## **Supporting Information**

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**Fig. S1.** Comparison of density of soft tissues between four Sooglossidae and three Eleutherodactylidae. (*Center*) Transparent volume rendering of *Sechellophryne pipilodryas*, showing the in situ position of the organs. Volume rendering of the holotomography of the body and tissues surrounding the ear. The skin is represented in violet, cartilage in yellow, the tongue in red, the brain is in pink, muscles in green, and the lens in blue. Boxplots (means, SD) present the density of tissues (in g/cm<sup>3</sup>) of Sooglossidae (earless, S) and of Eleutherodactylidae (eared, E). The results demonstrated no differences between eared and earless frogs ( $F_{1,30} = 1.84$ ; P = 0.19), yet showed significant differences between tissues ( $F_{5,30} = 14.46$ ; P < 0.001), with skin being significantly denser than all other tissues (Bonferroni post hoc tests, all P < 0.05). Cartilage, brain, and muscle showed the lowest density and were not different from one another. The tongue and lens were of intermediate density and different from other tissues.



Fig. S2. Schematic of the experimental setup used in synchrotron tomography and the numeric simulations. (A) European Synchrotron Radiation Facility is a source of synchrotron radiation of the third generation characterized by a low emittance and high brilliance. Electrons emitted by an electron gun are first accelerated in a linear accelerator (linac) and then transmitted to a circular accelerator (booster synchrotron) where they are accelerated to reach an energy level of 6 GeV. These high-energy electrons are then injected into a large storage ring 844 m in circumference with a current of 200 mA. (B) A high-intensity Xray beam is produced in one of the straight sections of the storage ring by an insertion device (wiggler). (C) A double crystal silicon monochromator selects the photons with an energy close to 20.5 keV from the synchrotron radiation emitted by the insertion device. (D) The experimental station ID19 of the European Synchrotron Radiation Facility (Grenoble, France), a long (150 m) imaging beamline, provides a large beam [40 (h) × 15 (v) mm<sup>2</sup>] with high spatial coherence [transverse coherence length ~30 µm (h) × 180 µm (v)]. (E) Experimental setup for holotomography. (F) The specimen (frog) is rotated around the vertical axis. (I) A high-resolution detector system records Fresnel diffraction patterns at varying distances (G) with respect to the sample (typically four distances). In the detection device used, visible light produced by an X-ray sensitive converter (H) is imaged onto a cooled CCD with 2,048 × 2,048 pixels in a (J) FReLoN camera 33. The effective pixel size was 7.5 µm, resulting in a field of view of 15 × 15 mm<sup>2</sup>. (M) Phase map retrieved from (K) radiographs at four sample-to-detector distances. (L) Absorption tomography slice through the frog head using the absorption radiographs (permitting to map the degree of mineralization) and (N) holotomography slice using the retrieved phase maps. The electron density of the holotomography map is in turn nearly proportional to the mass density. The relevant quantity, leading to the images, can be considered to be either mass or electron density. (O) Image segmentation at the level of the inner ears permitting to extract 3D morphometric data and volume rendering of the ear region. (Q) Speed of sound and (P) density maps where extracted from the highresolution tomography images. (R) Grid of propagation field of acoustic wave for numeric simulation in finite differences.



Fig. S3. Aerial transmission by bony conduction. Frames show the sound propagation (at 5 kHz) on a coronal section of the head of S. gardineri.



Fig. S4. Acoustic mode of the lung in S. gardineri. Results of a finite-element calculation of the acoustic mode of the lung in a ventral view. Color scale represents the acoustic pressure in Pascals.

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## Table S1. Acoustic parameters measured on the advertisement calls

Parameters	Average	SD	Minimum	Maximum	N 52
Call duration (s)	0.185	0.027	0.134	0.250	
Dominant frequency(Hz)	5,710	420	4,225	6,767	52
Fundamental frequency (fo) (Hz)	1,830	134	1,408	2,076	21
2fo (Hz)	3,700	268	2,812	4,157	25

## Table S2. Summary parameters of tissues measured surrounding the inner region in eared and earless frogs

Taxon	Minimum thickness of tissues between air and inner ear				Number of interfaces of tissues		Head (mm)	
	Dorsal (µm)	Lateral (µm)	Ventral (µm)	Ventral/dorsal	Dorsal	Ventral	Length	Width
Eared frogs								
Eleutherodactylus pinchoni	234.9 ± 10.1	822.0 ± 19.3	240.1 ± 10.2	1.0	3–4	3–5	6.01	5.54
Eleutherodactylus barlagnei	270.2 ± 21.3	1012.9 ± 11.6	416.7 ± 83.0	1.5	3	4–5	7.78	7.64
Eleutherodactylus martinicensis	326.6 ± 25.4	1217.5 ± 27.1	433.3 ± 61.0	1.3	3–4	3–4	8.89	8.78
Earless frogs								
Sechellophryne gardineri	112.1 ± 7.6	508.3 ± 14.2	81.0 ± 8.8	0.7	2–3	2	4.19	3.79
Sechellophryne pipilodryas	133.7 ± 14.6	588.0 ± 15.1	106.6 ± 4.7	0.8	2–3	2–3	4.47	3.87
Sooglossus sechellensis	179.5 ± 27.0	644.8 ± 29.6	140.7 ± 11.7	0.8	2–3	2–3	6.74	6.31
Sooglossus thomasseti	510.8 ± 35.4	2500.8 ± 55.3	358.7 ± 27.2	0.7	3–4	2–3	14.03	13.98

Ventral corresponds to tissue between the inner ear and the upper surface of oral cavity. The distance between the mouth resonator and the inner ears is compared with the distance of the outer surface of the head and the inner ears. To rescale frogs of different sizes, the ratio of distance between the mouth and the ear to the distance between the above head and the ear was calculated. This ratio is lower for earless frogs and thus contributes to a better transmission of sound from the mouth to the ears. In addition, this reduced thickness corresponds to fewer layers of tissue (two to three) separating the oral cavity from the inner ear compared with eared frogs (three to five). Alternating tissue materials constitute impedance jumps that are responsible for reduced acoustic transmission. Frogs without ears optimize transmission between the ear and the mouth in two ways: reduced total thickness and a reduced number of interfaces.

In eared species, interfaces consist of the roof of the mouth/muscle (or not)/first layer of bone/diploe with fat/second layer of bone. In contrast, in all of the earless frogs studied, the interfaces consist of the roof of the mouth/ muscle (or not)/one layer of bone or homogeneous cartilage.

Audio S1. Recording of S. gardineri advertisement call.

Audio S1

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