"The vocal apparatus of bats: an understudied tool to reconstruct the evolutionary history of echolocation?"

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Abstract:

Until recently, bat phylogeny separated megabats (laryngeally non-echolocators) and microbats (all laryngeal echolocators) into two distinct clades. This segregation was consistent with the assumption that laryngeal echolocation was acquired by a common ancestor and inherited by all microchiropterans. Thus, laryngeal echolocation was regarded to have evolved once. Recent advances in bat genome sequencing have added insights regarding the origin of bats and their phylogenetic relationships. The megabats previously thought to be phylogenetically isolated are now sharing a common ancestor with the superfamily Rhinolophoidea, which is constituted of laryngeal echolocators. This new relationship brings a counterpoint regarding the hypothesis of single origin of echolocation, which could have appeared several times independently. Concomitantly, recent studies in bat evolutionary developmental biology have illuminated the importance of characters from embryonic development to discussions of how and when echolocation evolved. Here, we describe how comparative chiropteran laryngeal morphology is a novel area of research that could improve the understanding of echolocation and may help resolve the evolutionary history of bats. This review provides morphological descriptions and comparisons of the larynx, bioacoustics interpretations, and newly developed visualisation approaches (i.e., contrast-enhanced computed tomography). We discuss the importance of understanding (1) laryngeal sound production so it may be linked with the evolution of the Chiropteran auditory system and (2) the evolution of laryngeal morphology to understand the ecological and behavioural aspects of bat biology.

(227 / 250 words)

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Main Text:

1) New phylogeny, new evolutionary scenario:

Bats are the second most diversified group of mammals (Simmons 2005), reflecting their spectacular evolutionary features: self-powered flight (Rayner 1988) and echolocation, a form of biosonar (Griffin 1944; Thomas et al. 2004). Most bats use laryngeal echolocation which requires three main capacities (Teeling 2009): production of high-frequency vocalisations with the larynx; reception of the echoes with the auditory apparatus; and processing of the acoustic information in dedicated brain areas, translating to different behaviour and feeding strategies in flight. Accordingly, these three anatomical regions should work in concert, illustrating the coevolution of how and when bats developed biosonar capabilities in their evolutionary history. The two sensory systems (nervous and auditory) that detect, transduce, and analyse echoes have already attracted considerable research interest (e.g., Baron et al. 1996; Hutcheon et al. 2002; Davies et al. 2013; Nojiri et al. 2021a; Sulser et al. 2022), whereas the evolution of the bat larynx has received far less attention. Essential anatomical and developmental work remains to be carried out on the range of laryngeal forms of the different bat taxa and close relatives.

Previous research on the bat larynx focused on understanding how they produce laryngeal echolocation signals in general (Novick and Griffin 1961; Roberts 1972; Suthers and Fattu 1973; Griffiths 1983; Griffiths et al. 1992). Bats' requirements to accommodate high subglottic pressure for laryngeal echolocation (Suthers 2004; Frey and Gebler 2010; Metzner and Schuller 2010; Metzner and Muller 2016) suggests considerable specialisation of the organ, especially regarding its size, in the process of call production. It is also known that bats' larynges are disproportionally large compared to other mammals of similar size due to sexual dimorphism in Pteropodidae (Langevin and Barclay 1990). Although there has been some recent focus on the larynx (Carter and Adams 2014, 2016; Carter 2020; Nojiri et al. 2021a; Snipes and Carter 2022), we suggest that further research is needed to unravel the extent and patterning of variation in this organ as it relates to echolocation capability.

This paper reviews current knowledge and understanding of the bat larynx. We illustrate that new studies on the laryngeal morphology of bats could significantly improve the understanding of laryngeal echolocation and expand the scope of evidence that can be brought to bear on the unsettled debate surrounding the evolutionary history of bats. The discovery and brief descriptions of laryngeal forms across bat phylogeny are essential to the assessment of a shared phylogenetic or functional relationship, potentially related to laryngeal echolocation (Dobson 1881; Robin 1881; Elias 1907; Denny 1976; Griffiths 1983; Harrison 1995). Therefore, a description of the laryngeal forms and a comparison of echolocation strategies is warranted to bring new insights into the much-debated topic of echolocation origins (Brudzynski 2010; Luo et al. 2017), and to provide evidence regarding whether the evolution of laryngeal features may be consistent with a single or multiple origins of laryngeal echolocation.

Regarding the evolutionary history of bats, controversies inside the Order Chiroptera started with classifying the five superfamilies (e.g., Simmons 2005; Tsagkogeorga et al. 2013). Primarily bats were divided into two clades, the Microchiroptera and the Megachiroptera (Simmons and Geisler 1998; Simmons 2005). With the development of molecular phylogenetics, bats have been regrouped into two suborders, Yinpterochiroptera and Yangochiroptera, which diverged around 57 to 55 mya (Teeling 2009; Teeling et al. 2012; Jones et al. 2013; Tsagkogeorga et al. 2013; Zhang et al. 2013; Teeling et al. 2016). This revised phylogeny created a morphological conundrum as it grouped some laryngeal echolocators with the non-echolocators. Inside the non-laryngeal echolocators, several species of fruit bats are reported to use echolocation by tongue-clicking and/or wing-flapping (Yovel et al. 2011; Boonman et al. 2014). Two main hypothetical models were assessed to account for the evolutionary history that resulted in separate vocal production mechanisms appearing in the same clade of bats (Simmons 2005; Teeling 2009; Davies et al. 2013; Jebb et al. 2020). Laryngeal echolocation might have evolved once at the origin of bats, followed by loss in Pteropodidae (single-origin hypothesis, H1), or laryngeal echolocation might have appeared multiple times independently in the different superfamilies of bats by convergence (independent origin hypothesis, H2). If we distinguish primitive echolocation from laryngeal echolocation, H2 can be divided into two more sub hypotheses (H2A & H2B) (Nojiri et al. 2021a). H2A proposes that laryngeal echolocation evolved independently in Rhinolophoidea and Yangochiroptera without the emergence of primitive echolocation such as tongue-clicking or wing-beating echolocation ability in the common ancestor. H2B suggests that the common ancestor developed primitive echolocation ability, and then Rhinolophoidea and Yangochiroptera developed laryngeal echolocation independently. To date, no consensus has been reached on a single hypothesis, because studies addressing this topic have focused on different areas of research (e.g., genomic, comparative anatomy, palaeontology) and have furnished different perspectives and interpretations (Veselka et al. 2010; Teeling et al. 2016; Fenton 2022).

Comparisons of fossils and extant adult morphology support the conclusion that laryngeal echolocation emerged from a common ancestor (H1) (Simmons et al. 2010; Veselka et al. 2010). Recent evidence detailing the morphology and prenatal development of the hearing apparatus supports the independent origin hypothesis (H2) (Davies et al. 2013; Nojiri et al. 2021a). Although the origin of laryngeal echolocation is still debated, the loss of the ability to echolocate by the Pteropodidae (H1, H2B) is supported by the retention of laryngeal echolocation-associated features such as relatively large cochlea compared to other mammals and some capacity to emit social calls, in a similar manner to laryngeal echolocators (Springer et al. 2001; Nojiri et al. 2021a). Novacek (1985) posits that biosonar likely arose in Eocene bats that could echolocate in a less advanced way than extant bats families (Simmons and Geisler 1998; Wible and Davis 2000). Schnitzler et al. (2004) and later Maltby et al. (2010) propose that the Eocene bat vocal apparatus could probably produce only relatively short, multi-harmonic, and quite narrow-frequency calls because specific calls were not needed during gliding onto vegetation and gleaning food from the surroundings. Despite its rudimentary capacities, the biosonar of primitive bats may have provided orientation, obstacle avoidance (Fenton et al. 1995), and foraging functionalities (Schnitzler et al. 2003; Jones et al. 2016). Unfortunately, some of these studies are based on fossils with damaged and possibly distorted anatomical features, therefore interpretations may not be conclusive (Veselka et al. 2010). Additionally, the fossil record of the Pteropodidae is poorly known (Teeling et al. 2005; Eiting and Gunnell 2009), so reconstructing the evolutionary history of this family remains challenging.

The molecular basis of echolocation has also been investigated (Li et al. 2007, 2008; Li et al. 2010; Liu et al. 2010, 2014; Parker et al. 2013). *Prestin, FoxP2, KCNQ4, TECPR2,* and *TJP2*, for example, are potential genes whose evolutionary history has been explored, of which *FoxP2* and *Prestin* have the strongest association with echolocation capacity. Those two genes (*FoxP2* and *Prestin*) have provided conflicting evolutionary scenarios supporting H1 or H2 (Li et al. 2007, 2008), such that it would be premature to make firm conclusions on the evolutionary history of echolocation in bats (Teeling 2009). Further exploration of the genetic basis of mammalian echolocation has been suggested to illustrate potential convergent phenotypes as illustrated by these genes (Liu et al. 2014). Recently, Jebb et al. (2020) generated the first reference-quality genomes of six bat species (*Rhinolophus ferrumequinum*, *Rousettus aegyptiacus*, *Phyllostomus discolor*, *Myotis myotis*, *Pipistrellus kuhlii* and *Molossus molossus*) and found three hearing-related genes (*LRP2*, *SERPINB6*, and *TJP2*) in the ancestral branch of bats. These results may support the single origin hypothesis (H1). To date, no specific evolutionary scenario is supported by genes related to sound production. Thus, an independent origin of laryngeal echolocation (H2A) cannot be rejected.

Ontogeny is another area that has been explored and which provided some arguments to the debate (e.g., Lancaster et al. 1990; Pedersen 1993; Carter and Adam 2014; Nojiri et al. 2021a). Often neglected, developmental variation among organs linked to laryngeal echolocation can reflect the evolutionary history and illustrate the phylogenetic links between the bat clades. Some research has focused on understanding laryngeal echolocation and the various strategies to produce these high-frequency sounds through observations of the larynx modifications during prenatal development and postnatal growth (Pedersen 1998, 2000; Carter and Adams 2014; Carter 2020). Recent studies have expressed the possibility that the independent origin hypothesis (H2) is a more realistic scenario than the single-origin hypothesis (H1) (Nojiri et al. 2021a; Sulser et al. 2022). The connection between the tympanic bone and stylohyoid chain, as well as turns in the cochlea shape, are similar in Rhinolophoidae and Yangochiroptera. The observation of different developmental patterns resulting in similar adult morphology of the organ provides evidence for the convergent evolution of laryngeal echolocation, supporting the H2 hypothesis (Nojiri et al. 2021a).

The conflicting hypotheses about the evolution of laryngeal echolocation express the complexity and high species diversity inside the bat phylogeny and each finds support in different research areas. To further test these hypotheses (between H1 and H2, and also H2A and H2B), one direction to be investigated further is the relatively under-studied larynx. Research on the vocal apparatus of bats has lagged far behind other morphological traits (e.g., the hearing apparatus) due to the non-representation of soft tissues (cartilages and muscles) in the fossil records. Additionally, prior to the advent of non-destructive soft-tissue visualization techniques such as contrast enhanced X-ray microtomography (Metscher 2009; Jeffery et al. 2011; Vickerton et al. 2013; Boyde et al. 2014; Gignac et al. 2016), studying the larynx of bats involved destructive techniques by opening and dissecting the throat of the animals. This has constrained earlier research drastically because museums are usually reluctant to destroy or damage their specimens. As a result, the discussion about the implication of the laryngeal forms in the evolution of laryngeal echolocation is still fully open to further research and discoveries.

2) Laryngeal anatomy:

a. General aspect:

Vocalisations are an essential factor in the evolution and survival of vertebrate species as they impact sexual competition and interactions between individuals through vocal sound production (Fitch and Hauser 2002; Taylor and Reby 2010; Bradbury and Vehrencamp 2011; Wilkins et al. 2013). Mammals produce vocalisations in a great variety of frequencies and amplitudes. Understanding sound production through the implication of different anatomical features is critical to build the evolutionary history of vocal communications in mammals and to explain the great diversity encountered (Borgard et al. 2020). There are two theories of sound production and control: the source-filter theory (Titze and Martin 1998; Taylor and Reby 2010) and the myoelastic-aerodynamic (MEAD) theory (van den Berg 1958; Titze and Alipour 2006; Švec et al. 2021).

The source-filter theory suggests that sound production needs independent contributions from two components. The larynx is the first component representing a source producing a fundamental frequency (F0). From the supralaryngeal space to the mouth or nose, the vocal tract forms a filter adding or cancelling harmonics and modifying the amplitude of the sound (Titze and Martin 1998; Taylor and Reby 2010; Brown and Riede 2017). In terms of the source, laryngeal morphology remains relatively constant through the different orders of mammals (Negus 1949; Harrison 1995; Saigusa 2011). Five intrinsic muscles (thyroarytenoid, lateral cricoarytenoid, transverse arytenoid, dorsal cricoarytenoid, and cricothyroid) originate and insert on four principal cartilages (one thyroid, one cricoid and a pair of arytenoids) (e.g., Negus 1949; Harrison 1995; Hoh 2005, 2010; Saigusa 2011; König et al. 2020). These laryngeal muscles tilt the thyroid and arytenoids cartilages during phonation, adducting and abducting a pair of multi-layered membranes, the vocal folds (e.g., Harrison 1995; Metzner and Muller 2016; Brown and Riede 2017). Two branches of the vagus nerve innervate the laryngeal muscles and control phonation: the cranial laryngeal nerve (also referred as the “superior laryngeal nerve” in physical anthropology and in some recent literature studying bats) commands the activity of the cricothyroid muscle, and the caudal (recurrent) laryngeal nerve controls the other intrinsic muscles (Harrison 1995; Brudzynski 2010; Hoh 2010; König et al. 2020). These synapomorphies of the mammalian larynx raise questions regarding how mammals are able to produce different F0 despite having similar laryngeal morphology. Bats are a perfect example with the production of highly specialised high-frequency vocalisations.

The MEAD theory provides complementary and detailed insights into understanding the variations of frequency in vocal production from a laryngeal source (Titze and Alipour 2006; Brown and Riede 2017; Švec et al. 2021). The theory states that the larynx's differential muscle activities control the airflow needed for vocalisations by opening and closing the glottis (the vocal folds and the space between them). The elasticity, together with the morphology of the vocal folds, are also the main components involved in the MEAD, as the vibratory properties of the membranes impact the sound production (Brudzynski 2009; Brown and Riede 2017; Švec et al. 2021). The size and strength of the lungs furnish air flow and thus will affect the amplitude and frequency of the calls. The frequency varies with coordinated action of the laryngeal muscles that abduct the vocal folds and stretch them. This muscle activity allows mammals to produce sound by suddenly releasing the subglottic air pressure and controlling the vocal folds' size and elasticity. Thus, the vocal folds vibrate and produce different F0 depending on the morphology and elasticity of the membranes and the strength applied by the laryngeal muscles (Finck and Lejeune 2010; Riede and Brown 2013; Titze et al. 2016). Therefore, the differential muscle activities and the physical properties of the vocal folds identified in the source-filter together with the MEAD theory explain the production of different F0 in mammals.

Laryngeal size scales with body size in most mammalian species (e.g., Bogdanowicz et al. 1999; Bowling et al. 2020), due to adaptation links between diet and ecology. Herbivores differ from carnivores by the size of their arytenoid cartilages (e.g., Negus 1949; Harrison 1995; Berke and Long 2010; Shiba 2010). Arytenoids have an essential role in protecting ruminants, because animals that have more risk of aspirating their food during the long rumination process. Adaptations to drastic changes in the environment are also visible in aquatic mammals, with some species presenting a larger larynx, air sacs and dorsal tracheal membrane (Harrison 1995; Thomas et al. 2004; Reidenberg and Laitman 2010). Lastly, bats being the only true flying mammals (Frick et al. 2013), present unique laryngeal features, as a reduction in length of the vocal folds and, in parallel, an extension of the posterior commissure of the glottis, allowing respiration in flight during vocalisation (e.g., Denny 1976; Harrison 1995; Thomas et al. 2004; Ratcliffe et al. 2013).

Bats' vocalisations are high-frequency pulses used specifically in echolocation behaviour (including reception and processing of their echoes) (e.g., Griffin 1944; Vater 2000; Maltby et al. 2010; Jones and Siemers 2011; Fenton 2013). Echolocation signal production in bats comprises frequencies from 11kHz to 212 kHz, with most bats emitting between 20 and 60 kHz (Jones and Holderied 2007; Maltby et al. 2010; Fenton 2013). These vocalisations are one of the highest-pitched sound productions among vertebrates and are considered to play a role in the contemporary laryngeal adaptations (e.g., Jones and Holderied 2007). Reinforced cartilage supports hypertrophied muscles that characterise the bat larynx (e.g., Denny 1976; Thomas et al. 2004). This feature reflects the strong support needed by the bats to create and maintain the required subglottic pressure inside the larynx during intense echolocation signal production. Bats' hypertrophied muscles have been characterised as superfast muscles due to some vocalisations reaching up to 220 calls/second (Elemans et al. 2011; Moss et al. 2011; Ratcliffe et al. 2013; Grinnell et al. 2016).

Bats are governed by the same voice production theories as other mammals, notwithstanding their highly specialised frequencies and amplitudes in sound production. Correspondence in sound production is illustrated by the similarities in laryngeal anatomy between bats and other mammals, presenting only a reinforced "frame" and hypertrophied muscles. Also, according to the MEAD theory, the influence of differential muscle activities is thought to be a significant component of echolocation signal production (Roberts 1972; Griffiths 1983; Fattu and Suthers 1981; Pedersen 2000; Kobayasi et al. 2012; Metzner and Muller 2016). However, assessment of variation in size and morphology of the different structures constituting the larynx could reveal new knowledge on the different echolocation types in bats (Brudzynski 2009; Kobayasi et al. 2012). Indeed, it has been shown that variations in subglottal air pressure could explain some changes in the frequency and amplitude ranges of the social vocalisations and echolocation calls produced by bats. Through the biomechanical properties of the larynx (undescribed in the mentioned paper), some vocalisations could be produced without direct neuromuscular implication of the brain (Kobayasi et al. 2012). Lastly, since echolocation behaviour, diet, ecology and the larynx of bats are intercorrelated (e.g. Schnitzler et al. 2003; Denzinger and

Schnitzler 2013), we can expect some adaptation of the larynx in relation to diet (as for other mammals; Harrison 1995) and, by inference, to the different echolocation strategies. As such, it is essential to investigate the laryngeal morphology on a macroevolutionary scale in bats to evaluate the extent to which features other than the muscle activity parameters could be responsible for the evolution and development of high-frequency sound production.

b. Limited macroevolutionary data on the bat larynx:

Research on vocal communication in mammals was first typically focused on the behavioural aspects of emitted sounds (e.g., McComb 1991; McElligott and Hayden 1999). Early bat echolocation research faced some of the same issues as we saw an abundance of publications about echolocating signals analyses like rate of calls by species but only a few about bioacoustic morphofunction (e.g., Griffin 1944; Novick 1971; Roberts 1972; Suthers and Fattu 1973; Pye 1979; Hartley and Suthers 1987). Understanding the specific laryngeal morphology of bats and comparing these laryngeal forms to comprehend echolocation strategies remains a complex exercise as there exist few publications on the topic.

Methods for literature review:

Literature selection as carried out using the free web search engine Google Scholar. The use of specific keywords such as “bat larynx”, “laryngeal anatomy”, “echolocation”, and “vocal folds” narrowed the research. We considered sixteen publications from Dobson (1881) to Carter (2020) as being sufficiently informative about the different laryngeal forms that can be seen in bats to be considered in this review (Table 1). We selected papers up to 1881 as the number of papers providing enough information about laryngeal anatomy in bats is reduced in recent years. The majority of the literature was found in English (fourteen out of sixteen papers), one in French (Robin 1881) and one in German (Elias 1907). The following criteria were used to include papers in this review: (1) studies with a clear description of an entire larynx from one or several species, (2) studies with a brief overview and/or comparison of different laryngeal forms encountered in bats, (3) anatomical studies mentioning the morphology of bat larynges. The small number of papers that met these three criteria, compared to the 2914 articles mentioning bats echolocation on the Web of Science from 1970 to 2021 (Cao et al. 2022), illustrates how modest the research on bat larynges has been and how underappreciated this topic remains. Additionally, the studies selected describe the larynges of bats by mainly reporting general aspects of the morphology in all species (e.g., Robin 1881; Denny 1976) or focusing on one or two species or families (Griffiths works between 1978 and 1994). Some publications also focused on the hyoid region and make only brief references to laryngeal morphology (Sprague 1943).

Published data on the anatomy of bat larynges is extremely limited. Out of twenty-one families of bats, only twelve have been detailed at the family level in terms of general morphology, with some details on particular traits (Table 1). Three other families (Table 1) have simply been briefly mentioned in a couple of studies but are not described (Elias 1907; Sprague 1943). Lastly, the remaining six families (Table 1) have not been mentioned in the laryngeal descriptive or comparative studies referenced to date.

Features of the bat larynx in comparison to other mammals

Bat laryngeal morphology presents a mammalian-like morphology with hypertrophied muscles supported by reinforced cartilages. This seems to be the evolutionary tendency that differenciates the larynx of bats from other mammals (e.g., Harrison 1995). Indeed, compared to mammals of the same body size (rodents, shrews), bat larynges are larger and present for most of the species an early mineralisation of the cartilages due to extremely developed muscles involved in the production of high-frequency calls. Despite a similar, roughly rounded shape of the cricoid cartilage, the presence on it of a dorsal crest in most bat families (extremely reduced on Pteropodidae, Figure 1) is a character usually found in much larger mammals, and this could explain the necessity for echolocating bats to support larger laryngeal muscles than in mammals of the same body size (e.g., Harrison 1995). Another key feature of bat larynges is the reduction in length of the vocal folds coupled with the extension of the posterior commissure in the glottis, allowing flight and vocalization to occur simultaneously (Harrison 1995). The vocal folds are attached to the thyroid and arytenoid cartilages like most other mammals and do not share the particularity of rodents, which emit high-frequency calls and have the vocal folds fixed on the cricoid cartilages (Harrison 1995). Also, it is not clear if compared to the other mammalian orders of the clade Scrotifera (Tsagkogeorga et al. 2013) bats present larger arytenoids as in the majority of the cetartiodactyls or smaller arytenoids as in carnivorans (e.g., Harrison 1995; Thomas et al. 2004). Lastly, one characteristic of some bat families that is different compared to most mammals (except for some marsupials and primates; Schneider 1964; Harrison 1995) is the presence of tracheal pouches or bulla potentially involved in echolocation (Figure 1 & 2A; Roberts 1972; Denny 1976; Griffiths 1994; Harrison 1995).

For many decades, research involving echolocation in bats considered differential muscle activity as the main factor of variation in sound production, whereas a detailed, macroevolutionary examination of how laryngeal anatomy varies in relation to ecology and echolocation has been neglected, although such relationships are likely to be present. Indeed, morphological variations have been reported in several studies considering 15 out of 21 families of bats (Table 1), and similar results could be expected from the six non-described families. These morphological variations are crucial to work on further determinations regarding the impact of the different echolocation strategies on the laryngeal anatomy and also the evolutionary history of echolocation.

Variations in the size and shape of laryngeal cartilage are visible when comparing the different families of bats. The Rhinopomatidae present larynges with a relatively basic morphology, with the caudal parts of the thyroid (cornus) relatively narrow and reduced in length, as well as the ventral part of the cricoid (Robin 1881; Denny 1976). The Emballonuridae are similar in morphology to the Rhinopomatidae and Vespertilionidae but more specialised than the former and less specialised than the latter (Robin 1881; Elias 1907; Denny 1976). This is illustrated by larger arytenoids than the

Vespertilionidae and wider cornus and cricoid than the Rhinopomatidae (Elias 1907). It must be noted that some genera of Emballonuridae, such as *Taphozous,* possess a cricoid with a "trunk" or "cone" shape that differentiates them from the other families of bats (Robin 1881; Elias 1907). The Vespertilionidae show the most specialised laryngeal morphology of all the families, with laryngeal cartilages being generally thinner than the Rhinolophidae and with thyroid divided into two parts for each lateral side of the cartilage, the most caudal part presenting a vertical wing shape (Robin 1881; Elias 1907). Two other families with a close phylogenetic position to Vespertilionidae, Miniopteridae and Molossidae (Teeling et al. 2012), have a laryngeal cartilage morphology similar to the one found in Vespertilionidae bats, and the Thyropteridae have a similar hyoid form to the three considered families (Robin 1881; Elias 1907; Sprague 1943).

Rhinolophidae and Hipposideridae families have a similar but shorter and more robust larynx than the Vespertilionidae, presenting more prominent cricoid cartilage (e.g., Elias 1907; Denny 1976; Griffiths and Smith 1991). Thus, it is contrasting with the families of Vespertilionidae, Miniopteridae, Molossidae, Emballonuridae and Rhinopomatidae that share similar form. Specifically, the prominence of the cartilages is due to the development of lateral "muscular wings" on the cricoid of the Rhinolophidae and a large sagittal crest on the dorsal aspect of the cricoid for both Rhinolophidae and Hipposideridae (Figure 1 & 2; Robin 1881; Elias 1907; Denny 1976; Harrison 1995). The Phyllostomidae present a larynx similar in shape to that of Rhinolophidae but thinner, especially in relation to thyroid and arytenoid cartilages (Robin 1881; Denny 1976; Carter 2020). Nycteridae, Megadermatidae, and Rhinolophidae have similar laryngeal morphology except that the larynx of the Nycteridae seems slightly longer and smoother than the larynx of the Rhinolophidae (Robin 1881; Sprague 1943; Denny 1976). In Megadermatidae, the larynx is more robust than in Nycteridae. The cricoid ring does not seem to present some notched or thin ventral part in Megadermatidae, which contrasts with all other laryngeal echolocators (Robin 1881; Denny 1976).

The larynges of three other families have been described relatively to those of other laryngeal echolocating bats. The Natalidae larynges are not currently known, but the hyoid region is similar to the Phyllostomidae (Sprague 1943). Conversely, the larynx of Mormoopidae have been described as very different from the one from Phyllostomidae, especially regarding the oval cricoid and the diamond shape of the thyroid (Griffiths 1978, 1983). The larynx of Mormoopidae appears unique in the form of its cricoid and thyroid when compared to other families. The Noctilionidae larynx seems to exhibit traits that are a combination of different families already described (Robin 1881; Denny 1976). The Noctilionidae larynx is shorter and larger than Emballonuridae and is similar to the Rhinolophidae in this aspect (Robin 1881), while some features (see Additional features below) like the ventricle-like air sacs below the vocal folds, are shared with the Rhinopomatidae and the Emballonuridae (Denny 1976).

The Pteropodidae being non-laryngeal echolocators have a larynx similar to the common mammalian morphology (Figure 1; Harrison 1995). Their larynx has a relatively smooth dorsal crest on the cricoid (Giannini et al. 2006) and they are the only known family with laryngeal sexual size dimorphism (Dobson 1881; Robin 1881; Langevin and Barclay 1990). These species possess a large thyroid in a shield shape and some pharyngeal air-sacs. The rounded shapes of the thyroid and cricoid ring observed in Pteropodidae are like those found in carnivorans or cetartiodactyls and contrast with the more compact and narrow cartilages of the laryngeal echolocating bats.

For the mineralisation or ossification of the different laryngeal cartilages, Carter (2020) is used as a reference, supplemented with additional information from selected research (e.g., Elias 1907; Griffiths 1983). Carter (2020) distinguished between three main patterns of calcification or ossification linked to bioacoustic requirements. The first pattern contains bat larynges with mineralised cricoid cartilage but no mineralisation of the thyroid and arytenoids. The Phyllostomidae are the main family belonging to this group (Figure 2; Denny 1976; Griffiths 1978, 1982; Carter and Adams 2014; Carter 2020). The second group of families includes as the Rhinopomatidae, Mormoopidae and

Vespertilionidae. Their cricoid is calcified like the Phyllostomidae, and their thyroid presents some important patches of mineralisation (Elias 1907; Griffiths 1978, 1983; Carter 2020). However, some modifications can occur, such as the uncalcification of the lateral border of the cricoid in *Pteronotus parnellii* (Griffiths 1978, 1983). Lastly, the third pattern of mineralisation patterns includes the Hipposideridae, Rhinolophidae and Emballonuridae. They possess a fully ossified cricoid and a partially ossified thyroid (Robin 1881; Elias 1907; Denny 1976; Carter 2020) in contrast to the larynx of the other two groups being fully cartilaginous with only some mineralisation (calcification) on the cricoid and sometimes patches on the thyroid (Harrison 1995; Carter 2020).

Pteropodidae are non-laryngeal echolocators and they have a larynx similar to the third pattern of mineralisation with an ossified cricoid and thyroid (Dobson 1881; Carter 2020). However, pteropodids possess a fully ossified thyroid, unlike the laryngeal echolocators of the third group that still have some patches of calcified cartilage. Pteropodids arytenoids are not mineralised, in contrast to the heavily mineralised arytenoids of the third group of bat families. Unfortunately, the families Megadermatidae, Miniopteridae, Molossidae, Natalidae, Nycteridae and Noctilionidae have not been described at a level of detail that would permit evaluation of the degree of mineralisation of their laryngeal cartilages.

Some laryngeal traits appear to be prominent in the more derived echolocators, apart from the general morphology of the three laryngeal cartilages. First, the "muscular wings" present on the cricoid of the Rhinolophidae (Harrison 1995) and second, the sagittal crest on the dorsal part of the cricoid in families like Rhinolophidae (e.g., Robin 1881), Hipposideridae (Denny 1976), Mormoopidae (*P. parnellii*; Griffiths 1983), and Vespertilionidae (Robin 1881; Elias 1907). Although these two features are morphological variations of the cricoid cartilage, they should be considered as anatomical entities distinguishing laryngeal forms (Figure 1; Robin 1881; Elias 1907; Harrison 1995).

Possibly the most important anatomical trait in bat laryngeal forms that should be discussed is the presence of enlargement in the laryngeal lumen as it may play the biggest role in the variety of echolocation signals found in laryngeal bats (e.g., Denny 1976; Harrison 1995). This enlargement has been achieved through various morphological forms. The first aspect is illustrated by the tracheal rings at the junction of the cricoid cartilage that are modified to form a pair of cartilaginous bullae. The Rhinolophidae and Hipposideridae larynges possess a pair of tracheal bullae on the first and second tracheal rings and third bullae in the dorsal position between the third and the fifth ring (Figure 1 & 2; Robin 1881; Denny 1976; Harrison 1995). The larynx of the Nycteridae also possesses a pair of large tracheal bullae extended to the tenth ring (*Nycteris macrotis*; Denny 1976). Second, the first tracheal rings can be enlarged under the cricoid cartilage, giving more volume to the larynx lumen. This morphology can be found in the Rhinopomatidae from the 5th to the 15th ring and in the Emballonuridae from the second to the tenth ring (Sprague 1943; Griffiths and Smith 1991). Tracheal enlargement (also called "tracheal pouches") is also visible for some Phyllostomidae and Mormoopidae from the cricoid to the fifth or eighth first rings (Denny 1976; Griffiths 1978, 1983). The larynges of the Rhinopomatidae and Emballonuridae also present a pair of ventricle-like air-sacs below the vocal folds, to which they are partially fused (Denny 1976). The Noctilionidae larynges do not possess tracheal pouches, but ventricle-like air-sacs have been described (Denny 1976). Another aspect of the laryngeal morphology is the elastic cricothyroid membrane (ECM), also called the saccus intercartilaginous anterior (Elias 1907). It is an elongation of the cricothyroid membrane composed of collagen and elastic fibres (Figure 1; Robin 1881). It appears to be a morphological adaptation of the Vespertilionidae and also potentially of the phylogenetically related Miniopteridae and Molossidae (Robin 1881; Elias 1907). This membrane stretches from the anterior lip of the cricoid cartilage to the thyroid cartilage and hypothetically plays the same role as the tracheal bullae or pouches (Robin 1881). Lastly, the vocal folds of all laryngeally echolocating bats possess thin extensions called vocal membranes that allow faster vibrations and the production of high-frequency vocalisations (Novick and Griffin 1961; Fitch 2006).

Bats commonly possess strong and well-developed laryngeal muscles (e.g., Harrison 1995). However, some qualitative differences have been noticed. The Hipposideridae and Rhinolophidae laryngeal muscles are similar and extremely powerful with large insertion areas, especially the thyroarytenoid and the cricothyroid muscle that cover the entire ventral part of the larynx (Robin 1881; Elias 1907). The Megadermatidae and Nycteridae larynges present similar forms with a complex cricothyroid (Griffiths and Smith 1991). The Rhinopomatidae only differ in the cricothyroid's attachment, which is more medial on the cricoid cartilage than on the Rhinolophidae (Elias 1907). The main difference between the laryngeal muscle morphology of certain families appears to be the relative size of different laryngeal muscles. Hypertrophied thyroarytenoids in Rhinolophidae differ from the hypertrophied cricoarytenoids in Vespertilionids (Figure 2). Indeed, the Vespertilionidae, Mormoopidae, and potentially the Miniopteridae and Molossidae present heavily developed dorsal cricoarytenoids on the dorsal side of the cricoid (Elias 1907; Griffiths 1978, 1983). The Pteropodidae possess muscles most similar to those of Vespertilionidae larynges but less developed when compared to all other bat families (Robin 1881). In contrast to these hypertrophied cricoarytenoid muscles, the laryngeal musculature of the Emballonuridae shows relatively underdeveloped and weak cricoarytenoid muscles even if the cricothyroid remains hypertrophied (Elias 1907; Griffiths and Smith 1991; Griffiths et al. 1991). Lastly, the Phyllostomidae have a relatively simple cricothyroid muscle that is weaker (smaller in mass) than all other bat families, which could be related to the lack of mineralisation observed in these larynges (Griffiths 1978, 1982).

The precise location and shape of the laryngeal muscle attachments to the cartilages have not been described, and nor has the composition in terms of muscle fibres, despite being essential to understanding the physiology and function of the laryngeal muscles for echolocation (Hoh 2005). In a general approach, six out of 21 families of bat (Teeling et al. 2016) are lacking laryngeal description and most of the other families involved in anatomical studies have only a brief description of their morphology. For example, the larynx of Nycteridae has been described in terms of hyoid morphology and drawings of the larynx were published in Griffiths study, but no description has been furnished (Griffiths 1994). Several families have been linked to each other by similar or different laryngeal forms and their biological adaptations. However, the studies are few and date back at least 50 and sometimes over 100 years (e.g., Robin 1881; Elias 1907; Denny 1976). Also, clear comparison of the laryngeal form of the different families one by one as it has been done with the hyoid bone (Sprague 1943) should be considered. Indeed, it is unknown whether the families similar to Rhinolophidae (e.g., Megadermatidae) present larynges with a dorsal crest on the cricoid cartilage because the descriptions are limited in terms of relative size, degree of mineralisation and some additional features that stand out.

Bioacoustic implications:

The distribution of morphological variation in size and shape for the three intrinsic cartilages appears to reflect a phylogenetic signal in bats. Indeed, most families that are phylogenetically close to the Vespertilionidae show a relatively similar laryngeal morphology, and the same applies for the families related to the Rhinolophidae. Potentially, two morphotypes would be identified regarding the conclusions brought from the studies describing bat larynges – one more related to Rhinolophoidea and a second form illustrated in a majority of Yangochiroptera. Nevertheless, some families present independent characteristics like the "trunk" shape of the cricoid of some Emballonuridae or the diamond shape of the thyroid of the Mormoopidae (Elias 1907; Griffiths 1978).

A link with sound production appears to be the most probable influence on the degree of mineralisation, on function to the different echolocation strategies (Figure 2). Bats producing vocalisations at a higher intensity and with higher rates (High Duty Cycle, High Intensity, e.g., Fenton 2013) possess the most mineralised or ossified larynges, while bats with the lowest intensity and rate of calls (Low Duty Cycle, e.g., Fenton 2013) have a weaker laryngeal structure with cartilages that are partially mineralised or unmineralised (Carter 2020). From a macroevolution perspective, it can be emphasised that the Phyllostomidae have the weakest laryngeal structure. This lack of mineralisation is linked to their echolocation behaviour which is for obstacle avoidance and not insect hawking and also their reliance on other senses (e.g., Denzinger and Schnitzler 2013). Therefore, their larynx does not require strong cartilages and muscles as they have reduced their use of echolocation (Griffiths 1982). In contrast, the Rhinolophidae and Hipposideridae cartilages have been replaced by bone through ossification to support hypertrophied muscles that produce high-rate calls of constant frequencies (Fenton et al. 2012).

Some laryngeal features like the tracheal bullae are possible adaptations to vocalisation requirements (Denny 1976; Harrison 1995; Metzner and Muller 2016), but this hypothesis remains unexplored. These structures are potentially seen as Helmholtz resonators implicated in the production of higher intensity calls (Roberts 1972; Denny 1976). The added air volume in the larynx could allow more pressure on the glottis and produce louder sounds (Roberts 1972). The elastic cricothyroid membrane could be employed for the same purpose in Vespertilionidae (Robin 1881). Additional features or elasticity of the membranes could be related to the variation of intensity in sound production.

The extreme development of the laryngeal muscles in bats seems to be related to the high requirements to produce high frequency echolocation signals (Figure 2). The three main structural variables of echolocation signals in bats are the change in frequency (constant frequency CF or frequency modulated FM, Vater 2000; Fenton et al. 2012), change in intensity, and the rate of calls. The intensity has been explained by potential gain in laryngeal volume thanks to added features such as the tracheal bulla, the tracheal pouches, the elastic cricothyroid membrane or the ventricle-like air-sacs (e.g., Robin 1881; Denny 1976; Griffiths 1983). Although the larynx is the principal component responsible for sound production, the coordinated action of the flight muscles and other musculature in the thoracic cage with those of the larynx are responsible of the high subglottic pressure generated in bats (Metzner and Schuller, 2010). The rate of call seems not only related to the cartilage reinforcement but also to muscle activity. High Duty Cycle (HDC) bats present both branches of a cricothyroid in the same direction but working with antagonist movement, one branch pulling the thyroid close to the cricoid cartilage and the other one pulling it back (Griffiths 1983). This coordinated action allows rapid creation and release of tension in the vocal folds. A relatively weak thyrohyoid muscle has been found also in HDC bats as the action of the two branches of the cricothyroid are sufficient to regulate the tension on the vocal folds. These antagonistic movements of the cricothyroid branches are important for the control of the third variable of echolocation. Bats emitting in constant frequency use the antagonistic movement of the cricothyroid branches for fine control of the frequency (Griffiths 1983). In FM bats, the cricothyroid pulls in only one direction, and it is the thyrohyoid muscle that releases the tension on the vocal folds and produces the drop in frequency. Therefore, differences in cricothyroid development and thyrohyoid size potentially impact the echolocation strategies (Griffiths 1983).

Unfortunately, as illustrated herein, knowledge about bat larynges is still partial and lacks understanding concerning the functional implications of structural variations with respect to echolocation. Additionally, no research hypotheses have been proposed to test the different associations (potential morphotypes, pattern of mineralisation, muscle development) and morphology observed. All these studies present another major issue: they all report qualitative results. Except for one study on Pteropodidae (Giannini et al. 2006), no quantitative descriptions have been published. As such, illustration of a potential phylogenetic or functional signal in laryngeal morphology through statistical analyses remains a challenge due to the absence of measurement data.

This information serves to highlight the opportunity for further quantitative research to uncover form-function relationships within laryngeal features and to assess their evolutionary patterning.

c. Advanced imaging:

Measurements of the different laryngeal components could extend the theoretical knowledge about echolocation in bats by comparing the various hypotheses about sound production to anatomical data. This could be achieved through different analytical methods, as has been implemented for other mammals (e.g., Nishimura 2003; Kim et al. 2004; Claassen et al. 2017; Bowling et al. 2020; Lesch et al. 2021). In that sense, the advent of X-ray microtomography (XMT; Elliott and Dover 1982; Davis and Wong 1996) has been a major step forward in biological research, opening many research avenues (Metscher 2009). Indeed, using X-rays enables the visualisation of in-situ organs and hard tissues like the larynx through its capacity to generate 3D reconstructions of these structures, facilitating detailed morphological description and quantification; especially regarding small specimens like bat larynges where dissections destroy the anatomical connections (Figure 3). Unfortunately, studies of the larynx are few due to the reconstruction of only hard tissues like the skeleton and fossilised remains. The development of contrast enhanced XMT based on X-ray attenuation by high atomic number elements such as tungsten or iodine allowed the visualisation and discrimination of soft tissues that are normally less attenuating and lack contrast, compared to mineralised tissues (Figure 3; Metscher 2009; Jeffery et al. 2011; Vickerton et al. 2013; Boyde et al. 2014; Gignac et al. 2016). Using an XMT scanner and iodine-stained specimens permits non-destructive "virtual dissection" of internal structures (Hedrick et al. 2018; Santana et al. 2019). This is highly valuable for anatomical research as it brings three main benefits: (1) the specimens studied would not be destroyed as in histological studies and anatomical relations between different tissues remain intact compared to gross dissection, (2) the specimens are reusable for further studies (especially important for rare species), and lastly (3) the data collected are reusable indefinitely and shareable within the scientific community (Santana et al. 2019; Smith et al. 2021a,b). The numerical aspect of these 3D data also allows precise acquisition of measurements in 3D, as well as the application of geometric morphometric methods, which explicitly analyse shape differences (Bookstein 1997; Slice 2007; Klingenberg 2016). Volumes and lengths of specimens can be precisely and rapidly obtained. This is particularly important for differentiating inter- and intraspecific measures variation, especially in bats where the order of magnitude can be small and the differences in size, subtle. In comparison, the conventional measurement approaches (e.g., measuring with a calliper) are usually less efficient in giving precise results.

Studies on mammalian larynges using iodine contrast-enhanced clinical computed tomography scans have been already made to observe laryngeal morphology of the orders Primates, Carnivora and Artiodactyla (e.g., Kim et al. 2004; Bowling et al. 2020). A similar protocol should be undertaken on bat larynges to provide a better illustration and knowledge of the potential morphotypes described in the literature. Studies focusing on other anatomical features of bats have been performed recently (Yohe et al. 2018; Santana 2018; Santana et al. 2019; Ito et al. 2021; Nojiri et al. 2021b; Smith et al. 2021; Sohn et al. 2021) and some studies used CT and iodine contrast-enhanced imaging techniques to answer hypotheses about the evolutionary history of bats, such as recent studies on the hearing apparatus (Nojiri et al. 2021a, b; Sulser et al. 2022). It will be highly valuable to illustrate the shape and size of the different cartilages, their degree of mineralisation, in addition to the size, shape and position of the different muscles involved in laryngeal echolocation, especially the cricothyroid and the thyrohyoid.

d. Evolutionary Development and Ontogeny:

During the last decades, research on the ontogeny of echolocation has been conducted through two main fields of expertise: bioacoustics and anatomy. In terms of bioacoustics, several studies focused on describing the development of the emitted sound for echolocation on post-natal specimens (e.g., Gould 1975; Brown and Grinnell 1980; Habersetzer and Marimuthu 1986; Moss 1988; Moss et al. 1997; Vater et al. 2003; Carter et al. 2014; Engler et al. 2017; Smarsh et al. 2021). Those studies state that bats vocalize from their day of birth and that FM and CF bats develop echolocation in different ways (Gould 1975; Brown and Grinnell 1980; Carter et al. 2014). FM bat pups develop echolocation by increasing the frequency and the rates of sound pulses, but the duration of these pulses is reduced during ontogeny (some being described as constant frequency signals; Gould 1975; Brown and Grinnell 1980; Habersetzer and Marimuthu 1986; Moss 1988; Moss et al. 1997; Carter et al. 2014). These low-frequency, low-rate calls potentially illustrate an immaturity of the larynx at birth and during the first postnatal week (Moss 1988). The same does not apply to the CF bat pups like Rhinolophidae and Hipposideridae. Their calls have the same constant frequency and rates of calls as the adult CF bats and the duration of those pulses increase with age (Brown and Grinnell 1980; Habersetzer and Marimuthu 1986). The variation observed in CF pups after several days concerned specifically the bandwidth and the suppression of the different harmonics to concentrate the energy of the sound on the second harmonic to produce less variable constant frequency pulses (CF_2; Habersetzer and Grinnell 1986; Vater et al. 2003; Carter and Adams 2016). It is interesting to notice that the only species of CF echolocators in the Yangochiroptera, *Pteronotus parnellii* (Mormoopidae), is not able to echolocate in CF during the first week, only producing some CF vocalizations as the other FM species of Mormoopidae (Vater et al. 2003). After several days these vocalisations are tuned on CF_2 and the pups can echolocate by maintaining this CF component that other FM pups are losing due to reduction of the duration in pulses (Vater et al. 2003). Therefore, the ontogeny of *P. parnellii* could potentially explain the evolutionary convergence of echolocation strategies with the Rhinolophids. Carter et al. (2014) and Engler et al. (2017) argued that social calls and echolocation signals in bats, do not have the same developmental pattern, and therefore have a different evolutionary history. *Rousettus* bats do not change the frequency or duration of their clicks during ontogeny due to an early development of the tongue morphology and neural innervation (Smarsh et al. 2021). All these observations of development in bioacoustics need to be compared with the ontogeny of the larynx itself to assess how the development of sound production relates to organ development in laryngeal echolocators.

In terms of anatomical research in the development of laryngeal echolocation, only a few research have been published regarding the relationship between anatomy and echolocation (e.g., Pedersen 1995; Carter et al. 2014; Nojiri et al. 2021b). Some of that research considered the evolutionary aspect of laryngeal echolocation (e.g., Nojiri et al. 2021a) and only the recent research in ontogeny focused on the laryngeal aspect of sound production and its implication in laryngeal echolocation development (e.g., Carter and Adams 2014; Carter et al. 2019; Carter 2020). These research highlighted that the ontogeny of the skull (Pedersen 1995) and of the cochlea (Carter and Adams 2016; Nojiri et al. 2021b) illustrated some constraints by the demands of vocalisations in bats.

By using a model integrating the development of the cochlea, the larynx, sound production, and flight in bats, Carter and Adams (2016) argued that hearing sensitivity developed before the production of high-frequency calls. Further, they suggested that sensitivity and high-frequency production both appeared before the ability to fly and then the three capacities coevolved through time. The ontogeny of the cochlea has been described as accelerated compared to non-echolocator mammals (Nojiri et al. 2021b) and among bats, specific developments of the hearing apparatus have illuminated several evolutionary pathways (Nojiri et al. 2021a). The ontogeny of the hyoid apparatus and the intrinsic laryngeal cartilages shows correlations with the development of echolocation, and the different echolocation strategies have been implicated in the reinforcement by mineralisation/ossification of some parts of the cartilages during postnatal development (Carter et al. 2019; Carter 2020). Also, these correlations between echolocation strategies and reinforcement of the larynx coupled to the ontogenetic pathways illustrated in the development of bioacoustics (Carter et al. 2014) indicate possible evolutionary scenarios. Therefore, understanding the development of laryngeal echolocation is essential as laryngeal morphology can inferentially explain the evolution of sound production. Unfortunately, as for the adult morphology, little is known about the different developmental forms of the laryngeal anatomy in bats. Future work on the ontogeny of the larynx across bat families could provide new insights about echolocation development and evolution pathways, into the resolution of the previous hypotheses tested on the ontogeny of the hearing apparatus (e.g., Nojiri et al. 2021a).

3) Conclusion: the larynx as a potential new proxy to elucidate the evolutionary history of echolocation.

This review has assembled different sources of information from over 140 years of publications to draw conclusions regarding the morphology of the bat larynx, the potential variations of form encountered, and the factors influencing variations. A phylogenetic signal has been found to potentially impact the forms of the laryngeal cartilages with two main morphotypes, illustrated in the two main superfamilies of bats (Rhinolophoidea and Verspertilionoidea). The three parameters of sound production for echolocation structure are correlated to different aspects of the laryngeal morphology. The differences in emitting calls with CF or FM appear to be related to muscle activity and the size of the muscles involved. The antagonist branches of the cricothyroid are the principal feature involved in the different frequencies emitted. The rate of calls (HDC/LDC) correlates the volume of muscles and the degree of reinforcement (mineralisation) of the different cartilages. Lastly, the intensity of the calls found in bats appears to be driven by modified features like the tracheal bullae or an elastic cricothyroid membrane in some Yangochiroptera. Unfortunately, these data are partial and based on a few species only, hence a comprehensive description and comparison among the high species diversity of bats remains to be undertaken. This will no doubt provide insights in the fields of bats bioacoustic and behaviour by discussing the morpho-function of the larynx and the special traits visible in bats.

The resolution of the evolutionary history of bats remains under debate and this also holds true for the origins of echolocation. The description and comparisons of laryngeal forms, as it is currently done for the hearing apparatus, represents a potentially fruitful avenue of further research that would advance our understanding of mammalian sensory evolution. Such an agenda would profit from the use of new technologies, allowing anatomical structures to be imaged, analysed, and visualised in a more detailed way. Data on the development of the larynx are also missing, and we know comparison of ontogenetic stages can deliver clues for the resolution of phylogenetic relationships and potentially illustrate convergence in evolution. Further research into laryngeal morphology will likely yield novel insights into the evolutionary history of echolocation and the correlations between morphology and echolocation strategies in bats.

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Author contributions:

Nicolas BRUALLA outlined the paper idea, performed the literature research, and drafted the work. All authors reviewed critically the work.

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Tables:

Table 1: Overview of descriptive studies of laryngeal anatomy in bats. **Generally Described**: the study describes broadly or fully the larynx of a species, a group of species, a family, or of several families; **Poorly Described**: the study briefly mentions the larynx and the descriptions are really succinct, but it brings some relevant information; **Not Described**: no study describing or mentioning the family in terms of laryngeal morphology.

Level of Morphological Description	Families	References
Generally Described	Pteropodidae, Hipposideridae, Rhinolophidae, Megadermatidae, Rhinopomatidae, Nycteridae, Emballonuridae, Phyllostomidae, Mormoopidae, Noctilionidae, Vespertilionidae and Molossidae.	Dobson (1881), Robin (1881), Elias (1907), Sprague (1943), Denny (1976), Griffiths (1978, 1982, 1983, 1994), Griffiths & Smith (1991), Griffiths et al. (1991, 1992), Harrison (1995), Giannini et al. (2006), Carter & Adams (2014), Carter (2020)
Poorly Described	Thyropteridae, Miniopteridae and Natalidae.	Elias (1907), Sprague (1943)
Not Described	Rhinonycteridae, Craseonycteridae, Furipteridae, Mystacinidae, Myzopodidae and Cistugidae.	None

Figures:

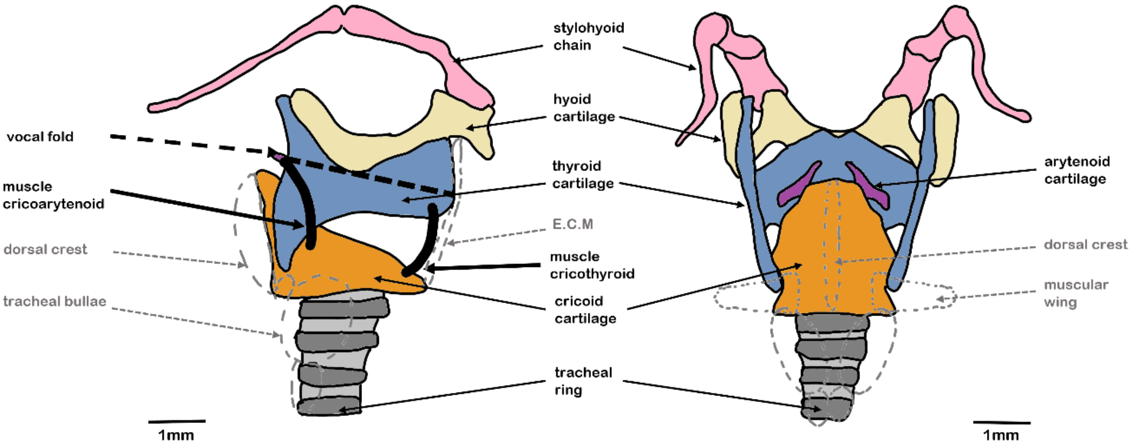
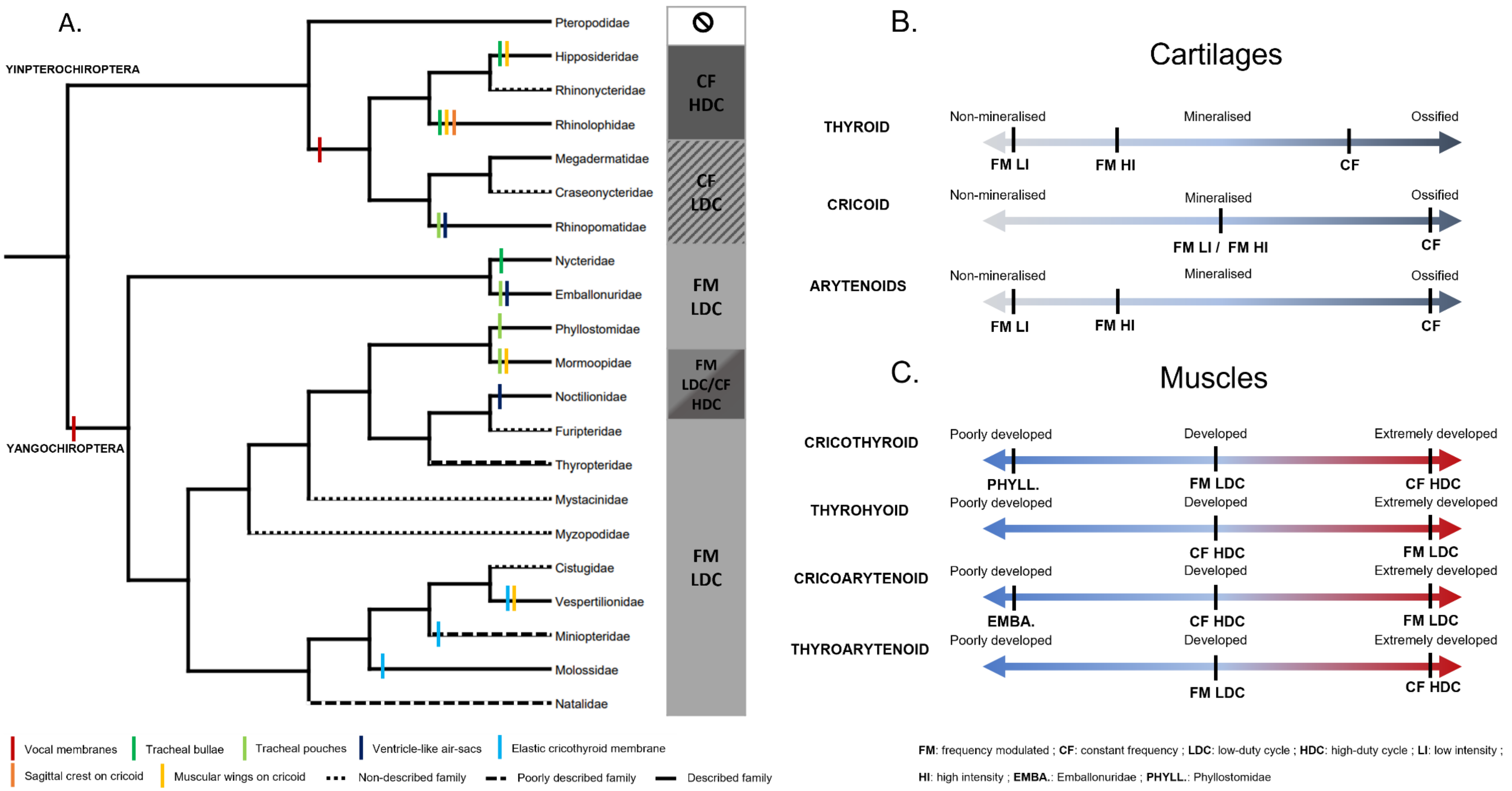


Figure 1: Left lateral (left) and dorsal (right) view of the schematized larynx of the cave nectar bat (*Eonycteris spalaea*), belonging to the family Pteropodidae (non-laryngeal echolocators). Dashed lines represent the features observable on specific families of laryngeal echolocators. **ECM**, elastic cricothyroid membrane.



FM: frequency modulated ; **CF**: constant frequency ; **LDC**: low-duty cycle ; **HDC**: high-duty cycle ; **LI**: low intensity ; **HI**: high intensity ; **EMBA.**: Emballonuridae ; **PHYLL.**: Phyllostomidae

Figure 2: Visual summary of the different laryngeal forms in bats described in the literature. **A.** Distribution along the bat phylogeny of the different traits observed in bat larynges; **B.** Characterisation of the different echolocation strategies regarding the degree of mineralisation of each laryngeal cartilage; **C.** Characterisation of the different echolocation strategies regarding the development of the different laryngeal muscles involved in echolocation.

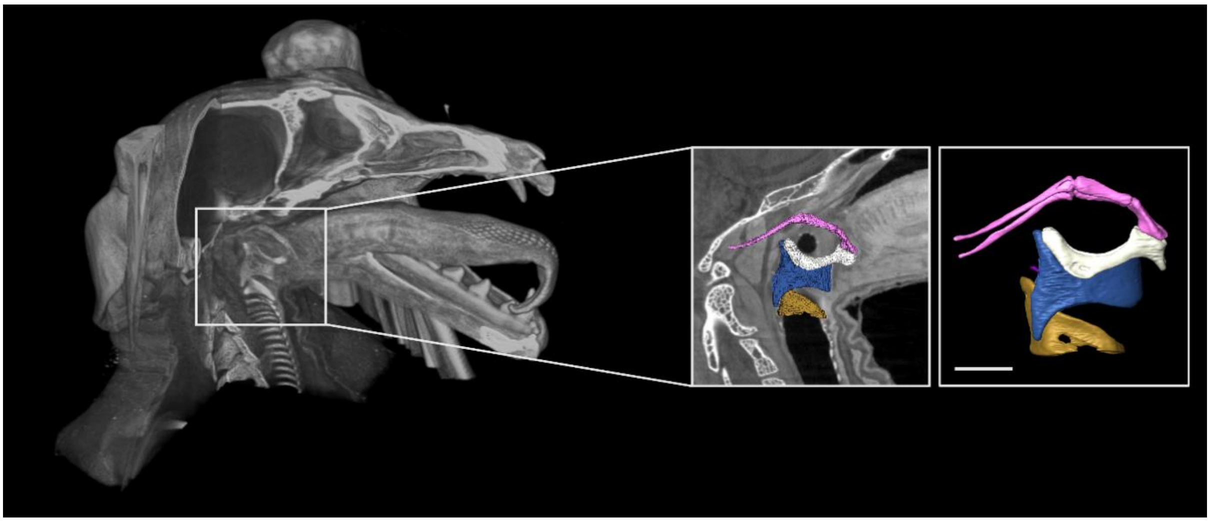


Figure 3: Sagittal and transversal biplanar cutaway view of a 3D visualisation of the skull and neck of an adult cave nectar bat (*E. spalaea*), using diffusible iodine contrast-enhanced CT scanning, showing digital reconstruction of the larynx with the cricoid (orange), thyroid (blue), arytenoids (purple), hyoid (white), and the stylohyoid chain (pink). Scale of 2mm.