Integrative Zoology 2017; 12: 172–184 doi: 10.1111/1749-4877.12249

ORIGINAL ARTICLE

A blind climber: The first evidence of ultrasonic echolocation in arboreal mammals

Aleksandra A. PANYUTINA,[1,2] Alexander N. KUZNETSOV,[2] Ilya A. VOLODIN,[2,3] Alexei V. ABRAMOV[4,5] and Irina B. SOLDATOVA[2]

[1]Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia, [2]Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia, [3]Scientific Research Department, Moscow Zoo, Moscow, Russia, [4]Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia and [5]Joint Vietnam–Russian Tropical Research and Technological Centre, Hanoi, Vietnam

Abstract

The means of orientation is studied in the Vietnamese pygmy dormouse *Typhlomys chapensis*, a poorly known enigmatic semi-fossorial semi-arboreal rodent. Data on eye structure are presented, which prove that *Typhlomys* (translated as "the blind mouse") is incapable of object vision: the retina is folded and retains no more than 2500 ganglion cells in the focal plane, and the optic nerve is subject to gliosis. Hence, *Typhlomys* has no other means for rapid long-range orientation among tree branches other than echolocation. Ultrasonic vocalization recordings at the frequency range of 50–100 kHz support this hypothesis. The vocalizations are represented by bouts of up to 7 more or less evenly-spaced and uniform frequency-modulated sweep-like pulses in rapid succession. Structurally, these sweeps are similar to frequency-modulated ultrasonic echolocation calls of some bat species, but they are too faint to be revealed with a common bat detector. When recording video simultaneously with the ultrasonic audio, a significantly greater pulse rate during locomotion compared to that of resting animals has been demonstrated. Our findings of locomotion-associated ultrasonic vocalization in a fast-climbing but weakly-sighted small mammal ecotype add support to the "echolocation-first theory" of pre-flight origin of echolocation in bats.

Key words: arboreal locomotion, reduced eyes, Rodentia, *Typhlomys,* ultrasonic echolocation

INTRODUCTION

Solid surfaces reflect both light and sound, which gives animals an opportunity to perceive out-of-touch surroundings using vision or echolocation. Use of vision is much more common because life on Earth is full of sunlight. Only under poor light conditions are animals forced to expend their own metabolic energy to

Correspondence: Aleksandra A. Panyutina, Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071, Russia.
Email: myotis@mail.ru

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

emit sounds and listen to echoes instead of using vision. To approach acuity of vision echolocation calls must be composed of as short as possible wavelengths to come into ultrasonic range. Among mammals, ultrasonic echolocation is used in water by odontocetes, from dolphins up to pygmy sperm whale (Madsen *et al.* 2005), and is used on land by chiropterans (excluding most fruit bats [Pteropodidae]) (Jones & Teeling 2006). Beyond these species no other mammal has yet been shown to rely on ultrasonic echolocation as the main means of orientation, either in water or on land.

Early reports on ultrasonic echolocation in shrews (Gould *et al.* 1964; Tomasi 1979; Forsman & Malmquist 1988) were subject to doubt (Konstantinov & Movchan 1985). Indeed, ultrasonic echolocation is likely disadvantageous as compared to touch in the typical environment of shrews. Inside leaf-bedding the high-frequency calls would be reflected as noise from all the nearby obstacles, which are readily accessible to vibrissae. These calls would not pass beyond these obstacles to provide far-away echoes for long-range orientation. Newer studies have failed to show ultrasonic echolocation in shrews (Catania *et al.* 2008; Siemers *et al.* 2009; Volodin *et al*. 2012, 2015; Zaytseva *et al.* 2015). It has been argued that lower-frequency calls (audible clicks and twitters) must be more appropriate for echolocation in cluttered substrates, and can be used by shrews to gain echoes for orientation (Siemers *et al.* 2009; Volodin *et al.* 2015; Zaytseva *et al.* 2015).

All available speculations on echolocation regarding small rodents come from behavioral experiments: dormice, hamsters, voles and rats were vision-deprived and forced to look for food (see review by Thomas & Jalili 2004). The only instance of potential ultrasonic echolocation was seen in Norway rats [*Rattus norvegicus* (Berkenhout, 1769)]. However, there was very limited acoustic analysis provided in the study (Thomas & Jalili 2004). The study concluded that the calls were echo-locating based on weak evidence including the pulse-like waveform of the calls, and on the fact that they were produced by rats left alone in the dark.

Therefore, neither shrews nor typical rodents aid to portray how a quadrupedal mammal can rely on ultrasonic echolocation. The issue is interesting not only by itself, but also because such an animal, if discovered, could serve as a model for the hypothesized ancestral state of bat echolocation. There are three currently debated theories on how echolocation in bats may have evolved (Speakman 2001; Jones & Teeling 2006; Simmons 2008; Maltby *et al.* 2010). According to the "flight-first theory," echolocation arose in bats after acquisition of flight ability (Speakman 2001, 2008; Simmons *et al.* 2010). The "echolocation-first theory" insists that echolocation was acquired earlier than flight by quickly moving small mammals adapted to complex but poorly-lit environments (Fenton *et al.* 1995; Jones & Teeling 2006; Teeling 2009; Teeling *et al.* 2012). According to the "tandem theory," the flight and echolocation developed simultaneously (Schnitzler *et al.* 2003). The "flight-first theory" is best supported by current evidence due to the fact that there is an absence of extant examples of non-volant mammals using ultrasonic echolocation. Followers of the "echolocation-first theory" must find a peculiar prototype: a mammal that has acquired ultrasonic echolocation and lives in an environment more appropriate as a runway for flight than the depth of leaf-bedding.

Zoologists have identified many mammals with reduced eyes and many fast-climbing mammals, but no mammal except the poorly studied *Typhlomys* (Fig. 1) combines these extreme features together. *Typhlomys*, which means "the blind mouse," belongs to an enigmatic rodent family, Platacanthomyidae (Jansa *et al.* 2009). The genus *Typhlomys* consists of two species, of which *Typhlomys chapensis* Osgood, 1932 (the Vietnamese pygmy dormouse) lives in northern Vietnam and *Typhlomys cinereus* Milne-Edwards, 1877 lives in southern China (Abramov *et al*. 2014). The pygmy dormice inhabit subtropical forests at the altitudes of 360–2000-m

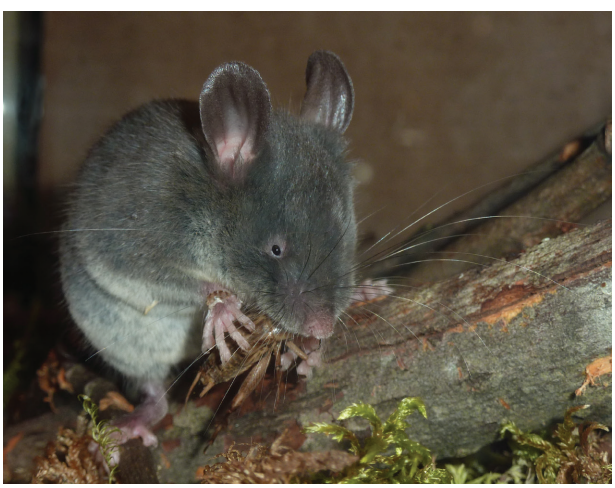

Figure 1 Vietnamese pygmy dormouse *Typhlomys chapensis*. Its reduced eyes are reflected in the generic name, which means "the blind mouse."

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

a.s.l. (Smith 2008; Abramov *et al.* 2012). The biology of these rodents is poorly studied; they are reported to be both terrestrial and burrowing (Smith 2008), and we trapped *T. chapensis* by live cage-traps not only on the ground but on tree branches too (Abramov *et al*. 2014), which indicates they have climbing ability. Keepers at the Moscow Zoo, which obtained 2 of these animals, confirm their outstanding agility, especially in an arboreal environment, and also report their strictly nocturnal activity and the absence of any vocalizations. One can hypothesize that with reduced eyes, the arboreal mobility in the dark and silence could be guided by ultrasonic echolocation.

In this study we have conducted the following experiments to provide evidence for use of ultrasonic echolocation in *Typhlomys*: (1) checked vision quality using histology to study the morphology of the eye structure; (2) investigated the presumed existence of ultrasonic vocalization with sensitive acoustic equipment and; (3) upon finding ultrasonic vocalization we used video and audio recordings to correlate vocalization with locomotion in an environment that is difficult to navigate using non-visual orientation.

MATERIALS AND METHODS

Eye morphology

Two ethanol-preserved adult specimens of *T. chapensis* from the Zoological Institute of the Russian Academy of Science (Saint Petersburg, Russia) were studied. They were collected in northern Vietnam in the mountain tropical forest in 2010–2012 by live cage-traps set up either on branches or on the ground. These specimens are: a 16.7-g female (ZIN 101563) and a 18.3-g male (ZIN 101566). In each specimen, both eyes were excised, and the eyeball axial length was measured externally with a vernier caliper (precision 0.1 mm) under a Stereomicroscope Carl Zeiss Stemi SV 11.

The expected eyeball axial length was calculated using an equation from Howland *et al.* (2004) for mammals in general:

Log (eye axial length in mm) = 0.9354+ 0.225*Log (body mass in kg), (1)

with coefficient of determination $R^2 = 0.835$.

In addition, we calculated the regression equation separately for rodents, using data on 28 rodent species from Howland *et al.* (2004) as:

Log (eye axial length in mm) = 0.9243+ 0.2161*Log (body mass in kg), (2)

with $R^2 = 0.650$.

We calculated the regression equation for bats, using data on 11 bat species from Howland *et al.* (2004) as:

Log (eye axial length in mm) = 0.9566+ 0.3255*Log (body mass in kg), (3)

with $R^2 = 0.745$.

After measurements were taken, all 4 eyeballs with a bit of surrounding tissues were embedded in paraffin, cut into 4 or 5-μm thick serial sections and stained according to Mallory or with hematoxylin and eosin using standard procedures. Sections were studied under a Leica DM 2500 M microscope.

Experimental design

Two adult males of *T. chapensis* (#1 and #2) were studied experimentally at the Scientific Department of the Moscow Zoo (Moscow, Russia). Like the specimens used in eye morphology research, they were collected in northern Vietnam in the mountain tropical forest in 2010–2012 by live cage-traps set up either on the branches or on the ground. In the Moscow Zoo, they were kept for 10 months before the acoustic experiments, under a natural light regime and room temperature (24–26 °C); individuals were isolated in glass-and-wire-mesh cages 40 × 40 × 80 cm with mulch bedding and various shelters and branches. The animals were fed with small rodent chow and water *ad libitum*.

Ultrasound-and-video recordings were carried out on 2 days in July 2013. Two adult males (#1 and #2) were tested singly in a separate room without other animals. Tests were performed in the experimental chamber (30 × 50 × 100 cm) having the back 50 × 100-cm wall made of smooth plastic, the front 50 × 100-cm wall and one 30 × 50-cm wall made of glass, and the other 30 × 50-cm and both 30 × 100-cm wall made of wire mesh (10 × 10 mm). The chamber contained straight and furcated dry branches 0.5–5.0-cm thick and the mulch bedding. During test trials the cage was set either vertically (see Supplementary_1.mov video) or horizontally (in respect of direction of its 100-cm side), with the branches being re-positioned from time to time (when exploratory activity of the animal ceased) so that the test animal remained unfamiliar with the environment. This was aimed at sustaining exploratory activity of the animals.

In total, 13 test trials (7 with male #1 and 6 with male #2) were conducted. A test trial lasted 2 to 12 min, depending on the behavior of the subject animal. All the test trials were performed between 1400 and 1800 hours at temperatures of 24–26 °C; there were two trials with

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

natural light from windows and 11 trials with bright light of halogen lamps (1.5 kWt in total) for better quality high-speed video recording. The test trials were audio and video recorded with an ultrasonic recorder and two camcorders simultaneously; the recordings were synchronized by clicker signals.

The Pettersson D 1000X recorder with a built-in microphone (Pettersson Elektronik AB, Uppsala, Sweden) was used for audio recording (sampling rate 768 kHz, 16 bit; frequency response from 5 to 235 kHz). During recordings, the distance from the hand-held microphone to a test animal varied from 10 to 50 cm. The microphone was oriented as far as possible directly to the muzzle of tested animal because the sector for ultrasound recording is rather narrow (see Supplementary_2.mov video). Each test trial was recorded as a WAV-file. The total duration of audio recordings was 62 min (30 min from male #1 and 32 min from male #2).

Two camcorders were used simultaneously for video recording: the JVC GC-PX10 camcorder (Victor Company of Japan, Yokohama, Japan) was used for a high-definition video (HD-video, 50 fps, shutter speed 1/1000 s, frame size 1920 × 1080 pixels, with soundtrack) and the Casio EX-F1 camcorder (Casio Computer, Tokyo, Japan) was used for a high-speed video (HS-video, 299.7 fps, shutter speed 1/2000 s, frame size 512 × 384 pixels, no soundtrack).

Acoustic analysis

All acoustic files were inspected for the presence of ultrasound by means of the Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany). Next, 540 pulses of good quality (325 pulses from male #1 and 215 pulses from male #2) were selected for acoustic analysis. After a high-pass filtration at 10 kHz (sampling frequency 768 kHz, Hamming window, FFT 512 points, frame 50%, overlap 93.75%, frequency resolution 1500 Hz, time resolution 0.04 ms), the maximum fundamental frequency (fmax) and the minimum fundamental frequency (fmin) were measured for each pulse, and the pulse bandwidth as bandw = fmax–fmin (Fig. 2b) was calculated. The pulse duration (dur), the duration to the pulse maximum amplitude in percentage of the entire pulse duration (disttomax) (Fig. 2b) and the peak frequency of a pulse (fpeak) (Fig. 2c) were measured by applying the automated parameter measuring option of Avisoft. The periods between pulses (period), from the beginning of a pulse to that of the next pulse (Fig. 2a)

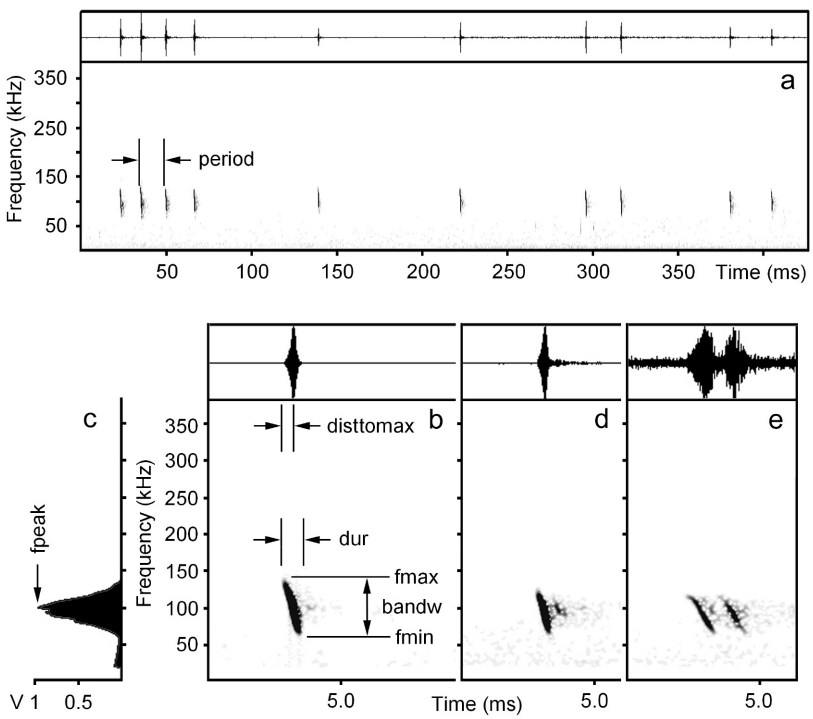

Figure 2 The waveforms, spectrograms and power spectrum (c) representing acoustic patterns and acoustic variables measured from the vocal pulses of *Typhlomys chapensis*. (a) Natural sequence of pulses, of which the first 4 comprise a typical bout; period, the period between pulses. (b) and (c) A pulse without echo; fmax, the maximum fundamental frequency; fmin, the minimum fundamental frequency; bandw, the pulse bandwidth; dur, the pulse duration; disttomax, the duration to the pulse maximum amplitude; fpeak, the peak frequency. (d) A pulse with weak echo. (e) A pulse with strong echo.

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

were also measured. Measurements were exported automatically to Microsoft Excel software (Microsoft, Redmond, WA, USA) for further processing.

As the study sample (only 2 animals) was too small for individual-based statistical analysis, we used the pooled samples from both individuals according to Leger and Didrichsons (1994). Analysis was carried out with STATISTICA v. 8.0 software (StatSoft, Tulsa, OK, USA). Significance levels were set at 0.05, and 2-tailed probability values were tested. Means are given as mean ± SD.

Video analysis

To estimate the probable bearing of orientation of experimental animals on the uncovered ultrasonic pulses, the relationship between vocalization and locomotion was studied. The respective ultrasonic audio-tracks and video-tracks were superimposed by using VirtualDub (http://www.virtualdub.org/) and AviSynth (http://sourceforge.net/projects/avisynth2/) software. Ultrasonic audio tracks and HD-video tracks were superimposed based on clicker signals. The HD-video tracks and HS-video tracks were superimposed based on animal postures. The total duration of the resulting HD-video or HS-video clips with the ultrasonic audio track was 57 min (see Online Resources Video for example).

The recordings were sampled by selecting the first 0.5-s fragment from every 5-s interval. Each selected 0.5-s fragment was classified into locomotion or resting state of the animal by viewing the video track in VirtualDub software. The audio track was analyzed using Syrinx software (http://www.syrinxpc.com/). The number of ultrasonic pulses was counted and their arrangement in bouts was registered for each 0.5-s fragment (detailed definition of a bout is presented in the Results section). The corresponding pulse rates (pulses per second) and numbers of single pulses and 2-pulse, 3-pulse etc. bouts per second were calculated. Fragments, in which the animal was beyond the video frame or in which ultrasonic pulses were overlapped with clicker strikes or other noises, were excluded from the analysis. In total, 531 0.5-s fragments (247 fragments from male #1 and 284 fragments from male #2) were analyzed.

To compare the pulse rates in the 0.5-s fragments during rest and locomotion, we used the online Kolmogorov–Smirnov test (http://www.physics.csbsju.edu/stats/KS-test.html). In addition, the medians ± quartiles were estimated for the 0.5-s fragments containing pulses (pulse rate >0).

Some ultrasonic pulses together with their echoes could have been missed by the microphone due to the narrow beam of ultrasound propogation and the movement of the subject animal outside of the audio recording reach. At the same time, virtually all pulses emitted by resting animals were collected. However, some underestimation of the pulses emitted during locomotion is unlikely to affect the validity of the present results.

RESULTS

Eye morphology

In each of the 2 studied specimens with an average body mass of 17.5 g, the eyeball axial length was measured as 1.4 mm. According to Equation (1), the expected eye axial length for a 17.5-g mammal should be 3.5 mm. According to Equation (2), the expected eye axial length for a 17.5-g rodent should also be 3.5 mm. According to Equation (3), the expected eye axial length for a 17.5-g bat should be 2.4 mm. Thus, the eye axial length of *Typhlomys* is very small, not only compared to an average mammal or rodent, but even to an average bat.

The measurement of the total eyeball inevitably included some soft tissue at its posterior side. Measurements on histological sections (Fig. 3) give even a smaller value of approximately 1 mm for the net eyeball diameter. The lens front surface, which faces the pupil, is somewhat irregular (Fig. 3b1). The lens matter is very damaged on our histological sections, but looks rather normally everywhere except for the pupil-facing portion, which is filled with irregularly packed fibers (Fig. 3b2).

Our histological sections show very restricted, if any, vitreous chamber between the lens and retina. Some parts of the retina tightly adjoin the lens, and the other parts form irregular folds (Fig. 3a). These folds are definitely not an artifact of hard ethanol fixation but the natural condition of the live animal, as is shown by the specific structure of the nerve fiber layer in the deep folds (Fig. 3b3). If they were an artifact the nerve fiber layer would be folded together with the other layers of retina to form a double lamina. Contrary to that, axons of ganglion cells converge from the opposite walls of the fold to form, inside it, a single lamina of the nerve fiber layer; if one decided that it was an artifact and tried to spread the fold, these axons would be inevitably torn. In the retina, ganglion cells are widely spaced from each other. In our series of sections, we did not find any local

© 2016 International Society of Zoological Sciences, Institute of Zoology/
Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

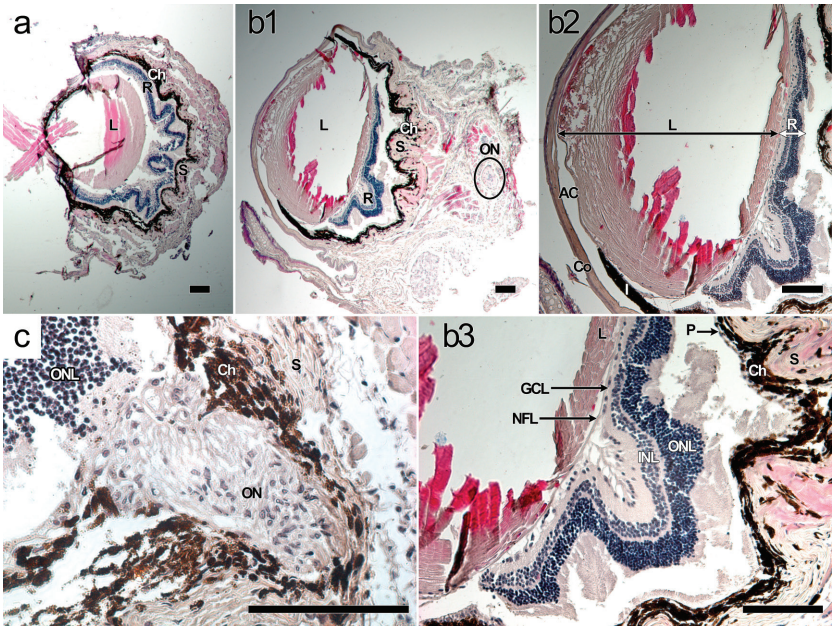

Figure 3 Eye structure in *Typhlomys chapensis*. (a) Section parallel to the optic axis stained according to Mallory represents extensive folding of retina. (b) Section through the optic axis stained according to Mallory at different magnifications: (b1) general eye composition, (b2) close-up view of the retina and (b3) close-up view of the retinal fold. (c) Longitudinal section of the optic nerve at its exit out of retina stained with hematoxylin–eosin shows gliosis of the optic nerve. AC, anterior chamber; Ch, choroid; Co, cornea; GCL, ganglion cell layer; I, iris; INL, inner nuclear layer; L, lens; NFL, nerve fiber layer; ON, optic nerve; ONL, outer nuclear layer (rod nuclei); P, pigment epithelium; R, retina; S, sclera. Scale bars 0.1 mm.

concentrations of ganglion cells. The section represented on Fig. 3b2 allows one to count them 1 by 1. Their number in this section is as few as 70–75, including 21–23 ganglion cells in the retinal fold. In accordance with the very small amount of the ganglion cells, the nerve fiber layer is very thin too (see again the nerve fibers in the deep retinal fold represented in Fig. 3b3). Finally, the optic nerve of *Typhlomys* is characterized by an irregular arrangement of nuclei inside it (Fig. 3c), whose pattern is indicative of reactive gliosis. We found no blood vessels supplying retina inside the optic nerve.

Locomotor behavior

In captivity, *Typhlomys* likes to dig in mulch and, at the same time, shows evidence of arboreal adaptation. Its agile movements over branches seem to indicate that its eyes function well (this cannot be true, however, with the given eye morphology). It moves with confidence in an unfamiliar environment over sloping and vertical branches, while also making rapid aimed leaps from branch to branch over gaps several times greater than the tactile reach of its vibrissae (see Supplementary_1.mov video). It can also upwardly jump 30–40 cm (body length being 7–9 cm, and vibrissae not longer than 5 cm). From time to time, the animal hangs for a while upside-down on its hind limbs and, detecting an appropriate surface below, beyond the reach of vibrissae, jumps down onto it (see Supplementary_3.mov video).

Acoustics

Almost all recordings, which we obtained in various experiments with *Typhlomys*, were surprisingly full of soft ultrasonic vocalizations composed of rather uniform units (Fig. 2). These units are pulses represented in most recordings by the fundamental frequency band only; the second (harmonic) frequency band was pronounced in less than 1% of pulse records. The fundamental frequency band shows a descending pattern of frequency modulation from the maximum at 127.3 ± 6.3 kHz to the minimum at 64.1 ± 4.6 kHz, resulting in pulse bandwidth of 63.2 ± 7.3 kHz. The pulse peak frequency is 93.4 ± 7.4 kHz, the pulse duration is 0.68 ± 0.15 ms, and the pulse amplitude reaches a maximum in the middle of the pulse (51.6% ± 11.1% from the start).

Single pulses alternate with double-pulse bouts (dyads), triple-pulse bouts (triads), and up to 7-pulse bouts. Bouts can be distinguished by shorter silent intervals between the pulses: intra-bout intervals were always shorter than 23 ms, while inter-bout intervals were always longer than 26 ms. In fact, an essential bout specificity exists (Fig. 2a): if there are 3 or more pulses in a bout, they are evenly spaced, so that the intervals are almost equal or change gradually differing from each oth-

© 2016 International Society of Zoological Sciences, Institute of Zoology/
Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

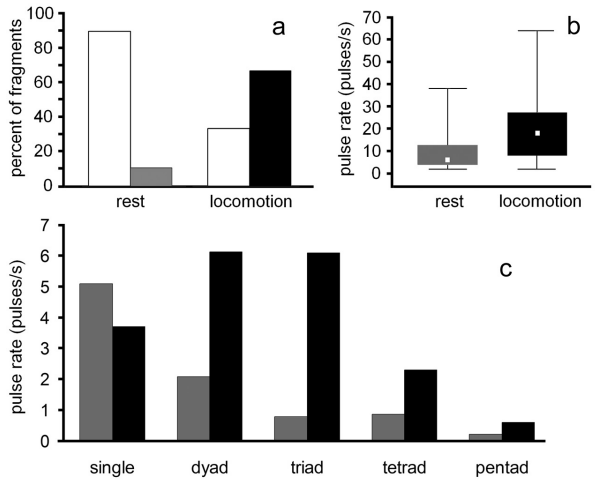

Figure 4 Relationship between vocalization and locomotion in *Typhlomys chapensis*. (a) Proportion of 0.5-s fragments without vocal pulses (white bars) and with pulses (grey or black bars) at rest and during locomotion. (b) Total pulse rates and (c) pulse rate distributions in different bouts, from single pulse to a 5-pulse bout (pentad), at rest (grey bars) and during locomotion (black bars) in 0.5-s fragments where the vocalization was present (pulse rate > 0). On (b) central points show medians, boxes show first and third quartiles, and whiskers show minimum and maximum values.

er only fractionally; contrary to that, the intervals before and after the bout are several times longer, reaching the full bout duration or exceeding it. The criterion of subequal intra-bout intervals is inapplicable to dyads, but they are well distinguished from 2 single pulses in that the total duration of the true dyad is less than that of triad and so on (Fig. 2a). The mean period in a bout is 13.02 ± 3.00 ms, with the minimum and maximum values of 8.70 and 23.27 ms, respectively. Further analysis of bouts is beyond the scope of this study.

Vocal activity

In 432 of the 531 0.5-s fragments, analyzed for a relationship between vocalization and locomotion, the test animals were resting, whereas in 99 fragments they were moving. At rest, vocal pulses were scored in only 10.6% of fragments (n = 46), whereas during locomotion the pulses were scored in two-thirds of fragments (n = 66) (Fig. 4a). At rest, the mean pulse rate was less than 1.0 pulse per second, whereas during locomotion it was more than 12.5 pulses per second. Finally, at rest, the maximum pulse rate was 38 pulses per second, whereas during locomotion it was 64 pulses per second. There is a significant correlation between increased pulse rate and locomotion (Kolmogorov–Smirnov P-value < 0.001). The Kolmogorov–Smirnov D-value reaches its maximum of 0.56 when the pulse rate is zero. Simply speaking, even at pulse rates as low as 1, the animal is likely to be in motion rather than at rest. On the whole, the vocal activity during locomotion is convincingly greater than at rest.

This does not simply mean that the more the animal moves, the more it vocalizes: the composition of calls changes abruptly with locomotion. We have compared the rest and locomotion pulse distributions in the subset of fragments where vocal activity was present (pulse rate > 0) (Fig. 4b,c). In this subset, the mean pulse rate at rest was 9.1 pulses per second (Fig. 4b), of which on average 5 pulses were single, 2 pulses were combined in a 2-pulse bout (dyad) and longer bouts were rare (Fig. 4c). Contrary to that, the mean pulse rate during locomotion was 18.8 pulses per second (Fig. 4b), of which on average less than 4 pulses were single, 6 pulses were combined in three 2-pulse bouts, another 6 pulses were combined in two 3-pulse bouts (triads), and a 4-pulse bout (tetrad) was rather frequent too (Fig. 4c). In the dataset considered, the top pulse rate (64 pulses per second) was registered in male #1, which produced three 4-pulse bouts and four 5-pulse bouts (pentads) during half a second.

DISCUSSION

Degree of blindness

Presented data on eye morphology (Fig. 3) strongly suggest vision degeneration in *Typhlomys*, which can be best appreciated by comparison with subterranean blind mammals such as the mole *Talpa* (Carmona *et al.* 2008; Carmona *et al.* 2010; Quilliam 1966) and the naked mole rat *Heterocephalus glaber* Ruppell, 1842 (Nikitina *et al.* 2004). Contrary to mice and similar to more mature naked mole rats (Nikitina *et al.* 2004), the lens surface in *Typhlomys* shows some wavy irregularities. The lens matter in *Typhlomys* looks rather similar to that in the mouse and the naked mole rat; it is definitely not as bad for light conduction as in the mole, whose lens is entirely filled with disorganized fibers and cell nuclei (Carmona *et al.* 2008; Quilliam 1966). In *Typhlomys*, the lens fibers are disorganized at the pupil-facing side

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

only and no cell nuclei are visible. On the whole, the lens of *Typhlomys* is diminished approximately like that in the mole rat but less than in the mole.

Irregular retinal folds are even more extensive in *Typhlomys* than in the naked mole rat (Nikitina *et al.*, 2004). Such folds protrude beyond the focal plane; they are ineffective with respect to object vision and destroy continuity of image projection on the retina. The reduction of vitreous chamber resulting in too short a distance between lens and retina is detrimental for focusing. Notably, the mole eye appears better suited for focusing than the eyes of *Typhlomys* and the naked mole rat in that it retains big depth of the vitreous chamber and does not have image-destructive retinal folds (Quilliam 1966; Carmona *et al.* 2008).

The acuity of the retina can be estimated by the number of its ganglion layer cells. Mice possess a single layer of tightly packed ganglion cells (Nikitina *et al.* 2004; Carmona *et al.* 2010). In the Iberian mole, a nearly blind rodent, this layer does not differentiate structurally from neuroblasts even in adults (Carmona *et al.* 2010). The naked mole rat (Nikitina *et al.* 2004) and *Typhlomys* show an intermediate state: the ganglion cell layer is present, but its cells are widely spaced from each other because their number is reduced. Our data allow us to roughly estimate that the retina of *Typhlomys* spread on a plane must have an area of a square of 50 × 50 ganglion cells, if the visually useless retinal folds are excluded. Therefore, in total there are approximately 2500 visually useful ganglion cells in the eye of *Typhlomys*. To appreciate the poor image quality of such an eye, imagine a digital photographic camera with a 2.5-kilopixel sensor which is pressed against the back surface of the lens. Note also that the common mole's eye is not much worse in this respect, bearing about 2000 ganglion cells (Quilliam 1966).

Typically, axons of the ganglion cells converge into the optic nerve over the concave lens-facing surface of the retina, lining it with a nerve fiber layer. Reduction of the ganglion cells' number in *Typhlomys* results in a very thin nerve fiber layer. In the naked mole rat, the nerve fiber layer tends to disappear after birth (Nikitina *et al.* 2004). As for the optic nerve, its normal structure is characterized by regular arrangement of glial cells, characterized by longitudinal rows of their nuclei (e.g. in a mouse; Nikitina *et al.* 2004). In the naked mole rat, as well as *Typhlomys* (Fig. 3c), this regular pattern is destroyed, and the optic nerve corridor is plugged with cluttering nuclei indicating reactive gliosis (Nikitina *et al.* 2004). In contrast, the optic nerve of moles looks more normal, with the blood vessels in the middle and nerve fibers on the periphery (Quilliam 1966).

On the whole, the type of eye diminishment observed is rather similar in *Typhlomys* and the naked mole rat. This is in contrast to the mole eye, which has a more destroyed light entrance (the lens) but has better retained those parts that are located downstream in respect of the signal propagation. We can conclude that *Typhlomys*, like the two subterranean mammals mentioned above, cannot focus on an obstacle or target such as a tree branch; at best, its eyes can distinguish dark from light or help photoperiodicity, like in the mole (Carmona *et al.* 2010). The retention of eyes for photoperiodicity may be crucial for survival of *Typhlomys* as an aid in avoiding vision-relying predators. Indeed, observations by the Moscow zookeepers indicate that *Typhlomys* avoids coming out of the shelter when the darkness is incomplete. Adhering to complete darkness, which was noticed by keepers in the Moscow Zoo, may help it in the wild to avoid any vision-relying predators.

Possible evolutionary scenario of *Typhlomys*

Generally, reduced eyes are characteristic of small subterraneous or leaf-bedding dwellers, who mainly rely upon smelling with whisking for orientation (Deschenes *et al.* 2012; Catania 2013) and also rely upon seismic sensitivity (Narins *et al.* 1997; Kimchi *et al.* 2005; Mason & Narins 2010). Therefore, the degenerated eyes along with the *Typhlomys*' digging ability suggest that this lineage may have evolved from a semi-fossorial animal residing in the leaf-bedding of tropical forests.

A peculiarity of *Typhlomys* is its unique juxtaposition of fossorial and scansorial adaptations. We hypothesize that the eyes of *Typhlomys* lost visual acuity in its leaf-bedding but non-arboreal ancestor, as in the mole rat. However, we can also hypothesize with confidence that high-frequency hearing did not degrade as it did in subterranean rodents and moles (Konstantinov & Movchan 1985; Heffner *et al.* 2006; Heffner & Heffner 2008). Once the chance to invade the arboreal niche in the tropical forest appeared, the almost blind emigrant from a leaf-bedding environment would only be able to crawl slowly, caring not to lose tactile contact with the tree surface. The re-acquisition of vision from a mole-rat-like degree of blindness is likely to have been difficult. If so, ultrasonic echolocation would have become the only available replacement of vision for long-range orientation and agile locomotion in the complex 3-D environment full of branches, which can suddenly become footholds or obstacles. Indeed, the definite posi-

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

tive correlation of ultrasound production with acrobatics on branches, as well as the serial arrangement of these short ultrasonic calls is definitively echolocation.

The ultrasonic vocalization of *Typhlomys* is reminiscent of bats' echolocation signals. Both are represented by pulses produced with very high peak frequency (93 kHz) and combined in bouts. Structurally, the pulses are most similar to frequency-modulated (FM) calls of bats, which are relatively short sweeps with fast descent of frequency from the start to the end (Schnitzler Kalko 2001; Maltby *et al.* 2010). In *Typhlomys* the frequency descends from 127 to 64 kHz during 0.68 ms. Yet, the sweeps of *Typhlomys* are much more faint than in bats, which is revealed by the fact that the vocalizations cannot be noticed at all with a common bat detector. The low intensity of echolocation sweeps may be explained by their use in relatively short-range orientation only, of approximately a few decimeters in front of the animal (it is a short range as compared to bat echolocation, but a long range as compared to the length of vibrissae). Note that signals of the so called "whispering bats" hunting among tree branches are poorly audible with a common bat detector as well (e.g. Griffin 2004).

Though faint, the sweeps of *Typhlomys* are much advanced with respect to their high frequency and, especially, in their frequency-modulated and bout-organized structure, which was yet unknown beyond bats. *Typhlomys*' sound-producing apparatus seems to have undergone some peculiar specialization in respect of the available frequency range. It has likely entirely lost the ability to produce any calls audible to the human ear. This follows from the fact that during several years in captivity neither keepers nor ourselves ever heard any calls of *Typhlomys*, which is in sharp contrast with the well-known ability of other small rodents, as well as bats, for both sonic and ultrasonic vocalizations.

The peak frequency of 93 kHz is just above the normal hearing range of non-chiropteran mammals of the size of *Typhlomys*, such as shrews, mice and dormice (Konstantinov & Movchan 1985; Heffner & Heffner 2008). These animals can hear well only at the lower end of *Typhlomys*' calls and echoes. It is known that the rise of call frequencies in bats is accompanied by respective improvement of high-frequency hearing sensitivity (Heffner *et al.* 2006), and the same must be necessary to *Typhlomys* to make use of the energy-expensive calls at the 93-kHz peak frequency. In this study, the perception of frequencies around 90 kHz by *Typhlomys* has not been shown directly: this could be the subject of future studies.

The higher the perceived echo frequency, the closer the use of sonar approaches vision with respect to acuity. Therefore we can definitely conclude that the ultrasonic activity of this unique rodent is rather advanced. Its ancestors could hardly hear above 70–90 kHz, like shrews (70 kHz limit), mice (80 kHz limit) and common dormice (90 kHz limit) (Konstantinov & Movchan 1985). They could have used primitive and less precise echolocation in the low ultrasonic range from 20 to 70 kHz, or even human-audible twittering as shrews in a leaf-bedding environment use (Siemers *et al.* 2009). The rise of produced and perceived frequencies must have accompanied the ascension from leaf-bedding onto trees: fossorial-to-scansorial transition.

Rethinking the evolutionary scenario of bats

Ultrasonic echolocation calls have never been discovered and recorded in any climbing mammals before. More than that, *Typhlomys* is the first non-volant mammal, which relies on on-air ultrasonic echolocation for long-range orientation during locomotion more than on any other senses. Thus, our finding allows us to reconsider a possible evolutionary scenario of bats; namely, the circumstances of echolocation origins. Based on molecular grounds, it is now clear that microbats, which are all characterized by echolocation, are paraphyletic relative to the almost exclusively non-echolocating megabats (Tsagkogeorgasend *et al.* 2013; see also pure molecular trees supplementary to O'Leary *et al.* [2013]). This new fact, that non-echolocating megabats are rooted inside echolocating microbats, should now be taken into account when answering an old question of whether or not the first ancestral bat, capable of flapping flight, was already able to echolocate. There two scenarios are possible: (1) the origin of echolocation followed that of flight several times in parallel and the absence of the former in megabats is plesiomorphic, or (2) echolocation was already developed in the flying ancestral bat and then secondarily lost in megabats. The *Typhlomys*' precedent seems to be good evidence in favor of the second alternative; that is, the so-called "echolocation-first theory." This theory implies that the origin of echolocation was in small quadrupedal mammals adapted to fast locomotion in complex but poorly-lit environments (Fenton *et al.* 1995; Teeling *et al.* 2012). Similar to *Typhlomys*, the non-flying bat ancestor could have been an arboreal animal with previously reduced eyes (most probably not impaired structurally as in *Typhlomys*), which developed ultrasonic echolocation for compensation. It must have been of great advantage when

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

leaping and gliding as a means to find the best surface to land upon in the dark. The initial function of echolocation was orientation rather than prey detection. First, this is due to the fact that for prey detection perfect echolocation is necessary, while even the most primitive usage of echoes, such as in humans (Rice *et al.* 1965) or in the piebald shrew (Volodin *et al.* 2012), is enough to aid orientation. The second reason is that the prey can effectively be detected by echo in a homogeneous medium, such as air or water. On solid surfaces, such as ground or branches, even bats must detect prey by other means than echolocation. Namely, most of the gleaning bats use echolocation for orientation, but locate the prey on the substrate by sounds produced by prey itself (e.g. Arlettaz *et al.* 2001; Jones *et al.* 2003). As to the hearing ability of ultrasonic echoes, the current understanding in the field is exemplified by this statement: "the typical high-frequency sensitivity of small non-echolocating mammals would have been sufficient to support initial echolocation in the early evolution of bats" (Heffner *et al.* 2006, p. 17).

In the course of flight development in the first true bats, the power of ultrasonic echolocation calls must have increased in order to satisfy the new speed of progression; flight is much faster than running or jumping over branches and so sensing echoes from the more distant obstacles would have become crucial. The secondary loss of ultrasonic calls, which we propose for Pteropodidae, may have directly resulted from their transition to the fruit diet. This is due to the associated increase of body size with this diet and of the larynx in particular. This results in a corresponding decrease of fundamental frequency of laryngeal vocalization, preventing echolocating. However, some of them (e.g. the cave-living genus *Rousettus*) developed ultrasonic vocalization again based on different morphological structure: by using brief, broadband tongue clicks, instead of laryngeal calls (Yovel *et al.* 2011). Note that non-echolocating Pteropodidae possess their best hearing sensitivities around 8–10 and 20–25 kHz (Heffner *et al.* 2006), while in *Rousettus* the second optimum of the hearing profile is shifted to 45 kHz (Yovel *et al.* 2011).

CONCLUSION

The major limitations of our study were the small number of live individuals to experiment with and the poor quality of dead specimens for histology. This is due to the extreme rarity of the Vietnamese pygmy dormouse, or "blind mouse" in nature. That is why our conclusions, although rather convincing, are still preliminary. Additional research is required to describe in detail the acoustic patterns of ultrasonic pulses and bouts in *Typhlomys* and to compare them with the known acoustics of bats and with non-echolocation ultrasonic calls of other rodents. A remaining question is the mechanism of signal production: Is it located in the larynx? In addition, is the animal entirely incapable of communicating in the human-audible range? It will be of interest to investigate the degree of eye degeneration and development of echolocation in a closely related and very similar species, the Chinese pygmy dormouse, *Typhlomys cinereus*.

The next very interesting direction of research is associated with the closest, and almost as rare, extant relative of *Typhlomys*: the Malabar spiny dormouse *Platacanthomys*. Scarce scientific data suggest their nocturnal activity in spite of rather small eyes and arboreal capabilities (Mudappa *et al.* 2001; Jayson & Jayahari 2009). Taken together, these traits are similar in the 2 related genera; this suggests that *Platacanthomys* may too use echolocation as a means for orientation. However, the eyes in this genus, although small, are much larger than in *Typhlomys*, judging from available photos. Therefore, there is a chance of finding a more basal stage of echolocation development. Perhaps *Platacanthomys* produces vocalizations in the low-ultrasonic or even the human-audible range, and its calls may not be yet organized in bouts. If this was true, *Platacanthomys* would fill the "evolutionary gap" at the very beginning of the suggested evolutionary scenario for *Typhlomys*, as well as of the grand scenario for bats.

ACKNOWLEDGMENTS

The field works in Vietnam were possible due to support of the Joint Vietnam–Russian Tropical Research and Technological Centre. We are sincerely grateful to V. V. Rozhnov for substantial organization efforts which allowed us to undertake this study, to A. V. Shchinov, Nguyen Dang Hoi and Vu Van Lien for their great help and scientific expertise during the field works, to the staff of the Scientific Department of the Moscow Zoo, and especially to O. G. Ilchenko, for the animal and room supply, to E. L. Yakhontov for assistance in experiments and work with literature, to B. D Vasiliev for his kind transport support in the crucial situation on the rainy 15 July 2013, our first shooting day, to A. G. Bush and E. D. Pavlov for assistance with microscopes, to O. Yu. Orlov and T. V. Khokhlova for consulting regarding eye morphology, to Al. B. Savinetsky for consulting in

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

statistics, to A. A. Lissovsky for help with Syrinx software usage, to C. Voigt and D. N. Lapshin for valuable discussion on echolocation, to E. V. Volodina for participation in English translation of the initial Russian text, and to M. Kaczmarek, D. V. Logunov and S. V. Kovalsky for improving the English of the final draft.

In all experiments we adhered to the "Guidelines for the treatment of animals in behavioural research and teaching" (2012, *Animal Behaviour*, **83,** 301–309). This study was approved by the Committee of Bio-ethics of the Lomonosov Moscow State University (research protocol no. 2011-36). Video processing was performed with support of the Russian Science Foundation (project 14-50-00029 "Scientific basis of the national biobank – depository of the living systems"). Acoustic analysis was supported by the Russian Science Foundation (project 14-14-00237) and the Program of Basic Research of the Presidium of the Russian Academy of Sciences "Wildlife: Current Status and Problems of Development."

All the other work, including experimental setting and recording, eye morphology research and manuscript preparation, was financed by the Russian Foundation for Basic Research (projects 14-04-01132 and 15-04-05049).

REFERENCES

Abramov AV, Aniskin VM, Rozhnov VV (2012). Karyotypes of two rare rodents, *Hapalomys delacouri* and *Typhlomys cinereus* (Mammalia, Rodentia), from Vietnam. *ZooKeys* **164**, 41–9.

Abramov AV, Balakirev AE, Rozhnov VV (2014). An enigmatic pygmy dormouse: Molecular and morphological evidence for the species taxonomic status of *Typhlomys chapensis* (Rodentia: Platacanthomyidae). *Zoological Studies* **53**, 34.

Arlettaz R, Jones G, Racey PA (2001). Effects of acoustic clutter on prey detection by bats. *Nature* **414**, 742–5.

Carmona FD, Jiménez R, Collinson JM (2008). The molecular basis of defective lens development in the Iberian mole. *BMC Biology* **6**, 44.

Carmona FD, Glösmann M, Ou J, Jiménez R, Collinson JM (2010). Retinal development and function in a 'blind' mole. *Proceedings of the Royal Society of London B* **277**, 1513–22.

Catania KC, Hare JF, Campbell KL (2008). Water shrews detect movement, shape, and smell to find prey underwater. *PNAS* **105**, 571–6.

Catania KC (2013). Stereo and serial sniffing guide navigation to an odour source in a mammal. *Nature Communications* **4**, 1441.

Deschenes M, Moore J, Kleinfeld D (2012). Sniffing and whisking in rodents. *Current Opinion in Neurobiology* **22**, 243–50.

Fenton MB, Audet D, Obrist MK, Rydell J (1995). Signal strength, timing and self-deafening: The evolution of echolocation in bats. *Paleobiology* **21**, 229–42.

Forsman KA, Malmquist MG (1988). Evidence for echolocation in the common shrew *Sorex araneus*. *Journal of Zoology* **216**, 655–62.

Gould E, Negus NC, Novick A (1964). Evidence for echolocation in shrews. *Journal of Experimental Zoology* **156**, 19–38.

Griffin DR (2004). The past and future history of bat detectors. In: Brigham RM, Kalko EKV, Jones G, Parsons S, Limpens HJGA, eds. *Bat Echolocation Research: Tools, Techniques and Analysis*. Bat Conservation International, Austin, Texas, pp. 6–9.

Heffner HE, Heffner RS (2008). High-frequency hearing. In: Dallos P, Oertel D, Hoy R, eds. *Handbook of the Senses: Audition*. Elsevier, NY, pp. 55–60.

Heffner RS, Koay G, Heffner HE (2006). Hearing in large (*Eidolon helvum*) and small (*Cynopterus brachyotis*) non-echolocating fruit bats. *Hearing Research* **221**, 17–25.

Howland HC, Merola S, Basarab JR (2004). The allometry and scaling of the size of vertebrate eyes. *Vision Research* **44**, 2043–65.

Jansa SA, Giarla TC, Lim BK (2009). The phylogenetic position of the rodent genus *Typhlomys* and the geographic origin of Muroidea. *Journal of Mammalogy* **90**, 1083–94.

Jayson EA, Jayahari KM (2009). Distribution of spiny tree mouse (*Platacanthomys lasiurus* Blyth, 1859) in the Western Ghats of Kerala, India. *Mammalia* **73**, 331–7.

Jones G, Teeling EC (2006). The evolution of echolocation in bats. *Trends in Ecology and Evolution* **21**, 149–56.

Jones G, Webb PI, Sedgeley JA, O'Donnell CF (2003). Mysterious Mystacina: How the New Zealand short-tailed bat (*Mystacina tuberculata*) locates insect prey. *Journal of Experimental Biology* **206**, 4209–16.

Kimchi T, Reshef M, Terkel J (2005). Evidence for the use of reflected self-generated seismic waves for

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

spatial orientation in a blind subterranean mammal. *Journal of Experimental Biology* **208**, 647–59.

Konstantinov AI, Movchan VN (1985). *Sounds in the Life of Animals*. Isdatelstvo Leningradskogo Universiteta, Leningrad, Russia. (In Russian.)

Leger DW, Didrichsons IA (1994). An assessment of data pooling and some alternatives. *Animal Behavior* **48**, 823–32.

Madsen PT, Carder DA, Beed-holin K, Ridgway SH (2005). Porpoise clicks from a sperm whale nose – Convergent evolution of 130 kHz pulses in toothed whale sonars? *Bioacoustics* **15**, 195–206.

Maltby A, Jones KE, Jones G (2010). Understanding the evolutionary origin and diversification of bat echolocation calls. In: Brudzynski SM, ed. *Handbook of Mammalian Vocalization: An Integrative Approach*. Elsevier, London, pp. 37–47.

Mason MJ, Narins PM (2010). Seismic sensitivity and communication in subterranean mammals. In: O'Connell-Rodwell CE, ed. *The Use of Vibrations in Communication: Properties, Mechanisms and Function across Taxa*. Transworld Research Network, Kerala, India, pp. 121–39.

Mudappa D, Kumar A, Chellam R (2001). Abundance and habitat selection of the Malabar spiny dormouse in the rainforests of the southern Western Ghats, India. *Current Science* **80**, 424–9.

Narins PM, Lewis ER, Jarvis JJUM, O'Riain J (1997). The use of seismic signals by fossorial Southern African mammals: A neuroethological gold mine. *Brain Research Bulletin* **44**, 641–6.

Nikitina NV, Maughan-Brown B, O'Riain MJ, Kidson SH (2004). Postnatal development of the eye in the naked mole rat (*Heterocephalus glaber*). *Anatomical Record Part A* **277A**, 317–37.

O'Leary MA, Bloch JI, Flynn JJ *et al (*2013). The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science* **339**, 662–7.

Quilliam AT (1966). The problem of vision in the ecology of *Talpa europaea*. *Experimental Eye Research* **5**, 63–78.

Rice CE, Feinstein SH, Schusterman RJ (1965). Echo detection ability of the blind: Size and distance factors. *Journal of Experimental Psychology* **70**, 246–51.

Schnitzler HU, Kalko EKV (2001). Echolocation by insect-eating bats. *BioScience* **51**, 557–69.

Schnitzler HU, Moss CF, Denzinger A (2003). From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology and Evolution* **21**, 386–94.

Siemers BM, Schauermann G, Turni H, von Merten S (2009). Why do shrews twitter? Communication or simple echo-based orientation. *Biology Letters* **5**, 593–6.

Simmons NB, Seymour KL, Habersetzer J, Gunnell GF (2008). Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. *Nature* **451**, 818–21.

Simmons NB, Seymour KL, Habersetzer J, Gunnell GF (2010). Inferring echolocation in ancient bats. *Nature* **466**, E8–E9.

Smith AT (2008). Family Platacanthomyidae. In: Smith AT, Xie Y, eds. *A Guide to the Mammals of China*. Princeton University Press, Princeton, pp. 208–9.

Speakman JR (2001). The evolution of flight and echolocation in bats: Another leap in the dark. *Mammal Review* **31**, 111–30.

Speakman JR (2008). Evolutionary biology: A first for bats. *Nature* **451**, 774–5.

Teeling EC (2009). Hear, hear: The convergent evolution of echolocation in bats? *Trends in Ecology and Evolution* **24**, 351–4.

Teeling EC, Dool S, Springer MS (2012). Phylogenies, fossils and functional genes: The evolution of echolocation in bats. In: Gunnell GF, Simmons NB, eds. *Evolutionary History of Bats. Fossils, Molecules and Morphology*. Cambridge University Press, Cambridge, pp. 1–22.

Thomas JA, Jalili M (2004). Review of echolocation in insectivores and rodents. In: Thomas JA, Moss C, Vater V, eds. *Echolocation in Bats and Dolphins*. The University of Chicago Press, pp. 547–64.

Tomasi TE (1979). Echolocation by the short-tailed shrew *Blarina brevicauda*. *Journal of Mammalogy* **60**, 751–9.

Tsagkogeorgasend G, Parker J, Stupka E, Cotton JA, Rossiter SJ (2013). Phylogenomic analyses elucidate the evolutionary relationships of bats. *Current Biology* **23**, 2262–7.

Volodin IA, Zaytseva AS, Ilchenko OG, Volodina EV, Chebotareva AL (2012). Measuring airborne components of seismic body vibrations in a Middle-Asian sand-dwelling Insectivora species, the piebald shrew (*Diplomesodon pulchellum*). *Journal of Experimental Biology* **215**, 2849–52.

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd

Volodin IA, Zaytseva AS, Ilchenko OG, Volodina EV (2015) Small mammals ignore common rules: A comparison of vocal repertoires and the acoustics between pup and adult piebald shrews *Diplomesodon pulchellum*. *Ethology* **121**, 103–15.

Yovel Y, Geva-Sagiv M, Ulanovsky N (2011). Click-based echolocation in bats: Not so primitive after all. *Journal of Comparative Physiology A* **197**, 515–30.

Zaytseva AS, Volodin IA, Mason MJ *et al*. (2015). Vocal development during postnatal growth and ear morphology in a shrew that generates seismic vibrations, *Diplomesodon pulchellum*. *Behavioural Processes* **118**, 130–41.

SUPPLEMENTARY MATERIALS

Supplementary_1.mov

Typical exploratory behavior of the Vietnamese pygmy dormouse *Typhlomys chapensis* (male #1) in the experimental cage set vertically. Natural speed playback, 50 fps. Note especially, 2 jumps from branch to branch.

Supplementary_2.mov

Blind climbing of the Vietnamese pygmy dormouse *Typhlomys chapensis* male #2. Soundtrack recorded at 768 kHz sampling rate is synchronized with videotrack recorded at 50 fps. Together, they are slowed down 10-fold, so that the resulting sampling rate is 76.8 kHz, fps is 5 and the peak frequency (fpeak) of ultrasonic pulses appears audible at approximately 9 kHz.

Supplementary_3.mov

Nine seconds of blind climbing of the Vietnamese pygmy dormouse *Typhlomys chapensis* male #1. Soundtrack recorded at 768 kHz sampling rate is synchronized with videotrack recorded at 299.7 fps. At the beginning the record is reproduced at normal speed; then videotrack and soundtrack are slowed down 20-fold, so that the resulting sampling rate is 38.4 kHz, fps is 14.985 and the peak frequency (fpeak) of ultrasonic pulses appears audible at approximately 4.5 kHz. Note especially, the non-stop vocalization of the animal hanging upside-down on its hind limbs.

Cite this article as:

Panyutina AA, Kuznetsov AN, Volodin IA, Abramov AV, Soldatova IB (2017). A blind climber: The first evidence of ultrasonic echolocation in arboreal mammals. *Integrative Zoology* **12**, 172–84.

© 2016 International Society of Zoological Sciences, Institute of Zoology/ Chinese Academy of Sciences and John Wiley & Sons Australia, Ltd