

# Nonecholocating Fruit Bats Produce Biosonar Clicks with Their Wings

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## Summary

Because evolution mostly acts over millions of years, the intermediate steps leading to a functional sensory system remain enigmatic [1–3]. Accordingly, there is an ongoing debate regarding the evolution of bat echolocation [4–10]. In search of the origin of bat echolocation, we studied how Old World fruit bats, which have always been classified as nonecholocating [3, 10–12], orient in complete darkness. We found that two of these nonecholocating species used click-like sounds to detect and discriminate objects in complete darkness. However, we discovered that this click-based echo sensing is rudimentary and does not allow these bats to estimate distance accurately as all other echolocating bats can. Moreover, unlike all other echolocating bats, which generate pulses using the larynx or the tongue, these bats generated clicks with their wings. We provide evidence suggesting that all Old World fruit bats can click with their wings. Although this click-based echo sensing used by Old World fruit bats may not represent the ancestral form of current (laryngeal) bat echolocation, we argue that clicking fruit bats could be considered behavioral fossils, opening a window to study the evolution of echolocation.

## Results and Discussion

The evolution of sensory systems has always intrigued scientists: how does a rudimentary sense evolve into a more sophisticated one [1–3]? Echolocation, the ability to perceive the world acoustically by emitting sound pulses and analyzing the returning echoes, has evolved in at least three different animal groups (birds [13], cetaceans [14, 15], and bats [16]), and possibly also other mammals such as in shrews [17] and tenrecs [18] (see Table 1). There is an ongoing debate as to whether bat echolocation evolved once or twice [4–10]. It is, however, agreed that more than 1,200 bat species [21] can be divided into ~85% echolocating species and ~15% Old World fruit bats (family Pteropodidae) that are currently classified as nonecholocating [3, 10–12], with the exception of a few species in the genus *Rousettus* that use lingual echolocation [22, 23]. Being nocturnal mammals, “nonecholocating” Old World fruit bats often fly at very low light levels. In 1988, Gould showed that one species of Old World fruit bat (*Eonycteris spelaea*) produces click sounds in the dark by wing clapping, but this study was not able to prove the functionality of the clicks [24]. Moreover, all previous studies have shown that

Old World fruit bats lack all of the genetic and physiological adaptations that are typically associated with echolocation [11, 12, 25]. We were therefore compelled to investigate how these bats orient in darkness.

We recorded individuals from three subfamilies that are widely spread within the Old World fruit bat phylogenetic tree [26] (eight *Eonycteris spelaea*, eight *Cynopterus brachyotis*, and three *Macroglossus sobrinus*) while they were flying in “complete darkness” ( $< 5 \times 10^{-6}$  lux), which precluded the use of vision, as we confirmed experimentally.

## Fruit Bats Emit Brief Clicks in the Dark

When flying in the dark, all 19 wild individuals of all three species emitted brief sound signals (300–350  $\mu$ s) with a click-like structure (only three to eight wave periods) that were audible to the human ear (Figure 1). The average peak frequency of the signals was  $17 \pm 1$  kHz (cross-species mean  $\pm$  SD), which is lower than that of *Rousettus* clicks [23, 27] but much higher than the click frequency of echolocating birds [28]. Moreover, clicks were wideband, with energy in high frequencies and a bandwidth of  $55 \pm 2$  kHz. At least one of our three species (*Cynopterus brachyotis*) has been shown to hear frequencies up to ~70 kHz [29]. The time interval between adjacent clicks in *Eonycteris* and *Cynopterus* was distributed around 100 ms, whereas in *Macroglossus* it was shorter (~80 ms; Figure S1 available online).

## Clicking Allows Functional Sensing in the Dark

We next aimed to reveal whether these clicks are used in a functional manner for sensing in the dark. We flew bats in the same tunnel as before, this time under light (~5 lux). For each individual, we ran four randomly ordered sessions: two in the dark and two in the light, with at least six trials per bat at each session (Supplemental Experimental Procedures). All *Eonycteris* and *Cynopterus* bats clicked significantly more in the dark. These bats significantly increased their click rate in the dark, by 3.5- to 5-fold on average (Figure 2A). *Macroglossus* bats also increased their click rate in the dark, but they clicked less than the other two species, and their increase was not significant (Figure 2A).

Moreover, in a second experiment in which two of the species flew in a large space in an attempt to discriminate between two objects (see details below), *Eonycteris* and *Cynopterus* bats increased their click rate in the dark even more dramatically, with *Eonycteris* clicking as much as 7.5 clicks per second—the click rate of the echolocating bat *Rousettus* [30] (Figure 2B).

These spontaneous adjustments in click rate in wild untrained bats strongly imply that clicking is a natural behavior for these bats. Are these clicks used for sensing in the dark? Because *Macroglossus* bats did not increase clicking significantly, we focused on the other two species. We first tested the bats with the classical wire avoidance paradigm. Here, 12 thick (20 mm diameter) cables were hung across the flight room. Although these cables were ~100 times thicker than echolocating bats are able to detect [31–33], our bats failed to detect them and constantly crashed into them. We therefore tested the bats in a more basic detection task. We trained 12 bats (six *Eonycteris* and six *Cynopterus*) to acoustically

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Table 1. Echolocation in the Animal Kingdom

Animal	Sound Type	Frequency (kHz)	Source	Functional Distance	Functionality	Ranging Ability	Reference
Shrews (Soricinae)	QCF pulses <sup>a</sup>	25–60	larynx	<0.3 m	holes, surfaces	?	[17, 19]
Tenrecs	clicks <sup>a</sup>	9–17	tongue	<0.3 m	surface detection	?	[18]
Rat	clicks <sup>a</sup>	40–50	teeth	<0.3 m	surface detection	?	[19]
Toothed whales	clicks <sup>b</sup>	30–160	sonic lips	0.05–150 m	full imaging <sup>c</sup>	proven	[20]
Swiftlets	clicks <sup>a</sup>	3–8	syrinx	0.1–8 m	large obstacles	?	[13]
Oilbird	clicks <sup>a</sup>	6–10	syrinx	0.1–8 m	large obstacles	?	[13]

<sup>a</sup>Uncontrolled/less stable.

<sup>b</sup>Controlled/stable.

<sup>c</sup>Sonar detection of prey demonstrated.

discriminate between two large (90 × 90 cm) objects: a highly acoustically reflective black board and a sheet of cloth that was identical in size, color, and odor but was 19 dB less reflective (Supplemental Experimental Procedures). The bats had to detect the objects, to discriminate between them, and then to land on the more acoustically reflective object in a completely dark (<5 × 10<sup>-6</sup> lux), large anechoic room. The objects were randomly repositioned in the room after each landing (or touching; Supplemental Experimental Procedures).

Our goal was to test whether bats can use these emitted clicks functionally as echolocation. We therefore ceased training after observing that all 12 bats quickly learned the task and that each of them landed on the correct target in at least 70% of the trials (see nights five to six in Figure 2C). However, only nine bats flew enough for us to assess their performance statistically (Supplemental Experimental Procedures). Seven of these nine bats (four *Eonycteris* and three *Cynopterus*) performed significantly above chance level ( $p < 0.05$  for each bat, binomial test). The ability of the bats to perform this task proves that they are capable of using biosonar to detect, localize, and discriminate large objects. Interestingly, many researchers have suggested that bats first evolved echolocation in order to detect large objects such as cave walls (see [34] for a review). Echolocation tasks that require fine detection and accurate localization, such as the wire avoidance task that we tested (above), are too difficult for these bats to solve. This is probably why Gould [24], who had *Eonycteris* bats flying through multiple bars, could not show that clicking was functional.

To further prove functionality, we also tried to disrupt the bats' ability to echolocate by playing loud white noise when they were performing the discrimination task. However, the bats refused to fly under these conditions. Plugging the ears of our bats proved very difficult. Moreover, this manipulation will mostly not completely eliminate hearing and might stress the animal, thus disrupting its behavior [22].

### Fruit Bat Clicking Represents a Less Sophisticated Form of Bat Echolocation

Although bats learned the task and were able to detect the objects and discriminate between them, in certain sensory aspects, mainly localization, their performance was poor in comparison to other echolocating bats. Various species of echolocating bats (including *Rousettus*; see Movie S1) have been shown to smoothly land on small targets while relying on echolocation [23, 35]. Despite the target being very large, our bats mostly required several attempts in order to land, often crashing into the target in an uncontrolled manner (Movie S2). In contrast, under light (even very dim light, <1lux), bats always landed smoothly on the landing platform on the first attempt (Movie S3).

It is extremely difficult to definitely prove that a bat cannot estimate range, because every experiment showing that an animal cannot perform a task might be a result of an inadequate experimental design. However, the bats' behavior strongly suggested that these fruit bats cannot estimate the accurate range of an object, based on the time delay between sound emission and echo reception, as all other bats can [36, 37]. The ability to detect and discriminate objects in the dark, but the inability to smoothly land on them, strongly implies that clicking is a functional, yet rudimentary form of echolocation. Estimating range is considered a hallmark of bat echolocation. The presumed inability of our bats to accurately estimate range therefore suggests that they represent a less sophisticated stage of echolocation.

### Clicks Are Generated with the Wings

We found that clicks were generated by the wings and not by the larynx or tongue as in all other echolocating bats. Several findings support this conclusion. (1) High-speed video of flying bats revealed that their mouth remained closed during sound production. In orally echolocating bats (including *Rousettus*), the mouth is open while emitting (Figure S2A). (2) Clicking was perfectly synchronized with wingbeat (Figure S2B). (3) The clicks' temporal structure varied greatly, suggesting a noisy sound production mechanism in contrast to laryngeal or lingual emission (Figure S2C). (4) Sealing the bats' mouths (two *Eonycteris* and one *Cynopterus*; Supplemental Experimental Procedures) did not stop clicking. (5) To further confirm that sound was produced by the wings, we impaired the right wing of the two best clickers (one *Eonycteris* and one *Cynopterus*) by taping three layers of insulating tape onto the leading edge of their right wing forearms. We flew these two bats in complete darkness. Both bats could fly in this situation, but the extra weight on their right wing made the wingbeat nonsymmetric, with the impaired wing lagging behind the other one. Both bats completely stopped clicking in this situation.

We confirmed that the bats' wingbeat rate did not differ in light versus dark, negating the possibility that the differences in click rate merely reflected a change in the wingbeat rate (Wilcoxon test,  $p > 0.05$  for all three species; Supplemental Experimental Procedures).

Gould [24] hypothesized that *Eonycteris* clicks were produced by wing clapping. Even though we prove that clicks are generated by the wings, we are not convinced that the act of the wings touching each other is the mechanism. Gould showed that the wings touch each other during flight; however, he did not establish a causal link between wing touching and clicking by manipulating the first to alter the latter. Moreover, he mentioned that touching occurred in the dark but sometimes also in the light. We analyzed high-speed videos of flying

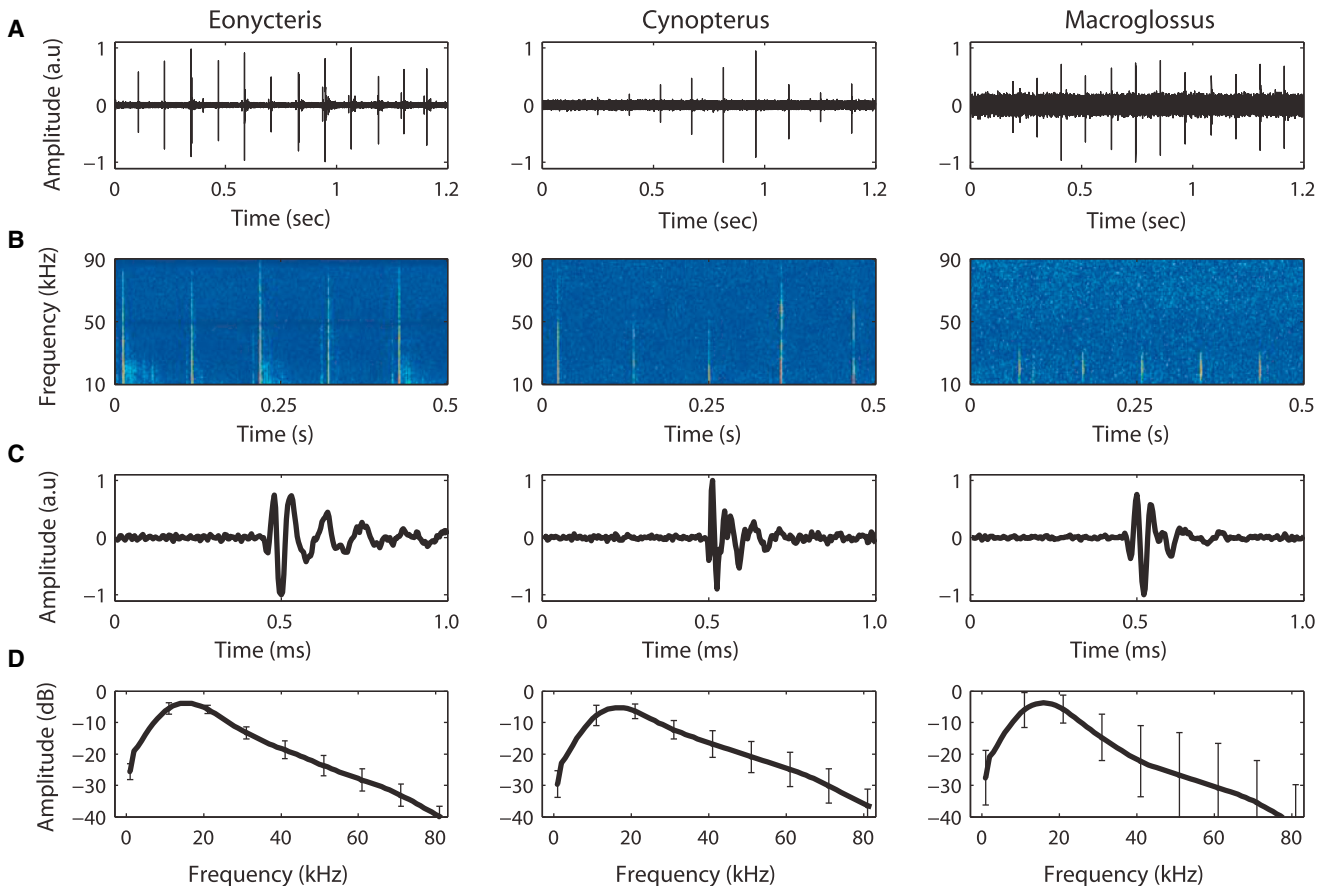


Figure 1. All Fruit Bats Emitted Brief Click-like Signals in the Dark

(A) Waveform example of a clicking sequence for each of the three fruit bat species (the sampling rate was 250 kHz). Such continuous clicking sequences for *MacroGLOSSUS* were rare.

(B) Spectrogram of a 0.5 s segment of a clicking sequence (calculated with a 512 sample window and 500 sample overlap).

(C) Example of a single click for each species.

(D) Average click spectrum for each species (the average of approximately 200, 1,000, and 2,000 click spectra for *MacroGLOSSUS*, *Eonycteris*, and *Cynopterus*, respectively, with at least 60 clicks for each individual bat). Notice the considerable energy content in high frequencies—up to at least 50 kHz. Spectra were calculated using the Fourier transform (256 sample window). Because this analysis is not always suitable for such brief clicks, we also analyzed the clicks using the instantaneous frequency method. With this method, the average peak frequency was  $12 \pm 1$  kHz, and the bandwidth was  $56 \pm 2$  kHz (mean  $\pm$  SD).

See also [Figure S1](#).

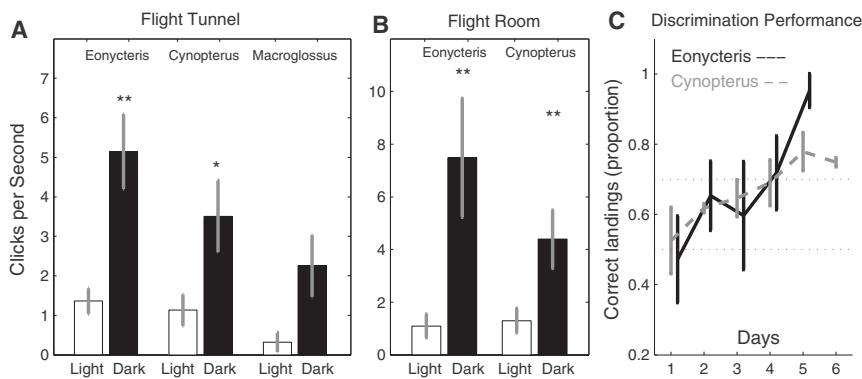
bats but were unable to find a clear difference between the wing cycle in dark versus light—the wings seemed to touch in both cases. Moreover, when we padded the forearms of (two *Eonycteris* and two *Cynopterus*) bats by taping foam on both sides, the clicking did not stop. There are many mechanisms to produce clicks by the wings, including (1) two parts of one wing touching each other, (2) a wing touching the body, or (3) snapping of bones. At least two of these mechanisms were shown to occur in birds [38]. More research is necessary in order to determine the exact sound production mechanism.

How is it possible that these clicks were overlooked during so many years of studying echolocation? The acoustic characteristics of such brief clicks make them very hard to record without an extremely quiet and anechoic environment and without highly sensitive equipment. The signals are much weaker than those of lingual or laryngeal echolocating bats [27, 39–41], and their click-like characteristics with energy that spreads into low frequencies make them difficult to

distinguish from noise and easy to overlook by echolocation researchers who are used to tonal signals. Moreover, unlike laryngeal emitters, which always echolocate, Old World fruit bats probably click only in specific situations, such as in extreme darkness.

### The Evolution of Echolocation

Rudimentary echolocation does not seem to require any adaptations beyond the ability to produce sound (e.g., clicks) and analyze sound (echoes). Other mammals (e.g., shrews [17] and tenrecs [18]; see [Table 1](#)) have been suggested to use echolocation, and many other mammals can probably be trained to use simple echolocation. This has been demonstrated in humans [42]. A main approach for studying the origin of echolocation is examining ancient bat fossils [43]. For instance, researchers compare the relative size of hearing-related organs (e.g., the cochlea, the stylohyal bone, etc.) in fossil bats with those of extant bat species in an attempt to infer use of echolocation by ancient bats [43–45]. Other studies



**Figure 2. Clicking Is Functional, Facilitating a Rudimentary Form of Echolocation**

(A and B) Clicking in light versus dark conditions (A) in the confined tunnel and (B) during the discrimination task in a large flight room. In both experiments, the average number of clicks per second was computed (Supplemental Experimental Procedures). In the tunnel (A), *Eonycteris* increased clicking from  $1.2 \pm 1.1$  to  $5.1 \pm 2.8$  and *Cynopterus* from  $1.3 \pm 1.0$  to  $3.5 \pm 2.4$  clicks per second (mean  $\pm$  SD for all eight bats of each species;  $p = 0.003$  and  $p = 0.02$ , respectively). *Macroglossus* increased clicking from  $0.3 \pm 0.4$  to  $2.1 \pm 1.3$  clicks per second ( $n = 3$  bats,  $p = 0.25$ ). In the discrimination task (B), *Eonycteris* increased clicking from  $1.1 \pm 0.7$  to  $7.5 \pm 3.8$  and *Cynopterus* from  $1.2 \pm 0.8$  to  $4.5 \pm 3.8$  clicks per second ( $n = 6$  bats for both species;  $p = 0.002$

and  $p = 0.004$ , respectively). In both (A) and (B), we ran the nonparametric Wilcoxon ranked-sum test for paired sampled data. \* $p < 0.05$ , \*\* $p < 0.01$ . (C) Landing performance (proportion of correct landings) in the object discrimination task. In total we monitored 433 landing events for all 12 bats. The mean and SD for all (six) individuals of each species are presented. Horizontal dashed lines depict the chance level (0.5) and learning criterion (0.7). See also Figures S2 and S3.

use a comparative approach and study relevant genes such as *Prestin* [12, 14] or search for adaptations in hearing related organs such as the cochlea [25]. None of these suggested adaptations, however, are present in Old World fruit bats, including the clicking *Rousettus* bats. These adaptations therefore do not allow distinguishing between echolocating and nonecholocating bats, but rather between laryngeal echolocating bats and the remaining species. It is therefore impossible to determine whether a fossil bat used (rudimentary) echolocation based on these adaptations.

Our results suggest that generating clicks with the wings is a general ability of Old World fruit bats (Figure 3). The three species that we studied do not represent a monophyletic group [26] within the Old World fruit bat tree, and clicking has also been recorded in another genus [46]. Moreover, we have recently recorded wing clicks produced by wild *Rousettus aegyptiacus* bats while they were flying in the field, in addition to their lingual echolocation clicks (Figure S3). A maximum-likelihood analysis (Supplemental Experimental Procedures) suggests a probability of 10% that wing clicking was a trait exhibited by the common ancestor of all Old World fruit bats. This analysis was performed with the most conservative assumption that all Old World fruit bats except for the five discussed above do not click. This probability is thus high when the high uncertainty is taken into account, and studying more species will likely reveal many more clicking species.

Even though it seems to be a common ability, differences in the tendency to click surely exist within the Old World fruit bat family, and they probably reflect different life styles. Being a typical cave dweller, *Eonycteris* often has to fly in complete darkness, and in accordance, it also exhibited the highest click rate. *Macroglossus*, on the other hand, is a tree-dwelling species and a very slow flier, giving it ample time to sense its surroundings with a low risk of collision. Accordingly, it exhibited fewer clicks than the other two and did not significantly increase its click rate in the dark.

The finding that Old World fruit bats use rudimentary echolocation challenges our current understanding of the evolution of bat echolocation. There is an ongoing debate as to whether bat echolocation evolved once or twice [4–10]. Bats of the genus *Rousettus* evolved their lingual echolocation independently according to both hypotheses. More research is necessary in order to determine whether the rudimentary

echolocation that we report here eventually evolved into the sophisticated echolocation of *Rousettus* bats. The fact that *Eonycteris*, the best echocator among the three species, is a close relative of *Rousettus* [26] supports this idea. Alternatively, if *Eonycteris* echolocation does not represent an intermediate stage of *Rousettus* echolocation, our results suggest that echolocation in bats has evolved more times than previously believed. The cost of shouting while flying has often been suggested as a bottleneck for the evolution of echolocation [47]. Wing clicking offers a simple mechanism by which echolocation could have evolved with minimal energetic costs, at least in Old World fruit bats. Interestingly, click-like signals have evolved at least four times to serve the function of echolocation: in toothed whales, in echolocating birds (where they evolved twice independently) [13, 14, 16], in bats [24, 27], and maybe even in other mammals (Table 1). We believe that fruit bats are behavioral fossils, presenting an ancient sensory behavior that (even if recently evolved) allows a rare glimpse at the evolution of a sensory system. We suggest that a deeper analysis of the (genetic and morphologic) differences between bats that rely on clicking to a different degree (e.g., *Eonycteris* versus *Rousettus*) might provide new insights on the evolution of (click-based) echolocation in nature.

#### Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and three movies and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2014.10.077>.

#### Author Contributions

Experimental data were collected by A.B., S.B., and Y.Y. Experimental data were analyzed by A.B. and Y.Y. All authors discussed the results. A.B. and Y.Y. wrote the paper.

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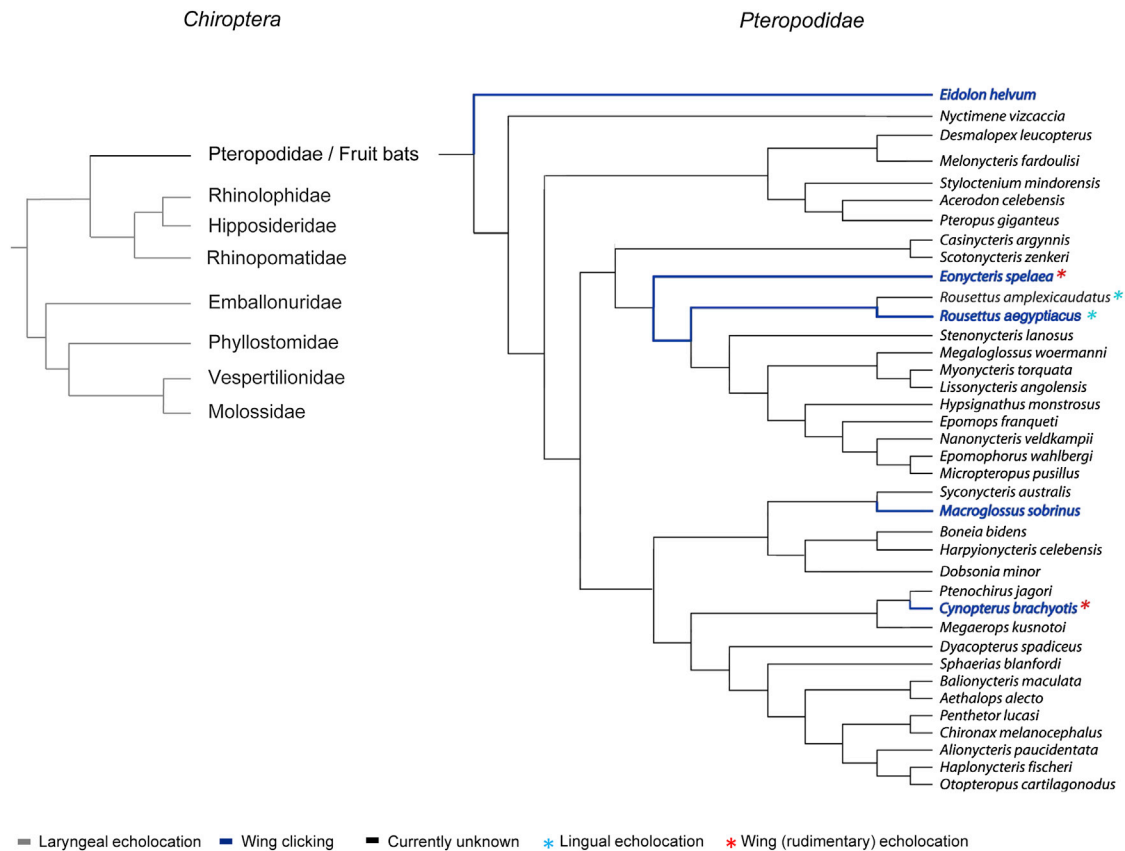


Figure 3. The Tree of Bat Echolocation

Current knowledge about the different types of Old World fruit bat echolocation mapped on top of their phylogenetic tree. The tree was adapted with permission from Figure 1 in [26]. Colors and symbols represent types of sound production and echolocation in genera in the fruit bat family. These include sophisticated echolocators (e.g., *Rousettus*), rudimentary (wing) echolocators (e.g., *Eonycteris* and *Cynopterus*), clicking bats that have not yet been studied (e.g., *Macroglossus* and *Eidolon*), and finally, many species for which nothing is known. Branches leading to ancestors that had a probability of more than 50% to click based on the maximum-likelihood analysis are colored blue. We suggest that comparison of closely related species with different levels of echolocation (e.g., *Eonycteris* and *Rousettus*) might provide new insights about the evolution of echolocation.

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