PNAS
www.pnas.org

Supplementary Information for:

Hearing sensitivity: An underlying mechanism for niche differentiation in gleaning bats

Authors:
Inga Geipel[1], Ella Z. Lattenkamp[1], M. May Dixon, Lutz Wiegrebe and Rachel A. Page

[1]Joint first authors

Corresponding author:
Inga Geipel, inga.geipel@gmail.com

Table S1: Summary of recorded ABR measurements and audio sequences. The number of individuals (n) recorded per species for two different sound stimuli (tone pips and clicks), on either a logarithmic or linear scale and the number of individuals (n) for which their echolocation calls where recorded are shown below.

Species	ABR measurements									Echolocation call recordings			
	Abbreviation of ABR files	Male (n)	Female (n)	Total per species (n)	Logarithmic 5-120 kHz (n)	Linear 5-117 kHz (n)	Linear 3-6 kHz (n)	Linear 0-5 kHz (n)	Clicks	Abbreviation of audio files	Male (n)	Female (n)	Total per species (n)
Gardnerycteris crenulatum	gd1-gd3	1	0	1	1	1	1	1	1	Gd_01	1	0	1
Glyphonycteris daviesi	lb10+lb11	1	0	1	1	1	1	1	1	Lamp_02	0	1	1
Lampronycteris brachyotis	lz1+bz1	0	1	1	1	1	1	1	1	LophoS_01	1	0	1
Lophostoma brasiliense	ls1-ls15	1	0	1	1	1	1	1	1	Ls_01-Ls_05	4	1	5
Lophostoma silvicolum	mama1-mama2	5	1	6	6	5	5	4	6	Mama_01	1	0	1
Macrophyllum macrophyllum	mh1-mh10	1	0	1	1	1	1	1	1	Mh_01-Mh_05	2	3	5
Micronycteris hirsuta	mimi1-mimi14	2	3	5	5	4	5	0	5	Mimi_01-Mimi_07	5	2	7
Micronycteris microtis	ms1-ms4	5	2	7	7	7	7	1	7	Micsch_01	1	0	1
Micronycteris schmidtorum	mc+mc2	1	0	1	1	1	1	1	1	Mc_01	1	0	1
Tonatia saurophila	ts1+ts2	1	0	1	1	1	1	1	1	Ts_01	1	0	1
Trachops cirrhosus	tc3-tc6, cp7, tc8-tc16	5	3	8	8	6	6	2	8	Tc_01-Tc_07	4	3	7
Trinycteris nicefori	lb1-lb9	4	0	4	4	4	3	0	4	Lb_01, Lb_02, Tn_03	3	0	3

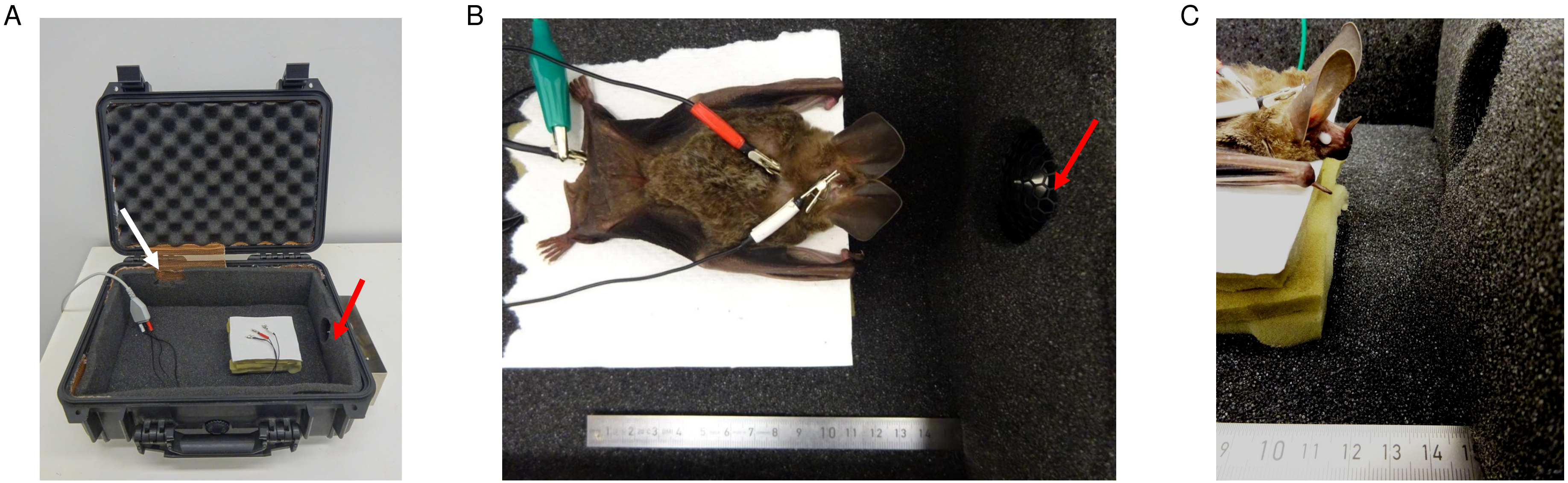

Fig. S1: ABR setup and positioning of the individuals. A) Photo of the open small sound-attenuating box, which was electrically insulated with a layer of copper mesh (see part of the mesh between lid and box, indicated by white arrow) and acoustically insulated with sound-attenuation foam. The loudspeaker was embedded in the right side of the box (indicated by red arrow). Small, soft foam pads were positioned in front of the loudspeaker to support the individual and covered with paper towels for hygienic reasons. B) Each individual was carefully positioned facing the center of the speaker (indicated by red arrow) with a 4 cm distance between its outer ear openings and the speaker. To adjust for the different sizes of the individuals, the small, soft, thin foam pats were stacked at different heights. The heads of the bats were always placed at the same level as the center of the speaker. We very diligently checked the head positioning to avoid an off-center angle towards the speaker. The recording electrode (red, plus) was placed at the caudal midline of the head, close to the brainstem and the reference electrode (white, minus) was placed at the dorsal midline of the head between the ears. The ground electrode (black) was placed on the tail membrane, carefully avoiding the calcar. To ground the bat, the ground electrode was connected to the copper mesh of the setup via a crocodile clamp (green). C) Side view: To prevent the bats' eyes from drying, a drop of eye cream was applied. *Lophostoma silvicolum* is shown as an example.

Table S2: Administered amounts of Fentanyl and anesthesia durations. For all measured species we show the body weight, amount of injected Fentanyl and duration of the anesthesia (given in min), i.e. time from the first injection until we observed the bat waking up or hanging from the side of the soft mesh cages the bats were placed in to fully recover from the anesthesia. Values represent the mean ± standard deviation (SD) for the total number of individuals. For the mean ± SD of the anesthesia duration, the number of individuals differs from the total number of individuals measured, as several individuals were used in an additional experiment and wakeup times for these individuals were not noted. The sample size (n) of the individuals for which anesthesia duration is known is given after the values, in parentheses.

Species	**Total number of individuals per species (n)**	**Weight [g]**	**Fentanyl injected [µg per g body weight]**	**Anesthesia duration [min] (n)**
Gardnerycteris crenulatum	1	15.1	0.017	376
Glyphonycteris daviesi	1	23.6	0.014	259
Lampronycteris brachyotis	1	11.5	0.017	249
Lophostoma brasiliense	1	8.1	0.012	
Lophostoma silvicolum	6	27.5 ± 2.7	0.013 ± 0.003	295 ± 81 (4)
Macrophyllum macrophyllum	1	9.4	0.013	141
Micronycteris hirsuta	5	16.1 ± 4.4	0.013 ± 0.006	188 ± 54 (4)
Micronycteris microtis	7	5.7 ± 0.3	0.014 ± 0.002	174 ± 19 (6)
Micronycteris schmidtorum	1	7.9	0.027	380
Tonatia saurophila	1	32.6	0.01	241
Trachops cirrhosus	8	33.7 ± 4.4	0.016 ± 0.005	248 ± 71 (5)
Trinycteris nicefori	4	9.2 ± 0.8	0.017 ± 0.002	276 ± 189

Table S3: Echolocation call parameter for 12 animalivorous gleaning bat species of the Neotropical leaf-nosed bat family (Phyllostomidae) from Gamboa, Panamá. Values are given as mean ± standard deviation, for species with more than one individual (n > 1) as the mean of the means.

Species	Individuals (calls)	Call duration [ms]	Peak frequency [kHz]	Bandwidth [kHz]	Min frequency [kHz]	Max frequency [kHz]	Sweep rate [kHz/ms]	Entropy
Gardnerycteris crenulatum	1 (50)	0.69 ± 0.20	71.1 ± 4.1	32.7 ± 4.8	63.2 ± 3.4	95.9 ± 4.8	47.6	0.293 ± 0.028
Glyphonycteris daviesi	1 (50)	0.55 ± 0.11	68.1 ± 4.8	36.2 ± 8.2	45.3 ± 4.9	81.5 ± 4.2	66.1	0.309 ± 0.041
Lampronycteris brachyotis	1 (50)	0.62 ± 0.21	88.1 ± 8.4	51.5 ± 13.9	68.9 ± 5.3	120.4 ± 12.5	82.5	0.367 ± 0.044
Lophostoma brasiliense	1 (50)	0.64 ± 0.10	69.1 ± 10.5	73.8 ± 12.2	58.2 ± 1.1	132.1 ± 12.4	115.5	0.398 ± 0.052
Lophostoma silvicolum	5 (250)	0.76 ± 0.08	73.7 ± 3.2	43.4 ± 3.4	56.4 ± 1.9	99.8 ± 2.2	57.0	0.336 ± 0.009
Macrophyllum macrophyllum	1 (50)	0.86 ± 0.14	77.2 ± 4.4	54.1 ± 15.8	53.8 ± 7.8	107.9 ± 14.3	63.0	0.329 ± 0.042
Micronycteris hirsuta	5 (250)	0.59 ± 0.03	80.4 ± 3.7	60.2 ± 3.4	55.9 ± 2.5	116.2 ± 3.4	101.6	0.390 ± 0.021
Micronycteris microtis	7 (350)	0.57 ± 0.04	97.6 ± 5.0	76.1 ± 5.6	60.3 ± 1.8	136.4 ± 5.0	132.6	0.430 ± 0.021
Micronycteris schmidtorum	1 (50)	0.64 ± 0.17	95.5 ± 16.9	78.5 ± 12.2	57.7 ± 6.7	136.3 ± 10.5	123.0	0.466 ± 0.041
Tonatia saurophila	1 (50)	0.69 ± 0.16	71.1 ± 8.9	64.3 ± 15.4	34.9 ± 10.4	99.2 ± 9.4	92.6	0.391 ± 0.045
Trachops cirrhosus	7 (350)	0.58 ± 0.05	69.6 ± 3.4	54.8 ± 3.5	51.5 ± 1.7	106.4 ± 3.6	94.5	0.394 ± 0.013
Trinycteris nicefori	3 (150)	0.69 ± 0.10	72.7 ± 7.7	43.3 ± 3.6	56.0 ± 6.7	99.4 ± 7.5	62.7	0.325 ± 0.016

Table S4: Comparative hearing thresholds of 12 gleaning bat species. Presented are the detected ABR thresholds (lowest sound levels (in dB peSPL) evoking a significant ABR signal) for all tested species measured in response to 29 tone pip frequencies. For species for which more than one individual was measured, the mean threshold is given and the lowest and highest measured threshold are highlighted (in green and yellow, respectively). This data is also presented in Fig. 2 of the main text.

Frequency [in kHz]	***M. microtis***	***T. nicefori***	***M. hirsuta***	***L. silvicolum***	***T. cirrhosus***	***L. brasiliense***	***L. brachyotis***	***T. saurophila***	***G. daviesi***	***G. crenulatum***	***M. macrophyllum***	***M. schmidtorum***
N	**7**	**4**	**4**	**5**	**6**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
5	75	**88**	79	56	**49**	64	82	46	64	64	88	94
9	51	**70**	38	**34**	42	40	46	34	NaN	46	70	46
13	37	**54**	39	**34**	40	40	40	34	NaN	40	46	28
17	38	**51**	42	**34**	38	40	34	40	34	34	34	34
21	**36**	**48**	40	**36**	**36**	34	34	40	34	34	NaN	40
25	36	**50**	40	**34**	36	34	34	28	34	40	40	34
29	37	**49**	39	**34**	39	40	34	40	NaN	34	34	34
33	38	**46**	**37**	**37**	38	52	40	34	34	40	40	34
37	38	**48**	39	**35**	40	64	40	34	34	40	40	52
41	39	**52**	43	**38**	**38**	76	40	34	34	52	40	34
45	42	**49**	43	**37**	39	64	40	34	40	46	40	40
49	**43**	**60**	49	46	47	64	52	40	46	40	52	46
53	**44**	**57**	49	50	47	64	52	40	46	52	52	64
57	43	**69**	49	46	**41**	76	52	40	46	52	52	46
61	47	**64**	54	50	**42**	76	58	40	46	58	52	52
65	47	**64**	58	53	**40**	82	52	40	46	52	58	58

Frequency [in kHz]	***M. microtis***	***T. nicefori***	***M. hirsuta***	***L. silvicolum***	***T. cirrhosus***	***L. brasiliense***	***L. brachyotis***	***T. saurophila***	***G. daviesi***	***G. crenulatum***	***M. macrophyllum***	***M. schmidtorum***
69	**46**	**70**	61	56	48	82	70	46	46	64	64	46
73	**45**	**70**	55	60	57	82	76	52	64	70	64	52
77	**47**	**72**	61	64	57	88	82	52	64	76	70	58
81	**49**	**73**	66	65	60	88	76	52	76	82	76	64
85	**53**	**82**	67	69	58	94	82	52	76	82	70	64
89	**55**	**79**	67	75	65	88	94	64	70	94	76	64
93	**60**	**84**	67	77	70	88	94	70	82	100	76	64
97	**62**	**85**	72	76	78	94	94	76	94	100	76	70
101	**66**	**88**	75	87	81	88	94	76	94	NaN	76	70
105	**71**	**88**	73	87	85	94	88	76	88	94	82	76
109	**77**	91	79	**93**	92	88	94	76	100	100	82	82
113	**79**	90	85	94	**95**	100	88	94	NaN	NaN	82	82
117	**84**	**100**	91	97	99	NaN	94	94	NaN	NaN	82	88

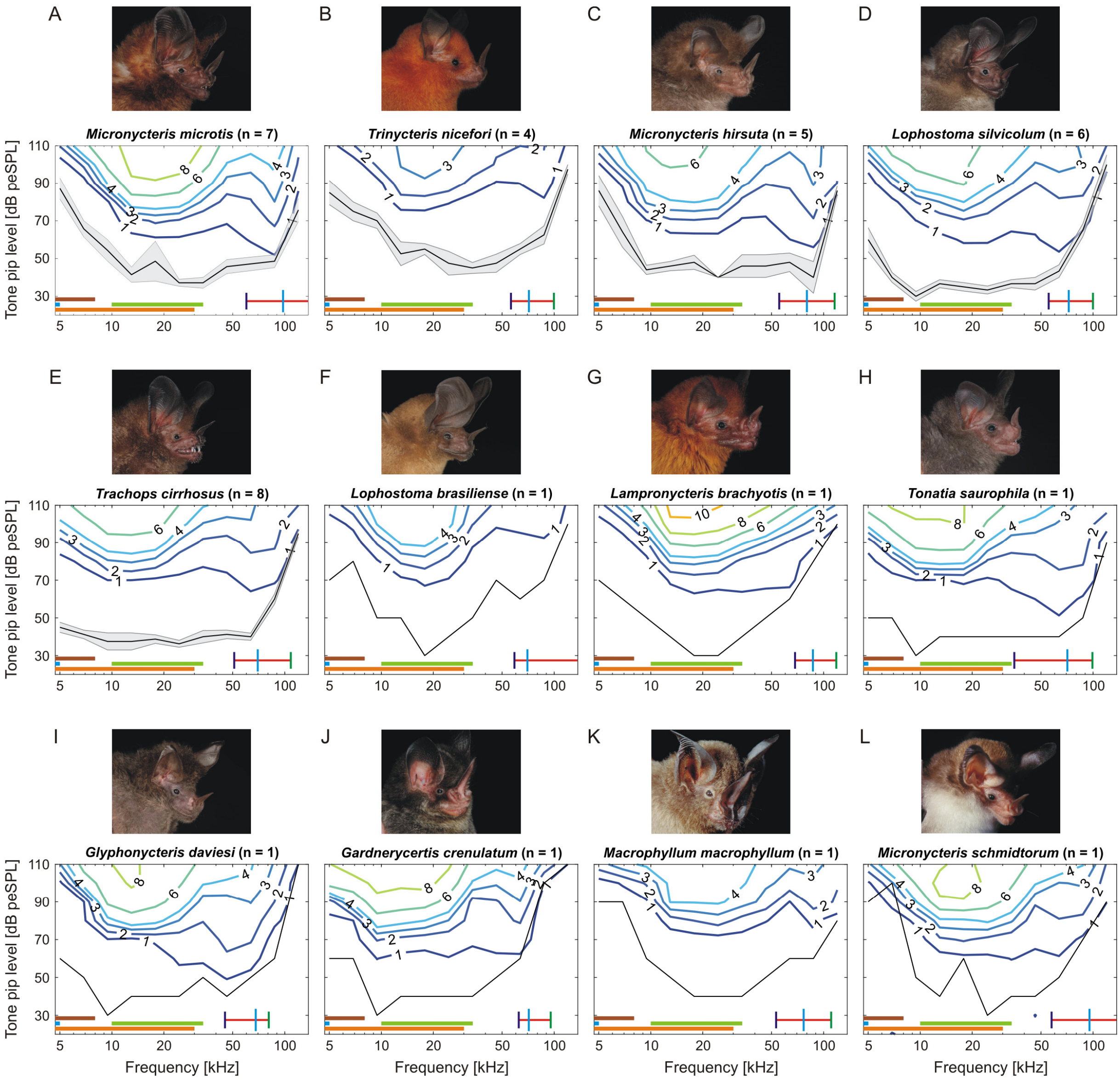

Fig. S2: Audiograms of 12 gleaning bat species. Graphs depict species-specific (mean) ABR thresholds (black lines; shading presents SEM for species with n≥4), which were calculated via bootstrap analyses. Measurements were conducted for 11 tone pip frequencies logarithmically distributed between 5 and 120 kHz and sound levels between 0 and 110 dB peSPL in 10-dB increments. The iso-response lines (colored lines) represent the different strengths of the measured ABR signal (numbers indicate ABR strength in µV). Species names and the number of measured individuals (n) are given in the respective plot title (A-L). Audiograms for single individuals (F-L) should be interpreted with care due to the low sample size (n = 1). Horizontal bars represent different prey frequency ranges: 2 - 8 kHz (crickets; brown), 0.4 - 5 kHz (frogs; light blue), 10 - 34 kHz (katydids; light green) and 3 - 30 kHz (prey rustling sounds; orange). The red horizontal bar in the audiograms indicates the recorded frequency bandwidth of the species-specific echolocation calls. Vertical bars represent minimum frequency (dark blue), peak frequency (light blue), and maximum frequency (green). For detailed echolocation call parameter measurements see SI Appendix Table S4. Bat portraits here and in Fig. S3, Fig. S4 and Fig. S5 with courtesy of Marco Tschapka.

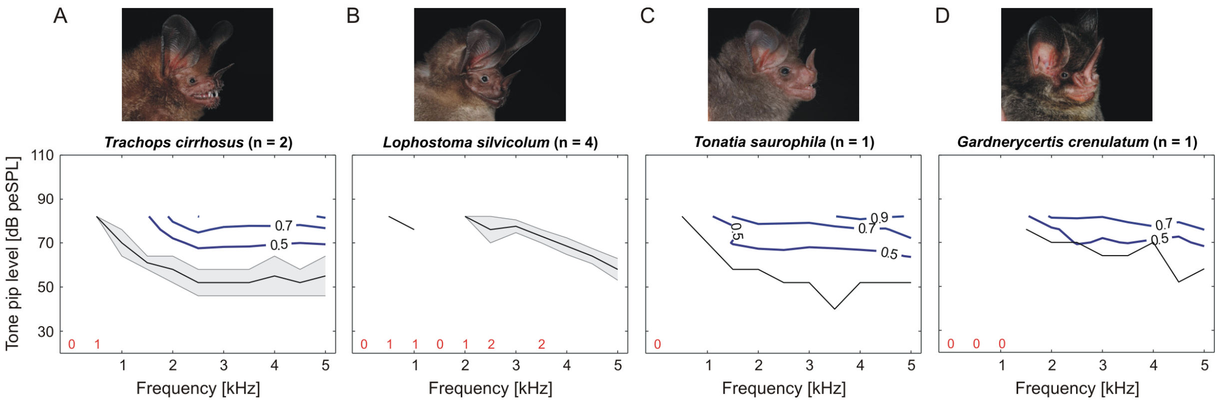

Fig. S3: Audiograms of four gleaning bat species with measurable ABR's below 5 kHz. Graphs depict species-specific (mean) ABR thresholds (black lines; shading presents SEM for species with n≥2), which were calculated via bootstrap analyses. Measurements were conducted for 11 tone pip frequencies linearly distributed between distributed between 0 and 5 kHz and 10 sound levels between 28 and 82 dB peSPL. The iso-response lines (colored lines) represent the different strengths of the measured ABR signal (numbers indicate ABR strength in µV). Species names and the number of measured individuals (n) are given in the respective plot title (A-D). The measurements should be considered with caution due to lack of calibration of the speaker below 2 kHz. Aside from *T. cirrhosus* and *L. silvicolum* (Fig. 3D,E), we found low auditory thresholds in the low frequency range (3 - 6 kHz) for *Tonatia saurophila* and *Gardnerycteris crenulatum* (Fig. 3H,J). For these four species we were also able to detect hearing thresholds below 3 kHz. The other eight species showed only weak responses to frequencies between 3 - 6 kHz (Fig. 3).

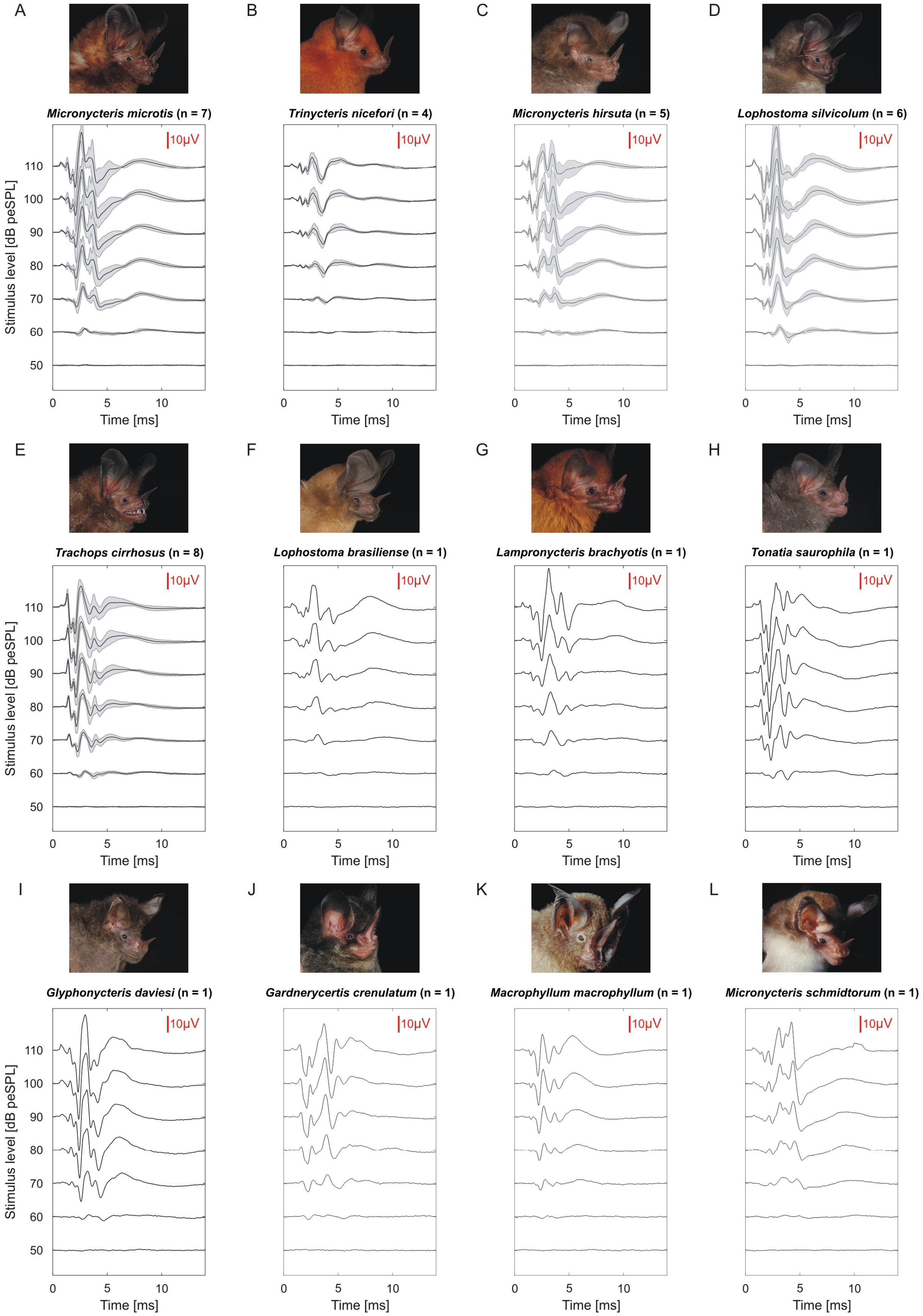

Fig. S4: Stimulus level dependent ABR waveforms of 12 gleaning bat species. The panels show the (mean) click-evoked ABR waveform (solid black line) and SEM (shading, for species with n>1). Displayed are ABRs recorded in response to broadband clicks (i.e. broadband impulses with a flat power spectrum between 3 and 120 kHz) presented at 50 to 110 dB peSPL stimulus level. The species show species-specific characteristics in the location of the waveform extrema, which correspond to variation in the neuronal activity in the different processing centers along the auditory pathway.

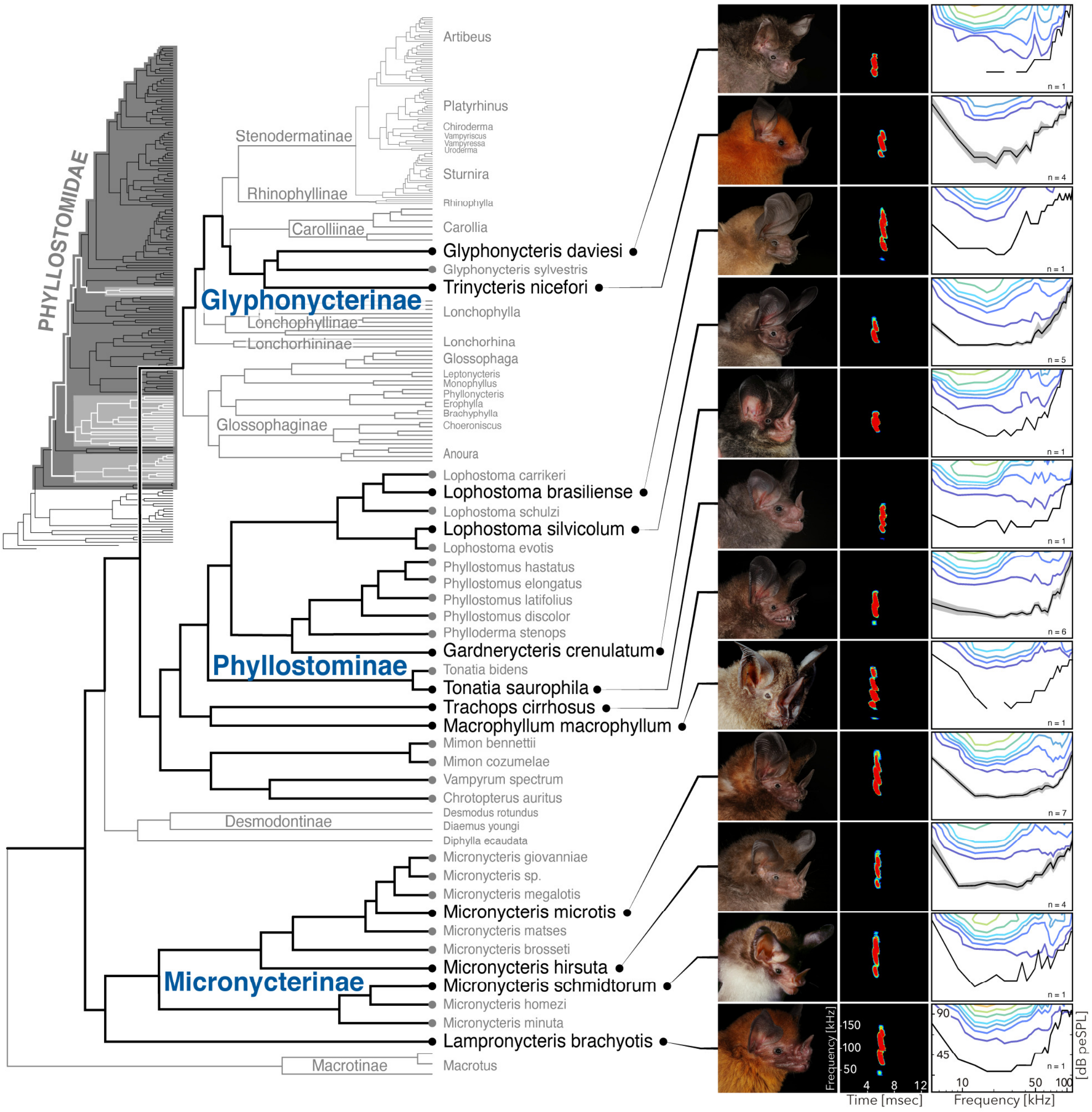

Fig. S5: Phylogeny, echolocation calls and audiograms of 12 gleaning bat species in the family Phyllostomidae. Phylogeny redrawn from Rojas et al. (1) highlights the 12 gleaning species included in our study. Adjacent to the bat portraits, spectrograms illustrate a representative echolocation call for the respective studied species. Frequency and duration are scaled for comparison. Calls were filtered with a noise reduction filter (see Methods). Spectrogram parameters: FFT size 1024, FlatTop window, 96.43 % overlap; frequency resolution: 488 Hz, temporal resolution: 0.064 ms. Furthest to the right, graphs depict species-specific (mean) ABR thresholds (black lines; shading presents SEM for species with $n \geq 3$), which were calculated via bootstrap analyses. Measurements were conducted for 29 tone pip frequencies linearly distributed between 5 and 117 kHz and 13 sound levels between 28 and

100 dB peSPL in 6-dB increments. The iso-response lines (colored lines) represent the different strengths of the measured ABR signal.

References:

1. D. Rojas, O. M. Warsi, L. M. Dávalos, Bats (Chiroptera: Noctilionoidea) challenge a recent origin of extant Neotropical diversity. *Syst. Biol.* **65**, 432-448 (2016).