Dataset

3D models related to the publication: Comparative anatomy of the vocal apparatus in bats and implication for the diversity of laryngeal echolocation.

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Abstract
The present 3D Dataset contains the 3D models analyzed in Brualla et al., 2024: Comparative anatomy of the vocal apparatus in bats and implication for the diversity of laryngeal echolocation. Zoological Journal of the Linnean Society, vol. zlad180. (https://doi.org/10.1093/zoolinnean/zlad180). Bat larynges are understudied in the previous anatomical studies. The description and comparison of the different morphological traits might provide important proxies to investigate the evolutionary origin of laryngeal echolocation in bats.

Keywords: Chiroptera, larynx, mammalian nasopharyngeal morphology, vocal tract, x-ray microtomography

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INTRODUCTION

Bats (order Chiroptera) are the second largest mammalian order in terms of species diversity, with over 1400 described species (Simmons and Cirranello 2020). They have the ability to fly and use echolocation to navigate in dark environments, allowing them to inhabit various ecological niches (Griffin 1944, Rayner 1988, Teeling 2009). Bats are classified into two suborders: Yinpterochiroptera (pteropodids and rhinolophoids) and Yangochiroptera (other recognized families) (Simmons and Cirranello 2020). Rhinolophoids and yangochiropterans use laryngeal echolocation, while pteropodids employ a primitive form of echolocation (Yovel et al. 2011, Boonman et al. 2014, Chaverri et al. 2018).

The origin of laryngeal echolocation in bats is debated. One hypothesis suggests a single origin followed by loss in pteropodids, while the other proposes multiple independent origins (Veselka et al. 2010, Wang et al. 2017, Davies et al. 2013, Nojiri et al. 2021). Limited research has focused on the larynx, but advanced imaging techniques such as X-ray microtomography enable detailed study of its anatomy (Brualla et al. 2023). The larynx plays a crucial role in vocalization, and its structure is relatively conserved across mammals (Harrison 1995, Saigusa 2011). Bats have unique laryngeal adaptations associated with high-frequency vocalizations and echolocation (Suthers 2004, Metzner and Schuller 2010, Metzner and Müller 2016, Brualla et al. 2023).

Previous studies have identified distinct laryngeal morphotypes in bats, correlated with variations in vocalizations (Brualla et al. 2023). However, there is still much to discover about the larynx and its relation to ecological diversity in bats (Brualla et al. 2023). Investigating laryngeal variation can provide insights into the evolutionary history and success of bats (Brualla et al. 2023).

In this study, iodine contrast-enhanced X-ray microtomography and virtual dissection were employed to describe and analyze the patterns and magnitude of laryngeal variation in bats (Brualla et al. 2023). The study aimed to determine if bat larynges exhibit unique features compared to other mammals and if discrete morphotypes are distributed across the bat phylogeny (see Figure 1; Brualla et al. 2023). The findings highlight the remarkable diversity and ecological success of bats within the mammalian world (Brualla et al. 2023).

METHODS

The study used X-ray microtomography (XMT) imaging data from a diverse collection of adult bat specimens, including 23 bat species. One outgroup species, Suncus murinus, from the Eulipotyphla group was also included. The sample represented various bat families, including non-laryngeal echolocating bats from the Pteropodidae family and laryngeal echolocating bats from the yinpterochiropteran suborder (Hipposideridae, Rhinopomatidae, Rhinolophidae, Megadermatidae, and Craseonycteridae) and yangochiropteran suborder (Emballonuridae, Phyllostomidae, Mormoopidae, Molossidae, and Vespertilionidae). The

Family	*Species*	Source	ID	Laryngeal echolocation strategy
Eulipotyphla	*Suncus murinus*	UMT	KATS_835A	Nil
Pteropodidae	*Eonycteris spelaea*	IER, VAST	VN18-026	Nil
Pteropodidae	*Macroglossus sobrinus*	IER, VAST	VN15-017	Nil
Hipposideridae	*Aselliscus dongbacanus*	IER, VAST	VTTu15-013	CF-HDC
Hipposideridae	*Coelops frithii*	IER, VAST	VN19-196	CF-LDC
Hipposideridae	*Hipposideros larvatus*	IER, VAST	VN18-209	CF-HDC
Rhinolophidae	*Rhinolophus cornutus*	IER, VAST	JP21-025	CF-HDC
Rhinolophidae	*Rhinolophus macrotis*	IER, VAST	VN11-089	CF-HDC
Megadermatidae	*Lyroderma lyra*	IER, VAST	VN17-535	FM-LDC
Emballonuridae	*Saccolaimus mixtus*	AM	A3257	FM-LDC
Emballonuridae	*Taphozous melanopogon*	IER, VAST	VN17-0252	FM-LDC
Phyllostomidae	*Artibeus jamaicensis*	ETSU	AJ001	FM-LDC
Vespertilionidae	*Kerivoula hardwickii*	IER, VAST	VN11-0043	FM-LDC
Vespertilionidae	*Myotis ater*	IER, VAST	VN19-016	FM-LDC
Vespertilionidae	*Myotis siligorensis*	IER, VAST	VTTu14-018	FM-LDC

Table 1. List of specimens and their source. Institution abbreviations: AM: Australian Museum, Sydney, Australia; ESTU: East Tennessee State University, Johnson City, Tennessee; IER, VAST : Institute of Ecology Resources, Vietnam Academy of Science and Technology; UMT: The University Museum, The University of Tokyo , Japan.

objective was to cover a wide range of bat diversity and gain morphological insights into different bat families. The sample included 86% coverage of yinpterochiropteran families and 36% coverage of yangochiropteran families, representing the majority of yinpterochiropterans and the families with high species diversity among yangochiropterans. Different laryngeal echolocation strategies, such as constant frequency (CF), frequency modulated (FM), high-duty cycle (HDC), and low-duty cycle (LDC) echolocators, were represented in the sample.

Here, we included 15 out of the 24 specimens (Table 1) due to licensing regulations of the XMT imaging data. Remaining species acquired from https://www.morphosource.org are available there. The iodine staining method involved using ethanol with 1% iodine for 14 days to enhance contrast and visualize soft tissues during scanning. XMT scanning was performed on all specimens using various scanners and protocols, with parameters and voxel sizes adjusted by the respective teams. Voxel sizes between 10 and 30 µm were chosen to ensure sufficient resolution for reconstructing different components. Laryngeal cartilage thickness ranged from 50 to 350 µm across most species. Manual reconstruction of the cricoid, thyroid, and arytenoid cartilages, as well as the entire hyoid apparatus, was conducted using AMIRA 5.3.3 software (ThermoFisher) and the brush tool and interpolation function. Intrinsic muscles, including cricothyroid, cricoarytenoid dorsalis, cricoarytenoid lateralis, oblique arytenoid, transverse arytenoid, thyroarytenoid, and vocalis muscles, were reconstructed consistently. The thyrohyoid muscle, connected to the hyoid apparatus and potentially involved in laryngeal echolocation, was also reconstructed. The resulting 3D surfaces were saved as STL and PLY files for anatomical comparisons in the study.

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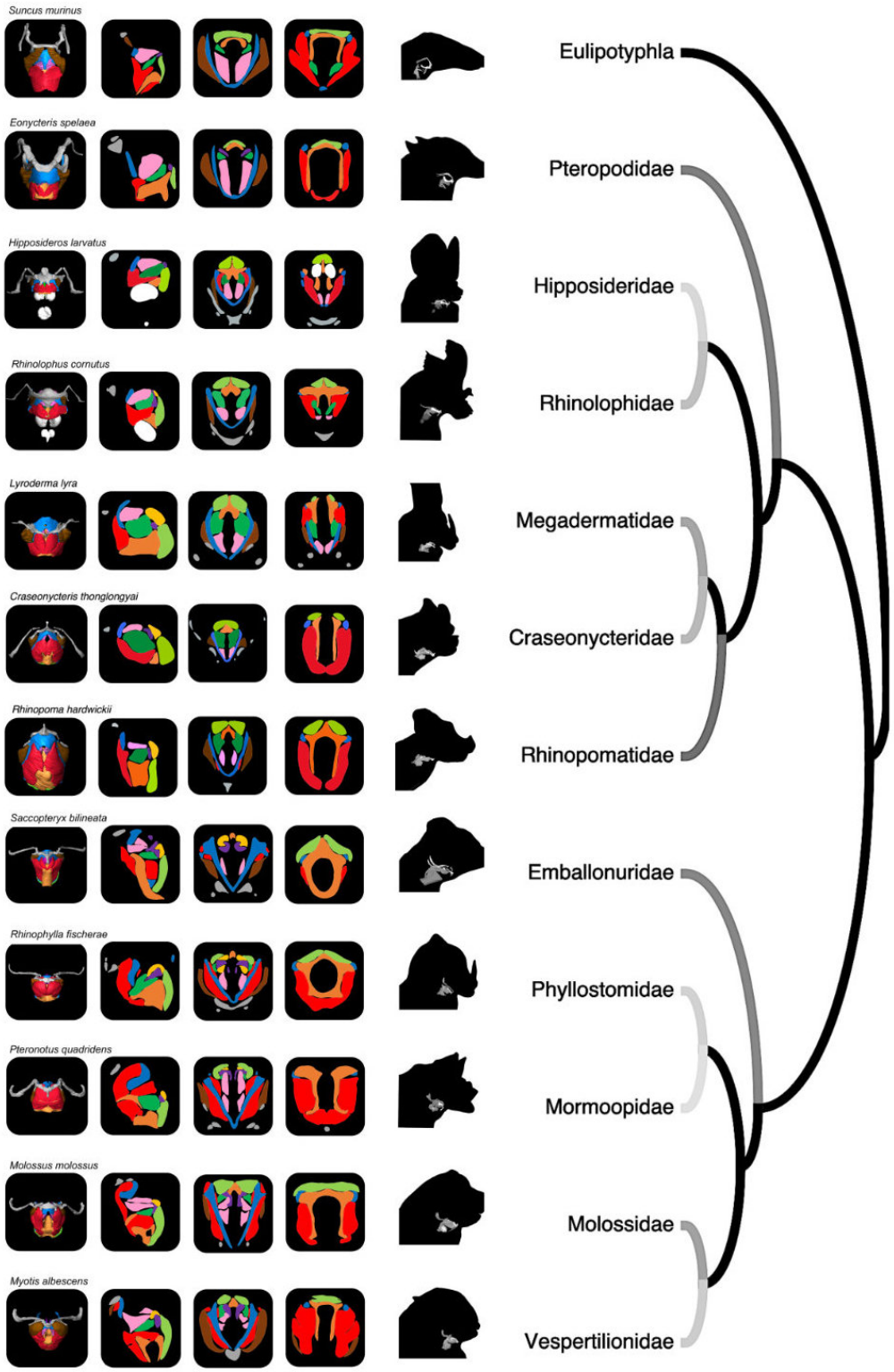

Figure 1. Distribution of the laryngeal morphology along the bat phylogeny. Color coding: blue, thyroid cartilage; brown, thyrohyoid muscle; dark green, cricoarytenoid lateralis muscle; grey, hyoid apparatus; light green, cricoarytenoid dorsalis muscle; orange, cricoid cartilage; pink, thyroarytenoid muscle; purple, arytenoid cartilage; red, cricothyroid muscle; yellow, arytenoid muscle; white, tracheal chambers.

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