a manually moved (M) target without wing beat, a stationary wing beat (WB) target, and a stationary control (C) without wing beat. The M target lacks the typical oscillating wing motion of a living moth, including prey-generated fluttering sounds, but produces a typical 'acoustic image' and motion. The offered WB target contains wing motion and produces a stationary 'acoustic image'. Therefore, the wing beat could be perceptible for the bat as a passive acoustic cue and/or through echolocation. The control also produces an 'acoustic image' but neither movement nor sound.

Preference

Both prey detection as well as prey cue selection can result in an observed preference for certain prey types. We saw *Lonchorhina aurita* approaching all targets offered, thus clearly demonstrating that it can detect all of them. Still, *L. aurita* consistently preferred by far the WB targets, as seen in a significantly higher number of approaches and attacks compared to those to the other targets. These results clearly show that *L. aurita* is able to differentiate between fluttering and non-fluttering prey types. Therefore, the observed result is not just a product of limited detectability, but rather reflects a preference for certain cues for the classification of potential prey.

The sensory abilities of an aerial predator such as *L. aurita* are particularly important, as they determine, among other things, why fluttering prey are preferred. This raises the question of which sensory cues are used by *L. aurita* for prey perception.

During our choice experiment *L. aurita* did not attack control moths (C). But we observed a total of seven approaches from different bats. The seven observed approaches to the control occurred at random

times during the experiments and therefore should not be the result of spatial learning. More likely, bats could actually perceive the control moth.

The ability to detect motionless prey using echolocation has been described for other bats as well: The phyllostomid *Micronycteris microtis* hunts in narrow space situations and collects silent and motionless insects from leave surfaces (Kalka and Kalko, 2006). It hovers in front of the leaves and uses echolocation to find silent and motionless prey (Geipel et al., 2013). Another species not relying on prey generated cues to detect, localize and classify prey is *Myotis auriculus* (Fenton and Bell, 1979). Instead it uses short, high frequency echolocation calls to localize moths on leaves foraging in an active mode (Fenton and Bell, 1979; Kalko and Schnitzler, 1998). Contrary to *L. aurita*, these two gleaning bat species use echolocation calls, which tend to be shorter, higher, broader in bandwidth and quieter just like described for typical gleaning bats (Fenton and Bell, 1979; Geipel et al., 2013; Hackett et al., 2014; Kalko, 2004).

While the control was detected but apparently never classified as prey, the manually moved moth (M) was occasionally approached and attacked. However, it was also clearly not the preferred target. Objectively, the M target offers more potential cues for the bats, though. Bats could perceive prey movement by echolocation through analysing the 3D-information from successive echoes (Jensen et al., 2001). Successive echoes from moving objects differ in their spatial information. Thus, movement was detected and the target was classified as prey. Therefore, *L. aurita*, as other aerial hawking bats (Gessinger et al., 2019) seems to be more sensitive to moving than to stationary targets (Anderson and Racey, 1993; Jensen et al., 2001). The swinging movements of the moved moth may resemble cues available also in natural prey. This might be especially important in dense forest to distinguish prey from motionless obstacles, such as small leaves caught by spider webs.

Finally, the experiment showed clearly that *L. aurita* consistently preferred targets with wing beat (WB), as the number of approaches and attacks were significantly higher than those on the other targets. This result shows that *L. aurita* is able to distinguish between fluttering and non-fluttering prey. *L. aurita* repeatedly aborted attacks when fluttering prey items stopped flapping their wings. Theoretically, a WB target should be perceptible to bats by active echolocation, but under certain circumstances also by passive listening. The abortion of attacks could occur both because the passive listening cue and/or echolocation cue ceased. Because the bats often ignored moths at proximity unless they were moving, we suggest that the presence of the wing beat might promote the classification of targets as potential prey. In the following we will discuss both possible perception modes.

Passive listening

Wing movements of a moth are potentially perceptible by passive listening. Moth wing beat sounds are characterized by low intensities and frequencies often below 20 kHz (Anderson and Racey, 1993; Coles et al., 1989). Listening to low-intensity wing beat sounds of moths can potentially be crucial during prey detection (Anderson and Racey, 1993; Faure and Barclay, 1992; Simmons et al., 1979). And indeed, the

hearing of some gleaning bat species is well adapted to frequencies below 20kHz (Anderson and Racey, 1993; Coles et al., 1989; Geipel et al., 2021). Passive listening could be used to assess prey size and a rough species identification, due to relatively constant wing beat frequencies of moths (Anderson and Racey, 1993; Kober and Schnitzler, 1990). *L. aurita* has enormous tragus and large ears (Mantilla-Meluck and Montenegro, 2016). These structures act as sound pressure transformers amplifying low frequency sounds, and are therefore often found in bats adapted to passive listening (Anderson and Racey, 1993; Coles et al., 1989; Faure and Barclay, 1992). A number of these species are able to detect low frequency fluttering sounds (Coles et al., 1989; Faure and Barclay, 1992; Siemers and Swift, 2006). Because the insect was attached to a wire that was occasionally struck by its wings, the fluttering sound in our experiment may have been occasionally altered. We were able to record these sounds with our microphones while having difficulties recording the sounds of free flying moths. In contrast to mating calls that are often used by passive listening gleaning bats to find insect and/or vertebrate prey (e.g., Jones et al., 2016; Tuttle and Ryan, 1981), all in all the sounds of wing beats appear to be very silent. Therefore, in a loud and noisy environment such as a nocturnal tropical rainforest they should be rather unlikely cues.

Echolocation

We were able to record echolocation calls during approaches, independent of the target, suggesting that echolocation is used for prey localization, like in other echolocating bats (Thies et al., 1998). *Lonchorhina aurita* emits 5-7 ms long, constant-frequency echolocation calls during the search phase, ending with a frequency-modulated down-sweep (Gessinger et al., 2019; Chapter 2). While this is a unique call design for a phyllostomid bat, it is similar to the short CF-FM calls used by Old World leaf-nosed bats (Hipposideridae), as well as to an inverted FM-QCF call, as used by other edge space echolocators such as *Pipistrellus pipistrellus* (Kalko and Schnitzler, 1993). While long constant-frequency calls, such as those of *Rhinolophus ferrumequinum* or *Rhinolophus paradoxolophus* may cover several wing beat periods of prey insects per call and the returning echo may encode characteristic wing beat rate, e.g., in rhythmic amplitude peaks, wing size and other prey-specific information (Emde and Schnitzler, 1986; Schnitzler et al., 2003; Schoeppler et al., 2023), bats using short CF-FM calls, might perceive wing beats through small amplitude and frequency modulations, so-called ‘acoustic glints’ (Emde and Schnitzler, 1986; Schnitzler et al., 2003), occurring in the echoes of successive calls.

Insects with a pair of stiff wings produce one dominant glint per wing beat cycle. Most moths (Arctiidae, Noctuidae, Notodontidae) have wing beat rates between 30 and 50 Hz (Kober and Schnitzler, 1990). The higher the duty cycle (= ratio of pulse interval and pulse duration) of a calling bat, the higher the probability that an echo may contain a glint (Fenton et al., 2012). Therefore, high duty cycles (≥ 25%) allow bats to better detect fluttering prey in dense vegetation (Fenton et al., 2012; Lazure and Fenton, 2011). However, in flight cage experiments *L. aurita* showed a duty cycle of only 6 % during search phase and thus must be classified as a low duty cycle bat (Chapter 2). Due to the shorter calls compared to other constant-frequency bats, combined with longer intervals of silence, *L. aurita* may receive only

few call echoes containing a glint, which may still be sufficient to identify targets as prey by glints. However, our results do not allow to conclusively decide whether the bats used echolocation to classify WB targets as prey, if they used passive listening for prey classification or perhaps used a combination of these two options.

Conclusion

The results of the multiple-choice experiment suggest that cues associated with fluttering wings are especially important for the prey detection system of *L. aurita*. To the best of our knowledge, this is the first time this has been shown for a phyllostomid bat. As *L. aurita* also approached moving targets and occasionally also stationary dead prey items, the species does not rely exclusively on insect wing movement to detect prey, however, this may be one of the clearest prey cues. Depending on distance to prey and the acoustic background *L. aurita* might use a combination of echolocation and passive listening as described for other bat species (Faure and Barclay, 1992; Simmons et al., 1979). Entirely insectivorous families, such as Rhinolophidae and Hipposideridae, may solely utilize echolocation during hunting of aerial prey (Bell and Fenton, 1984; Emde and Schnitzler, 1990; Heller and Helversen, 1989). However, our results from a phyllostomid with unusual echolocation and hunting strategy, once more demonstrate that bats from this family may flexibly utilize several cues, especially if sensory modes other than echolocation may provide greater acuity for the perception and location of food. When foraging in dense forests, species of the phyllostomid family may use scent, or prey-generated acoustic cues (Bruns et al., 1989; Korine and Kalko, 2005). Therefore, the extremely high foraging diversity of phyllostomid bats compared to other bat families may be due to a higher sensory flexibility used for food acquisition (Bruns et al., 1989; Korine and Kalko, 2005; Winter et al., 2003), which ultimately allows the family to use an extremely wide range of resources and has led to the impressive adaptive radiation of the family.

Acknowledgements

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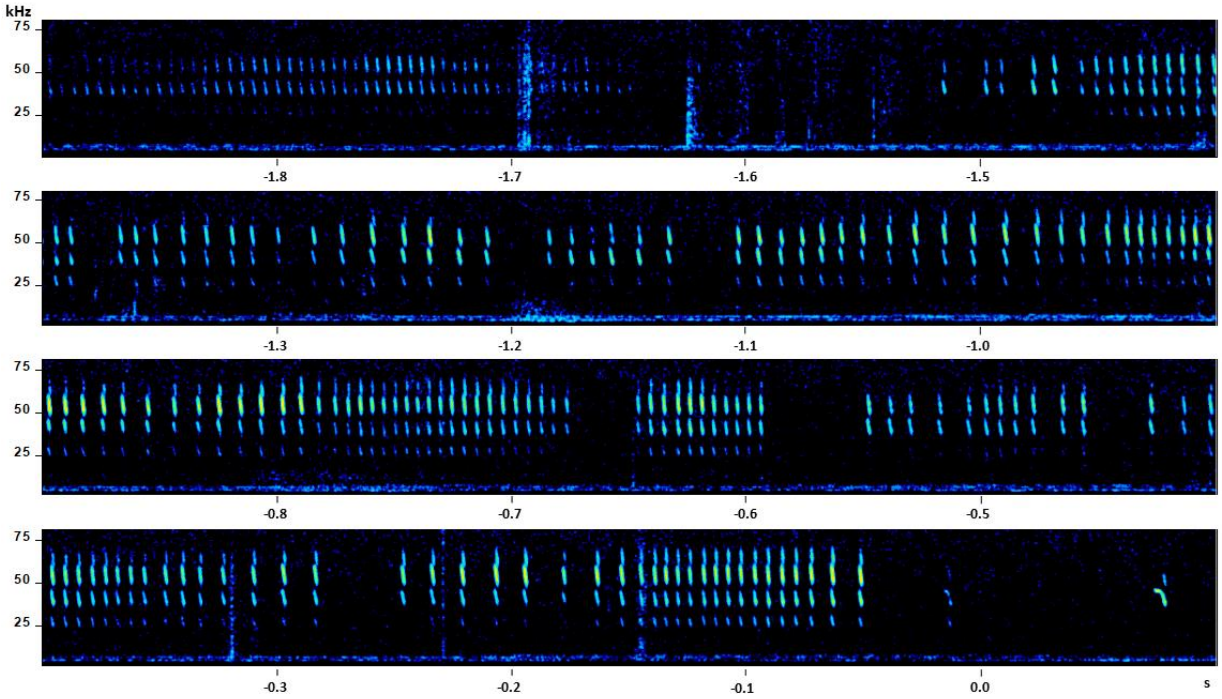
Supplementary material

Supplementary Video 1: High speed video of L. aurita attacking a tethered moth. The bat removed the moth from the wire, guiding it into its uropatagium and grabbed it with the mouth before taking off.



Supplementary Video 2: High speed video of an aborted attack after the moth stopped flapping its wings.





Supplementary Figure 1: Spectrogram of the echolocation calls emitted by Lonchorhina aurita hovering in front of a wing beat target after an unsuccessful attack (t=0.3). FFT 512, Hamming window, 75 % overlap.

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2020 Bernal Rodriguez-Herrera, Gloria Gessinger, Marco Tschapka**.** The masked seducer: Lek courtship behaviour in the Wrinkled-faced bat *Centurio senex* (Phyllostomidae), *Tagung Deutscher Fledermausforscher*. Fraueninsel im Chiemsee, Deutschland. Vortrag

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Declaration of Autonomy for written assignments, especially theses

Declaration of autonomy

I, Gloria Gessinger, hereby declare, that I have written this thesis entitled

“On the sensory ecology of phyllostomid bats”

independently and without outside assistance and have fully acknowledged all sources and tools used in this thesis.

Regarding the use of tools based on Artificial Intelligence (e.g., DeepL), I declare that the use of AI-based tools was permitted by the examiners. My examination performance or any part of it was not replaced by these tools. I am prepared to explain the possible use of AI-based tools with appropriate documentation (e.g., chat logs) if requested by the examiners in case any clarification is needed.

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