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Title: Vocal development during postnatal growth and ear morphology in a shrew that generates seismic vibrations, Diplomesodon pulchellum

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Abstract: The ability of adult and subadult piebald shrews (Diplomesodon pulchellum) to produce 160 Hz seismic waves is potentially reflected in their vocal ontogeny and ear morphology. In this study, the ontogeny of call variables and body traits was examined in 11 litters of piebald shrews, in two-day intervals from birth to 22 days (subadult), and ear structure was investigated in two specimens using micro-computed tomography (micro-CT). Across ages, the call fundamental frequency (f0) was stable in squeaks and clicks and increased steadily in screeches, representing an unusual, non-descending ontogenetic pathway of f0. The rate of the deep sinusoidal modulation (pulse rate) of screeches increased from 75 Hz at 3-4 days to 138 Hz at 21-22 days, probably relating to ontogenetic changes in contraction rates of the same muscles which are responsible for generating seismic vibrations. The ear reconstructions revealed that the morphologies of the middle and inner ears of the piebald shrew are very similar to those of the common shrew (Sorex araneus) and the lesser white-toothed shrew (Crocidura suaveolens), which are not known to produce seismic signals. These results suggest that piebald shrews use a mechanism other than hearing for perceiving seismic vibrations.

Dear Dr. Shannon Kundey, We revised the Ms. Ref. No.: BEPROC-D-14-00269R1 Title: Vocal development during postnatal growth and ear morphology in a shrew that generates seismic vibrations, Diplomesodon pulchellum, in accordance to the comments by the reviewer. Please find the detailed list of responses below. All coauthors approved the submission of the revised version. Sincerely Yours, Ilya Volodin, corresponding author,

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Ms. Ref. No.: BEPROC-D-14-00269R1,

Title: Vocal development during postnatal growth and ear morphology in a shrew that generates seismic vibrations, Diplomesodon pulchellum

Responses to the comments by the Reviewer

Reviewer #2: This already excellent manuscript has been improved appreciably via the revision process, and as such, I recommend its publication in Behavioral Processes without further review. I encourage the authors, however, to take the following minor suggestions into account:

1. Introduction (lines 87-88): revise the phrase "so it is possible that they might detect reflected vibrations from e.g. substrate heterogeneities related to the presence of their prey.", which contains the unnecessary double caveat "possible that they might" and an awkwardly placed "e.g." to read "so it is possible that they detect reflected vibrations from substrate heterogeneities such as those related to the presence of their prey."

Response

The text was corrected as recommended.

2. Materials and Methods (line 184); where > 20 calls of a given type were available for analysis, was the method of selecting 20 calls from different section truly "random", or was it arbitrary? If truly at random, some formal method of randomization should be reported.

Response

"randomly" was changed to "arbitrary"

3. Results (line 323): should read "...weakly, although significantly, increased...".

Response

The text was corrected as recommended.

Highlights

Piebald shrews may vibrate the entire body resembling 'phone vibratory mode'.

Vocal development highlights the ontogenetic pathway of this sensory modality.

Unchanged ear morphology implies that vibration cues are not perceived auditorily.

Vocal development during postnatal growth and ear morphology in a shrew that generates seismic vibrations, *Diplomesodon pulchellum*

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1 Abstract

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3 The ability of adult and subadult piebald shrews (Diplomesodon pulchellum) to produce 4 160 Hz seismic waves is potentially reflected in their vocal ontogeny and ear morphology. In this 5 study, the ontogeny of call variables and body traits was examined in 11 litters of piebald 6 shrews, in two-day intervals from birth to 22 days (subadult), and ear structure was investigated 7 in two specimens using micro-computed tomography (micro-CT). Across ages, the call 8 fundamental frequency (f0) was stable in squeaks and clicks and increased steadily in screeches, 9 representing an unusual, non-descending ontogenetic pathway of f0. The rate of the deep 10 sinusoidal modulation (pulse rate) of screeches increased from 75 Hz at 3-4 days to 138 Hz at 11 21-22 days, probably relating to ontogenetic changes in contraction rates of the same muscles 12 which are responsible for generating seismic vibrations. The ear reconstructions revealed that the 13 morphologies of the middle and inner ears of the piebald shrew are very similar to those of the 14 common shrew (Sorex araneus) and the lesser white-toothed shrew (Crocidura suaveolens), 15 which are not known to produce seismic signals. These results suggest that piebald shrews use a 16 mechanism other than hearing for perceiving seismic vibrations.

17

18 Key words: acoustic communication; ontogeny; middle-ear structure; separation calls;
19 Soricidae

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1. Introduction

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24 Some small mammals, such as golden moles (Afrosoricida: Chrysochloridae) and spalacid 25 mole-rats (Rodentia: Spalacidae), appear to respond both to airborne sound and to seismic 26 vibrations; the interrelationships between these sensory modalities and their potential use in 27 communication have been of research interest for over two decades (Burda et al. 1990; Willi et 28 al. 2006b; Mason and Narins 2010; Bednářová et al. 2013). Shrews (Soricomorpha: Soricidae) 29 are less well-studied, but recent investigations of the piebald shrew (*Diplomesodon pulchellum*) 30 have revealed interesting bioacoustic parallels. Captive adult and subadult piebald shrews may 31 vibrate the entire body when held, when lifted up in their plastic pipe shelters or when placed on 32 a drum membrane in behavioural experiments (Volodin et al. 2012). These vibrations, at a 33 frequency of 160 Hz, resemble the mobile 'phone "vibrate" mode and are apparently produced in 34 response to a change in the substrate under their feet. These vibrations are always produced by non-vocalizing, silent animals (Volodin et al. 2012). At the same time, adult piebald shrews 35 36 produce loud screech vocalisations with a deep, repetitive, sinusoid-like frequency modulation 37 (pulse rate), coinciding in rate with the vibrations of the body (Volodin et al. 2012). In pups, the 38 pulse rate of screeches is much lower than in adults (Volodin et al. 2015b). Body vibrations of 39 160 Hz are documented in piebald shrews from 34 days post-partum (Volodin et al. 2012), but 40 have not been investigated in pups, so it is not known whether the screech pulse-rate increase, 41 from pups to adults, is reflected in the ontogeny of body vibration too.

Piebald shrews represent a convenient model for studying vocal ontogeny, because the same call types and call variables can be measured across ages (Volodin et al. 2015b), and because of their rapid growth (Zaytseva et al. 2013). In mammals, body size and body mass increase progressively during ontogeny (Gaillard et al. 1997), whereas pathways of vocal ontogeny differ across species, call types and call variables (Ey et al. 2007; Matrosova et al. 2007). The fundamental frequency (f0) is typically higher in juvenile mammals than in adults

48 (Morton 1977). This is because acoustic differences between juveniles and adults primarily result 49 from the differences in sizes of sound-producing structures (Fitch and Hauser 2002). In humans 50 and many other mammals, the f0 is inversely related to mass and length of the oscillating 51 portions of the vocal folds (Titze 1994), and both mass and length increase together with the 52 growth of the larynx (Kahane 1978, 1982). In most mammalian species, the growth of these 53 sound-producing structures is related to the growth of the body, which results in a steady descent 54 of f0 with age (for instance, Briefer and McElligott 2011; Efremova et al. 2011, Campbell et al. 55 2014). In humans, this pattern is complicated in males by an abrupt fall of f0 due to accelerated 56 growth of the larynx at puberty (Fitch and Giedd 1999; Lee et al. 1999). Nevertheless, in a few 57 species of ground squirrels, the fOs of alarm calls are indistinguishable between pups and adults, 58 in spite of much larger bodies and larynges in adults than in pups (Matrosova et al. 2007; Swan 59 and Hare 2008; Volodina et al. 2010). The f0 also increases with body growth in bat pups, in both echolocation calls and social calls (Jones et al. 1991; Hiryu and Riquimaroux 2011; Jin et 60 61 al. 2011, 2012).

An ontogenetic study of body traits and body mass in 18 litters of piebald shrews demonstrated that they grow very rapidly (Zaytseva et al. 2013), similarly to other shrews (Dryden 1968; Vlasák 1972; Michalak 1987). In piebald shrews, weaning is at about 20 days of age (Vakhrusheva and Ilchenko 1995) and first copulations were recorded at 27 days in females and at 40 days in males (Ilchenko et al. 2011). At separation from the mother at 22 days, shrews are comparable in body length to reproductively mature adults (62 mm and 70 mm respectively, Zaytseva et al. 2013).

In captivity, piebald shrews produce eight call types, all within the human audible frequency range (< 20 kHz): short and long low-frequency squeaks with nearly flat contour, high-frequency squeaks with modulated contour, high-frequency squeaks with fractured contour, short screeches and screeches, clicks and whimpers (Fig. 1). Seven of the eight call types are shared by pups and adults, suggesting that this vocal repertoire is established at birth (Volodin et al. 2015b). The previous cross-sectional study revealed that the f0 of all four types of squeaks were indistinguishable between pups and adults, whereas the f0 of screeches was higher in adults than in pups (Volodin et al. 2015b). However, the rate of deep sinusoidal frequency modulation (hereafter "pulse rate") of screeches was found to be substantially and significantly lower in pups than in adult piebald shrews. While an ontogenetic study of body features and a cross-sectional study of call types and call variables already exist, an ontogenetic study of the acoustics is lacking for this species.

81 Body vibrations are produced by silent piebald shrews, independent of any vocalizations 82 (Volodin et al. 2012). Perhaps they generate these vibrations for communication purposes, for 83 example as a warning aimed either at conspecifics or potential predators. Alternatively, they might use 'seismic echolocation' for spatial orientation purposes, analogous to what has been 84 85 proposed for subterranean Spalax mole-rats (Kimchi et al., 2005). During the night, piebald 86 shrews patrol their semidesert habitats and dig up invertebrates from depths of 2-3 cm in sand (Dubrovskij et al. 2011), so it is possible that they detect reflected vibrations from substrate 87 88 heterogeneities such as those related to the presence of their prey. Hypotheses relating to 89 intraspecific communication and seismic echolocation demand that the shrews have a means of detecting seismic vibrations. In principle, seismic vibrations could be detected following 90 91 radiation into the air, resulting in airborne sound which is detected by the ear in the normal way. 92 Alternatively, vibrations could be detected directly if the body or head is in contact with the 93 vibrating substrate, by somatosensory receptors or by a form of bone-conducted hearing (see 94 Mason and Narins 2010 for a review). Bone-conducted hearing may be the sensory modality 95 employed by golden moles, in which the-hypertrophied mallei may be used as inertial sensors 96 (Mason 2003a, 2003b; Willi et al. 2006a); some unusual features of the ear of the mole-rat 97 Spalax ehrenbergi have also been interpreted as adaptations to promote bone conduction (Rado 98 et al. 1989), although this view has been challenged (Mason et al. 2010). Although piebald shrews are not subterranean mammals, their ability to produce seismic vibrations might similarlybe reflected in their ear morphology: this possibility has not been investigated previously.

101 The general focus of this study was to consider the relationship between vocal and body 102 ontogeny, and to explore whether functional associations exist between vocalizations, vibration 103 generation and ear morphology. The particular aims were (1) to define the relationships between 104 changes in acoustic variables, body mass and head length during the ontogeny of piebald shrews, 105 and (2) to examine the ear morphology to see if there are any obvious adaptations promoting 106 vibratory sensitivity.

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110 2.1. Study Site and Subjects

2. Materials and Methods

111 Calls as well as measurements of body mass and head length were collected from members 112 of a captive colony of piebald shrews at Moscow Zoo, Moscow, Russia, from 1 June to 22 113 August 2011. Our live subjects were 40 piebald shrews (24 males and 16 females from 11 litters) 114 examined from birth to separation from the mother at 22 days of age. All study animals (3rd-6th 115 generations in captivity) were descendents of 27 animals collected in 2008 in the Astrakhan 116 Region, Russia (47°12′33″N; 48°18′45″E).

117 The animals were kept under a natural light regime at room temperature (24–26°C), in 118 family groups consisting of a mother and littermates. The animals were housed in plastic cages 119 of 53x76x42 cm, with a bedding of sand and dry moss, various shelters and running wheels. 120 They received custom-made small insectivore chow with insect and calcium supplements, and 121 water *ad libitum*. Before parturition, females were checked twice a day for the appearance of a 122 litter, and birth dates as well as the number of pups were recorded. Litter size varied from 3 to 6 123 pups (mean $\pm SD = 3.9 \pm 1.1$), with 43 pups in total being born. Three pups died at 1 or 2 days of 124 age. The registered first day post-partum was considered to be the first day of pup life for the 125 chronological splitting of age groups along ontogeny. The 11 study litters originated from 11 126 different mothers. For each mother, the study litter was her first litter in the breeding season. 127 Study pups were sexed between 3 and 9 days of age based on the appearance of nipples in 128 females (Vakhrusheva and Ilchenko 2010). The small size of pups during the study period (the 129 mean body mass of a 5 day-old pup was 2.83 g and body length was 38.5 mm: Zaytseva et al. 130 2013) prevented individual marking for ethical reasons. The definitive pinnae appear only at day 131 9-10 of pup life (Zaytseva et al. 2013), so newborn piebald shrews could not be individually 132 marked by cuts on ears.

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134 2.2. Call and body growth data collection

135 Calls were recorded collectively from all pups of each litter (i.e. the calling pups within a 136 litter were not identified individually), whereas body mass and head length measurements were 137 taken from each littermate separately. Pups were weighed and measured for the first time on the 138 first post-partum day of their lives, if they were born in the morning or during the day, or 139 otherwise on the second day. After that, body mass and head length measurements were taken 140 every two days up to the age of 22 days, when pups were separated from the mother. For 141 weighing, we used G&G TS-100 electronic scales (G&G GmbH, Neuss, Germany), accurate to 142 0.01 g. For head length measurements, we used electronic calipers (Kraf Tool Co., Lenexa, 143 Kansas, US), accurate to 0.01 mm. When making these measurements, the litter was separated 144 from the mother and returned within 40 minutes. Mothers were tolerant of the temporary absence 145 of their pups and no pup appeared to suffer from this experience. Pups younger than 10 days 146 were placed for weighing directly on the scales; pups older than 10 days were placed for 147 weighing in a transparent plastic can. After weighing, we measured head length of the hand-held 148 pup from the tip of the snout to the occiput. This measurement was repeated three times and the 149 mean value was taken for analysis.

Pup calls were recorded from individuals separated from their mother and placed on a clean, smooth table-top area (60x60 cm) or in a clean plastic enclosure (28x43x14 cm), depending on the age. In addition, some calls were recorded when pups were together with the mother (primarily during caravanning at 12-16 days of age). Caravanning represents a behaviour whereby a pup catches the fur at the base of the mother's tail, and then the rest of the litter attaches one to another in the same way (Vlasák 1972; Tsuji and Ishikawa 1984). Caravanning could only be elicited in the presence of the mother (Schneiderová 2014).

157 Acoustic recordings were made on the day of weighing, but at least 5 hours after the 158 individuals were weighed. All acoustic recordings were conducted in a separate room where no 159 other animals were present, at room temperature (24-26 °C) during daytime. For sound 160 recordings (sampling rate 96 kHz, 24 bit resolution) we used a Fostex FR-2LE professional 161 digital recorder (Fostex Company, Tokyo, Japan) and a Sennheiser K6-ME64 condenser 162 microphone (Sennheiser electronic, Wedemark, Germany). In addition, to monitor the potential 163 presence of ultrasound, half of the recordings were made also in the ultrasonic range with 164 sampling rates of 192 kHz or 768 kHz, 16 bit resolution, using a Pettersson D 1000X recorder 165 with built-in microphone (Pettersson Electronik AB, Uppsala, Sweden). All microphones were 166 kept at distance of 5-15 cm from the animals. Each trial lasted 7-14 minutes and was recorded as 167 a wav-file.

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169 2.3. Call samples

We chronologically subdivided acoustic recordings made from the 11 litters into 11 agegroups (hereafter "ages") of 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18, 19-20 and 21-22 days post-partum. This provided matched data on the vocalizations, body mass and head length measurements during the development of each litter. The percentages of recording trials in which a given call type was emitted were used as measures of the occurrence of different call types, for pups at different ages. For some litters at some ages, recording trials were missing (due to delayed detection of the litter or for other reasons). As a result, only 105 trials (81 without
mother and 24 with mother) of the potential 121 recording trials were made, and therefore from 7
to 11 recording trials per age were available for acoustic analyses of 7 call types (Volodin et al.
2015b).

180 If calls of the given type were emitted in the given recording trial, we took for acoustic 181 analysis 1-20 calls (mean $\pm SD = 7.00 \pm 5.62$; n = 464) per call type (excluding whimper) per 182 recording trial: 3095 calls in total. If the number of calls per trial of a particular type was ≤ 20 , 183 all available calls were included in the analysis; if it was > 20, we selected 20 calls per call type 184 per trial for analysis, taking calls from different parts of a trial, arbitrary, to decrease 185 pseudoreplication. When this was impossible, we took calls separated by at least one call of 186 another type. In total, we selected for analysis 1270 short LF squeaks, 303 long LF squeaks, 568 modulated HF squeaks, 256 flat HF squeaks, 323 screeches, 110 short screeches and 265 clicks 187 188 (Fig. 1). We excluded the whimper from acoustic analyses, as this call type was made only 189 within a very short period of development, from the second to the sixth day post-partum 190 (Volodin et al. 2015b).

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192 2.4. Call analysis

193 Inspection of spectrograms of the acoustic files recorded in the ultrasonic range (> 20 194 kHz), using Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany), did not 195 reveal any ultrasonic components. All spectrographic analyses were performed with Avisoft 196 SASLab Pro (sampling frequency 96 kHz) and the results were exported to Microsoft Excel 197 (Microsoft Corp., Redmond, WA, USA). As minimum fundamental frequency (f0min) of calls 198 always exceeded 1 kHz (Volodin et al. 2015b), before measurements all wav-files were 199 subjected to 1 kHz high-pass filtering and 30 kHz low-pass filtering, to remove low-frequency 200 noise and to avoid aliasing (high-frequency aberrations) respectively. For all call types, we 201 measured the duration and the frequency of maximum amplitude (fpeak) from the call's mean

202 power spectrum, using the option "Automatic parameter measurements" in Avisoft (Fig. 2). For 203 all call types excluding clicks, we measured the maximum fundamental frequency (f0max) with 204 the reticule cursor in the spectrogram window (sampling frequency 96 kHz, Hamming window, 205 FFT 1024 points, frame 50%, overlap 96.87%, providing frequency resolution 93 Hz and time 206 resolution 0.3 ms) (Fig. 2). For screeches and short screeches, we additionally measured the 207 mean rate of the deep sinusoidal frequency modulation (thereafter "pulse rate") as the inverse 208 value of the mean modulation period, using the standard marker cursor in the main window of 209 Avisoft (Fig. 2). For clicks, we measured the click fundamental frequency as the inverse value of 210 the mean f0 period, visible on the extended (zoomed-in) waveform, using the standard marker 211 cursor in the main window (for details, see Volodin et al. 2015b). This f0 value was used as 212 f0max of clicks in subsequent statistical analyses.

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214 2.5. Anatomical specimens and ear scans

215 CT-scans of two Diplomesodon specimens, which died by accident in the study colony of 216 the Moscow Zoo, were made at the Berlin Museum of Natural History. These specimens were a 217 subadult female (body mass 7.1 g) and an adult male (body mass 17.2 g). The head was not 218 prepared in any way: the entire animal was in each case placed into a General Electric Phoenix 219 Nanotom X-ray Micro-CT scanner at the Museum für Naturkunde, Berlin. The scanner was 220 operating at 80-90 kV and 150-350 µA. 800 or 1400 projections were taken over 360° total 221 rotation, each with 750 msec exposure and 3 frames averaged per projection. Voxel linear 222 dimensions were 6-8 µm for close-ups of the head, or 30 µm for whole-body scans. Scan data were 223 processed using Datos|x software (GE Sensing & Inspection Technologies GmbH - phoenix|x-ray).

For comparative analyses of ear morphology, the ear regions of two other shrew species were examined. Two specimens of *Sorex araneus* (body mass 5.9 and 7.3 g) were found as corpses in Cambridgeshire, U.K.; one specimen of *Crocidura suaveolens* (body mass 5.4 g) was found as a corpse on Gugh, Isles of Scilly, U.K. These shrews were probably killed by cats. One 228 Sorex and the Crocidura specimen were preserved by freezing prior to examination while the 229 other Sorex was fresh. The heads of all of these animals were removed, skinned, covered in 230 cellophane to reduce the rate of drying and taken for CT scanning. The ear region of one of the 231 Sorex specimens was later dissected out, allowed to dry and scanned again at higher 232 magnification. The CT-scans of the Sorex and Crocidura specimens were made using a Nikon 233 XT H 225 scanner at the University of Cambridge. The scanner was operating at 106-120 kV and 234 148-183 µA for the whole head scans, or at 79 kV and 246 µA for the auditory region scan. 720 235 or 1080 projections were taken over 360° total rotation, each with 1000 msec exposure and 1 or 236 2 frames averaged per projection. Voxel linear dimensions were 8-12 µm. The software used in 237 the processing of the scan data included CT Agent XT 3.1.9 and CT Pro 3D XT 3.1.9 (Nikon 238 Metrology 2013, Tring, UK).

Exported tiff stacks from the scans were converted to jpg files using IrfanView 4.37 (www.irfanview.com/). MicroView 2.1.2 (GE Healthcare, 2000-2006) was used to visualise skull structure for orientation purposes, while WinSurf 4.0 (www.surfdriver.com/) was used to construct three-dimensional images of the ear, following visual identification and tracing of the borders of relevant structures. The inner walls of the bony labyrinth were traced to make the reconstructions of the inner ear.

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246 2.6. Statistics

Statistical analyses were conducted using STATISTICA (StatSoft, Tulsa, OK, USA). Means are presented as mean \pm *SD*, all tests were two-tailed and differences were considered significant whenever p < 0.05. Distributions of all measured parameter values did not depart from normality (Kolmogorov-Smirnov test, p > 0.05). We used the General Linear Model (GLM) to compare the effects of age, sex and litter identity on body mass and head length. Because body mass should, under the assumption of isometry, be proportional to the cube of a linear dimension like body length, we used log body mass for the analysis. We calculated

254 Pearson correlation coefficients with Bonferroni corrections to examine the correlations between 255 body mass, head length, age and the acoustic variables (using mean values of call variables per 256 litter per age-group). Because some litters did not produce some call types at some ages, not 257 every litter was represented at every age-group. Therefore, we used a two-factor General Linear 258 Mixed Model (GLMM), with age as fixed factor and the litter identity as the random factor, appropriate for analysis of data with missing values, to compare the effect of age on acoustic 259 260 structure. For the analysis with GLMM, we used the mean values of each acoustic variable for 261 each litter in each age-group. This decreased the number of degrees of freedom and allowed a 262 more robust examination of the effects of age.

263

3. Results

265

266 3.1. Age effects on call type occurrence

Seven call types (all four types of squeaks, both types of screeches and the clicks) were detected among pups of all 11 litters, whereas the whimpers were detected in just 3 of the 11 litters. Short low-frequency (LF) squeaks were emitted in most recording trials (99.0%) (Fig. 3); next in order of occurrence were the modulated high-frequency (HF) squeaks (72.4% of recording trials) and then the long LF squeaks (63.8% of recording trials). Flat HF squeaks, screeches, short screeches and clicks were present in 48.6% to 57.1% of recording trials. Whimpers were detected in only 5.7% of recording trials.

Analysis of percentages of recording trials containing the given call type, when separated by age-group (Fig. 4), revealed that short LF squeaks were emitted at high rates (from 88.9 to 100% of recording trials) at all ages. The percentage of trials containing long LF squeaks increased steadily to 5-6 days (up to 80% of recording trials), remained at this level to 15-16 days and then abruptly fell. The percentage of trials containing modulated HF squeaks was high from birth to 17-18 days (57.1 - 100% of recording trials), but then fell abruptly to zero at 21-22 days. Notably, flat HF squeaks were relatively rare before 11-12 days, then frequent up to 17-18 days (70.0% - 81.8% of recording trials), and then became rare again.

282 Screeches appeared only at 3-4 days of age. The percentage of trials with screeches 283 increased steadily up to the maximum of 90.0% at 11-12 days and then decreased, varying from 284 54.5% to 70% of recording trials (Figure 4). The occurrence of short screeches matched those of screeches. They also appeared at 3-4 days, increased steadily to 10-12 days, then steadily 285 286 decreased, but rose up again to 71.4% at 21-22 days of age. Clicks were most frequent at early 287 ages, but the percentage of recording trials with clicks decreased towards maturation, so after 11-288 12 days of age, clicks were detected in less than 50% of recording trials. Whimpers occurred 289 very rarely and only in 4-6-day pups.

Overall, squeaks were found more often in recording trials compared to screeches and clicks. The use of screeches increased to 10-12 days of life and then decreased, whereas clicks were most frequent at early ages and showed a steady decrease in occurrence with advancing age.

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3.2. Age and sex effects on body mass and head length

296 GLM revealed strong and significant effects of age ($F_{9,318} = 310.3$; p < 0.001) and litter 297 identity ($F_{10,318} = 56.2$; p < 0.001), but not pup sex ($F_{1,318} = 3.6$; p = 0.06) on body mass. GLM 298 similarly revealed strong and significant effects of age ($F_{9,318} = 352.9$; p < 0.001) and litter 299 identity ($F_{10,318} = 22.9$; p < 0.001), but not pup sex ($F_{1,318} = 0.01$; p = 0.95) on head length. As 300 sex effects on body mass and head length were not significant, we could use a pooled sample of pups of both sexes and operate with mean values of body mass and head length per litter for each 301 302 age. Body mass and head length both increased significantly with age (r = 0.916, p < 0.001 and r303 = 0.912, p < 0.001 respectively) (Fig. 5).

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305 3.3. Age effects on acoustic variables

The age effects on acoustic variables are presented for four call types (short LF squeaks, modulated HF squeaks, screeches and clicks), as these four calls are produced most frequently and are representative of the four main structural patterns (Table 1, Fig. 6). For the remaining three call types (long LF squeaks, flat HF squeaks and short screeches), age effects on the acoustics are presented in Supplementary Material 1. Descriptive statistics of acoustic variables for all the seven call types are also presented in Supplementary Material 1.

312 For short LF squeaks, GLMM revealed a significant effect of age on fpeak, but not on f0 or 313 duration (Table 1). Duration of LF squeaks showed a weak although significant decrease with 314 age, whereas fpeak increased significantly up to 15-20 days of age and then significantly and 315 noticeably decreased (Table 1, Fig. 6). The f0 and fpeak of modulated HF squeaks increased 316 with age weakly although significantly, whereas the duration was not related to pup age (Table 1, 317 Fig. 6). For screeches, GLMM and Pearson's correlation revealed a strong and significant 318 increase of f0 and fpeak from 3-4 to 13-14 days of age, followed by a plateau. The duration of 319 screeches decreased significantly up to 13-14 days of age, also followed by a plateau (Table 1, Fig. 6). The pulse rate of screeches steadily and significantly increased with age ($F_{9,41} = 30.54$, p 320 321 < 0.001; r = 0.884, p < 0.001), from 74.8 \pm 13.4 Hz at 3-4 days to 138.4 \pm 11.3 Hz at 21-22 days 322 of age (Fig. 7). For clicks, the f0 was not related to pup age, whereas the duration significantly 323 (although inconsistently) changed with age, and fpeak weakly, although significantly, increased 324 with age (Table 1, Fig. 6).

325

326 3.4. Body mass and head length effects on acoustic variables

As body mass and head length are positively and highly significantly correlated with age (Fig. 5), correlation coefficients of acoustic variables with body mass and head length (Table 2) were similar to correlation coefficients of acoustic variables with age (Table 1). The maximum f0 was significantly positively correlated with log body mass and head length for screeches but only with head length for modulated HF squeaks. The durations of squeaks and clicks did not show significant relationships with body size, and only the duration of screeches showed a significant decrease with increasing log body mass and head length. However, in all four call types, fpeak increased significantly with increasing pup log body mass and head length (Table 2). The pulse rate of screeches was significantly positively correlated with log body mass and head length (r = 0.816, p < 0.001 and r = 0.813, p < 0.001 respectively).

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338 3.5. Ear structure

339 CT reconstructions showed that all three shrew species examined (Diplomesodon 340 pulchellum, Crocidura suaveolens and Sorex araneus) lack bony bullae: the tympanic cavity 341 rostral to the cochlea is separated from the cranial cavity above only by a thin layer of fibrous 342 tissue, and the ectotympanic bone, which supports the tympanic membrane, takes the form of an 343 incomplete ring which is not fused to the surrounding bones. The middle ear bones and bony 344 labyrinth of *Diplomesodon*, *Crocidura* and *Sorex* are very similar in shape and size (Fig. 8); the 345 adult male *Diplomesodon* specimen had ear structures which were only slightly larger than those 346 of the subadult female. The ossicles of all three shrew species are of a 'microtype' morphology 347 featuring a large orbicular apophysis of the malleus (bulkiest in *Sorex*), a long anterior process 348 and a thin manubrium which is roughly parallel to the anterior process. The anterior process runs 349 in a groove in the ectotympanic bone and may be synostosed to that bone towards its tip. In 350 Sorex, the malleus and incus were indistinguishably fused, but this was not the case in either 351 Diplomesodon or Crocidura.

The gross morphology of the inner ear is also very similar in all shrews examined. The cochlea forms a short coil. The oval window accommodates the small, elongated stapes footplate; the much larger round window is found within a deep but narrow fossula fenestrae cochleae, the entrance to which is just caudomedial to the stapes. The anterior semicircular canal is longer than the other two canals. In *Diplomesodon* and *Crocidura*, this semicircular canal lies in a plane approximately perpendicular to the ectotympanic bone, whereas the angle between thetwo structures is greater in *Sorex*.

359

- 360 **4. Discussion**
- 361

362 4.1. Vocal ontogeny with body growth

Juveniles produced eight call types, all in the human audible frequency range. All call types were produced in the same context of separation from the mother and from the nest. In addition, some calls were also recorded during the caravanning behaviour displayed by piebald shrews at 12-16 days post-partum. None of these call types showed an ontogenetic decrease of fundamental frequency, as would be expected among mammals in general (Morton 1977; Fitch and Hauser 2002). The pulse rate of screeches steadily increased and became more regular with age.

370 Of the eight call types recorded in piebald shrews, six call types (four types of squeaks, 371 clicks and whimpers) were made from birth, whereas screeches and short screeches were only 372 registered from 3-4 days post-partum. Seven of the call types found in the young (the exception 373 being the rarely-occurring whimper) were found also in adult piebald shrews (Volodin et al. 374 2015b). This is different from the ontogeny of the vocal repertoire in the Asian house shrew 375 *Suncus murinus*, in which some call types disappear and other call types appear during postnatal 376 development (Schneiderová 2014). Only five juvenile call types of Asian house shrews persisted 377 among adults: two call types disappeared and 10 call types appeared anew. In the piebald shrew, 378 in contrast, vocal development does not involve changes in the overall set of call types, but there 379 are stepwise changes in their structural characteristics.

The use of different call types by piebald shrews differed according to postnatal age. The study animals mainly used various squeaks. The occurrence of long LF squeaks was maximal from 5-6 to 15-16 days, which coincides approximately with the time of opening of ears and eyes 383 and the development of caravanning behaviour in this species (Zaytseva et al. 2013). In adult 384 piebald shrews, the long LF squeaks are often recorded when a male chases a female during 385 courtship, a behaviour which is followed by copulation (our unpublished observations). It is 386 practically impossible to identify an individual caller during courtship and sexual interaction, so 387 it is unclear whether the male or the female is producing these calls. Similar changes in the 388 context of calls of adults were found in Asian house shrews. Chirps of pups and adolescents of 389 this species when caravanning did not differ in structure from adult male chirps made when 390 courting females (Schneiderová 2014). In an earlier study of Asian house shrews, Gould (1969) 391 considered that chirps at courting were produced by receptive females, not by males.

392 Screeches and short screeches were recorded only from the 3rd day post-partum in piebald 393 shrews. These were the only call types that were not emitted from birth. The structure of 394 screeches of 3-4 day old pups appeared imperfect, with breaks in the sinusoidal frequency 395 modulation which was also considerably slower than in adults, showing an irregular pulse rate. 396 With increasing age, the pulse rate steadily increased from 75 Hz at 3-4 days to 138 Hz at 21-22 397 days and became more and more regular. It may well be that the maximum contraction rate of 398 certain body muscles, involved in the deep sinusoidal modulation of their screeches, increases 399 with postnatal age. In a study of the greater white-toothed shrew Crocidura russula and the 400 Etruscan shrew Suncus etruscus, the deep sinusoidal modulation of screeches was found to slow 401 progressively with cooling, leading to torpor (Hutterer et al. 1979), which represents indirect 402 evidence of the relationship between the pulse rate of screeches and the work of body muscles. A 403 direct relationship between pulse rate of loud trilled calls and the performance of trunk muscles 404 (external obliques) was demonstrated using high-speed video and electromyography (EMG) in 405 vivo in two related species of North American gray tree frogs (Girgenrath and Marsh 1997). Both 406 species produce trilled calls with high sound intensity, but the sound pulse frequency within calls 407 in Hyla chrysoscelis is twice that in H. versicolor. In both species, sound pulse rate directly 408 correlated with the active contractions of the trunk muscles. It should be noted that sound 409 production mechanisms in frogs and mammals differ considerably, as in frogs the air is forced 410 from the lungs into the air sac(s) with trunk muscles, which results in coincidence of the sound 411 pulse frequency with contractions of the trunk muscles (Wells 2007).

As the rates of screech modulations and of vibrations in piebald shrews exceed 100 Hz, the possibility that their muscles are capable of 'superfast contractions' should be investigated. Such contractions have been found in syringial muscles of songbirds (Elemans et al. 2008), laryngeal muscles of buzzing bats (Elemans et al. 2011; Ratcliffe et al. 2013), swimbladder muscles of some fishes (Rome et al. 1998; Rome 2006) and tail shaker muscles of rattlesnakes (Conley and Lindstedt 1996; Rome et al. 1996). In all cases, these superfast contracting muscles are associated with acoustic signal production.

In *Diplomesodon*, there is a consistent increase in the pulse rate of screeches during ontogeny. Two species of flat-headed bats, *Tylonycteris pachypus* and *T. robustula* (Zhang et al. 2005), and Laxmann's shrews, *Sorex caecutiens*, also show an increasing pulse rate of screeches with maturation. In Laxmann's shrew pups, the pulse rate of screeches increased during postnatal development from 78 Hz at 9 days to 173 Hz at 25 days (Movchan and Schibkov 1983; Konstantinov and Movchan 1985) and reached 212 Hz in adults (Movchan and Schibkov 1982, 1983).

426 In this study, clicks were found to be most frequent soon after birth, and then became less 427 frequent with age and nearly ceased by 22 days. Piebald shrews produce only audible calls below 428 15 kHz; we found no ultrasonic components within their vocalizations. They therefore appear 429 not to produce the ultrasonic orientation clicks described for other species of shrews (Gould et al. 430 1964; Tomasi 1979; Forsman and Malmquist 1988; Thomas and Jalili 2004) and for tenrecs 431 (Gould 1965). Clicks were most commonly made by helpless, blind newborns with closed ears, 432 usually when placing the pups on the flat surface of a table. If pups use clicks for orientation, the 433 low fundamental frequency of these signals might be related to the lack of a patent external 434 auditory meatus. However, the helpless and blind pups hardly need orientation calls, as their ability to move is very limited even if they had information about their environment. A more
likely explanation is that clicks are normally used for attracting attention of the mother: the f0 of
newborn clicks found in this study (9.2 kHz in 1-2 day-old pups) falls within the optimum
hearing range of adult piebald shrews, which is approximately between 7 and 30 kHz
(Konstantinov et al. 1987). The clicks are probably made with the tongue, as has been reported
for other species of shrews (Gould 1969). In adult piebald shrews, clicks are very rare and were
documented only when the animal was placed on an elevated disk (Volodin et al. 2012, 2015b).

442 In piebald shrews, the f0 of squeaks and clicks remained unchanged in spite of the age-443 related increase in body mass and head length; moreover, the f0 of screeches increased with age. 444 This pathway of f0 ontogeny contrasts with the steady decrease of f0 with age which is more 445 typical of mammals, an effect of the growth of sound-producing components of the vocal 446 apparatus (Morton 1977; Fitch and Hauser 2002; Matrosova et al. 2007). A descending f0 during 447 postnatal development was found in primates (Inoue 1988; Hammerschmidt et al. 2000, 2001; 448 Pistorio et al. 2006; Ey et al. 2007), elephants (Stoeger-Horwath et al. 2007), rodents (Owings 449 and Loughry 1985; Nesterova 1996; Blumstein and Munos 2005), bovids (Briefer and 450 McElligott 2011; Efremova et al. 2011; Volodin et al. 2014) and cervids (Torriani et al. 2006; 451 Volodin et al. 2015a).

452 An increase of f0 of echolocation and social calls with age is known for many species of 453 bats (Jones et al. 1991; De Fanis and Jones 1995; Moss et al. 1997; Zhang et al. 2005; Liu et al. 454 2007; Hiryu and Riquimaroux 2011; Monroy et al. 2011; Jin et al. 2011, 2012; Funakoshi et al. 455 2013; Wang et al. 2014). This might result from selection pressures for avoiding obstacles or 456 spotting prey items. The higher the f0 of the echolocation pulse, the higher the directionality of 457 the sound wave and the higher the precision of location of the object by the reflected echo 458 (Madsen and Surlykke 2013). Throughout maturation, bat pups produce signals of an 459 increasingly high fundamental frequency using the thin vocal membranes on their vocal folds 460 (Novick and Griffin 1961; Suthers and Fattu 1973).

The f0s of screams of the mothers and 1-4 month old pups of sea otters (*Enhydra lutris*) were indistinguishable (McShane et al. 1995). That study was devoted to description of the vocal repertoire and individual traits in calls of sea otters, and the indistinguishable f0s between pups and adults were not discussed by the authors. In contrast, in the Asian small-clawed otter *Aonyx cinerea*, the f0 in contact calls was lower in adults compared to adolescents (Lemasson et al. 2014).

467 Among tonal calls of Asian house shrews, only twitters and chirps were found in both pups 468 and adults. In twitters, the f0 is halved from pups to adults, whereas the f0 of chirps remains 469 unchanged throughout ontogeny (Schneiderová 2014). For five species of ground squirrels 470 (genus *Spermophilus*), the fOs of alarm calls were indistinguishable between pups and adults 471 (Matrosova et al. 2007, 2012; Swan and Hare 2008; Volodina et al. 2010). Playbacks confirmed 472 that adult Richardson's ground squirrels S. richardsonii did not discriminate between calls of 473 pups and adults (Swan and Hare 2008). It has been proposed that pups mimic calls of adults, in 474 order to decrease the age-dependent risk of predation and infanticide (Matrosova et al. 2007; 475 Volodina et al. 2010).

476 We have shown that shrews represent another group of mammals which do not show a 477 descent in call fundamental frequency as they grow from pups to adults. The available data are 478 insufficient to explain the reason underlying this phenomenon. It is doubtful that similar f0s of 479 pups and adults result from an accelerated growth of the pup larynx compared to the growth of 480 the rest of the body. In speckled and yellow ground squirrels (Spermophilus suslicus and S. 481 *fulvus*) the size of the larynx is proportional to the condylobasal length of the skull, across both 482 pups and adults (Matrosova et al. 2007), despite the alarm call f0s being indistinguishable. 483 Although mammalian juveniles have relatively larger heads compared to body size than adults, 484 their absolute head sizes differ considerably: in the piebald shrew, the newborn head length 485 comprises only 48.6% of the adult head length (Zaytseva et al. 2013).

486

487 4.2. Vibration production and ear morphology

488 A previous study demonstrated the abilities of adult and subadult piebald shrews from the 489 34th day post-partum to produce seismic vibrations, the frequency of which was the same as the 490 pulse rate of screeches in the same individuals (Volodin et al. 2012). Although the relationship 491 between vibration frequency and the rate of the deep sinusoidal modulation (pulse rate) of 492 screeches has not yet been directly confirmed for piebald shrews, the developmental pathway of 493 the pulse rate of screeches might point to the developmental pathway of vibration production in 494 this species. We did not measure seismic vibrations in the present study, but the vibrating 495 behaviour of piebald shrews is very characteristic and obvious, and this was not observed in our 496 animals (our observations extended up to the 22nd days of their lives). Vibrating behaviour 497 therefore must appear later in the ontogeny of piebald shrews.

498 The ears of *Sorex* and *Crocidura* shrews have been fairly well described, one of the most 499 comprehensive accounts being the paper by Burda (1979). Our anatomical findings for these 500 genera are consistent with Burda's report. In contrast, the only information relating to the ear of 501 Diplomesodon that the authors are aware of is a single illustration of the auditory region of the 502 skull, minus ossicles, in McDowell (1958). We found the ear structures of Diplomesodon to be 503 very similar to those of *Crocidura* and *Sorex*; such differences that did exist tended to separate 504 Sorex from the other two. The apparent fusion of malleus and incus in Sorex is an unusual 505 feature of shrews of this genus (Mason and Farr 2013).

Diplomesodon has been found in molecular studies to emerge from within the monophyletic genus *Crocidura* (Dubey et al. 2008). From a phylogenetic point of view, the close similarity between the ear structures of *Diplomesodon* and *C. suaveolens* is therefore unsurprising. However, the skull of *Diplomesodon* is relatively shorter and broader than that of other shrews, perhaps relating to its burrowing habits (Heptner 1939), and *Diplomesodon* is the only shrew so far known to produce vibratory signals. There was, then, reason to suspect that the ear region of this animal might be specially adapted to reflect these behaviours, but our morphological comparison has failed to show that the *Diplomesodon* ear differs substantially
from that of other shrews, at least in terms of the hard structures visible in CT scans.

515 Among rodents, the mole-rat Spalax (Spalacidae) communicates by means of seismic 516 vibrations of a few hundred Hertz generated by "head-drumming" on the burrow roof (Heth et al. 517 1987; Rado et al. 1987); bathyergid mole-rats (Bathyergidae) and kangaroo rats (Heteromyidae) 518 also communicate using seismic signals (Narins et al. 1992; Randall 2010). Although it is not yet 519 known for sure whether the middle ear represents the means of detection of these signals, all of 520 these rodents have a "freely mobile" ossicular morphology, associated with low-frequency 521 hearing (Mason 2015). The Namib desert golden mole *Eremitalpa* is believed to be able to detect low-frequency seismic vibrations generated by grassy tussocks in the wind, and by the insect 522 523 prey that live therein (Narins et al. 1997; Lewis et al. 2006). This Afrotherian insectivore has 524 extraordinarily enlarged middle ear ossicles which appear to be suited to vibration detection 525 through a form of inertial bone conduction (Mason 2003a, 2003b; Willi et al. 2006b). In contrast, 526 the 'microtype' ear morphology of *Diplomesodon*, also found in other shrews, bats and mice, is 527 associated with good high-frequency hearing (Fleischer 1978; Mason 2013). Consistent with 528 this, evoked potentials in response to airborne sound were found in *Diplomesodon* and several 529 other shrew species at frequencies from around 1 to 70 kHz (Konstantinov et al. 1987). The ear 530 morphology of the piebald shrew is therefore quite unlike the ear morphology of mammalian 531 species which are known to make use of seismic vibrations, and it shows no clear signs of being 532 tuned to the low seismic frequencies that this shrew produces.

Although it is possible that the ears of *Diplomesodon* in particular and perhaps shrews in general have some alternative and as-yet unidentified means of augmenting seismic sensitivity, there is currently no evidence for this. Somatosensation has been implicated in the detection of seismic cues in certain subterranean mammals which lack enlarged middle ear ossicles (see Mason and Narins 2010, for a review), and it may well be that seismic sensitivity in *Diplomesodon* is based on this sensory modality. Although controversial (see Rado et al. 1998),

539 some believe that Spalax uses a somatosensory rather than an auditory route for seismic 540 detection (Nevo et al. 1991). The lamellated corpuscles found in the skin of the paws may 541 subserve this sensory modality when the animal is performing 'seismic echolocation' (Kimchi et 542 al. 2005) and similar receptors have also been found in the skin of the rhinarium of this species 543 (Klauer et al., 1987). Piebald shrews have ridges of fine hairs on their feet (Heptner 1939), which 544 start growing from 7 days of age (Vakhrusheva and Ilchenko 1995, 2010) and are well-545 developed at 34 days, when vibratory behaviour has been recorded (Volodin et al. 2012). It is 546 possible that such hairs might mediate vibrational sensitivity in the shrew, the auditory route 547 being used mainly for perception of higher-frequency, vocal cues. However, ridges of hairs on 548 the feet are quite common in desert species and they may help *Diplomesodon* to walk on the sand 549 (Heptner, 1939): they are not necessarily related to seismic sensitivity.

550 It is interesting to speculate that the vibratory behaviour of *Diplomesodon* might relate to 551 its semidesert habitat, an unusual environment for a shrew. Sand is regarded as a favourable 552 medium for the propagation and localization of biological signals (Brownell, 1977); many desert 553 animals from diverse groups including the scorpions studied by Brownell, desert ants 554 (Buehlmann et al. 2012), the sandfish lizard (Hetherington, 1992) and the desert golden mole 555 discussed above appear to be able to detect and make use of vibrations in sand. While it remains 556 to be proven that *Diplomesodon* can detect the vibrations that it generates, which might 557 potentially be used for prey detection, orientation or intraspecific communication, it would be 558 surprising if it could not. A histological examination of the feet of this desert shrew is required to 559 look for the presence of lamellated receptor organs, which might subserve vibratory sensitivity.

560 While many studies have related vertebrate seismic sensitivity to ear morphology, far 561 fewer have considered the structural relations between the spectra of seismic and acoustic signals 562 (Volodin et al. 2012, 2015b, Bednářová et al. 2013). Bednářová et al. (2013) discuss the possible 563 structural and functional relationships between seismic and acoustic components of mechanically 564 produced sounds in giant mole-rats (*Fukomys mechowii*) in relation to differential propagation of these two components in the environment, which might underlie different communication functions. However, these proposals have not yet been tested experimentally. In the case of the piebald shrew, high-frequency acoustic cues may be perceived in the normal way by the apparently unmodified auditory system, while seismic vibrations could potentially be detected using the somatosensory system.

- 570
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- 572

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References

587	Bednářová, R., Hrouzková-Knotková, E., Burda, H., Sedláček, F., Šumbera, R., 2013.
588	Vocalizations of the giant mole-rat (Fukomys mechowii), a subterranean rodent with the richest
589	vocal repertoire. Bioacoustics 22, 87–107.
590	Blumstein, D.T., Munos, O., 2005. Individual, age and sex-specific information is
591	contained in yellow-bellied marmot alarm calls. Anim. Behav. 69, 353-361.
592	Briefer, E., McElligott, A.G., 2011. Indicators of age, body size and sex in goat kid calls
593	revealed using the source-filter theory. Appl. Anim. Behav. Sci. 133, 175-185.
594	Brownell, P.H., 1977. Compressional and surface waves in sand: used by desert scorpions
595	to locate prey. Science 197, 479–482.
596	Buehlmann, C., Hansson, B.S., Knaden, M., 2012. Desert ants learn vibration and
597	magnetic landmarks. PLoS ONE 7(3):e33117 doi:10.1371/journal.pone.0033117.
598	Burda, H., 1979. Morphology of the middle and inner ear in some species of shrews
599	(Insectivora, Soricidae). Acta Scientiarum Naturalium Akademiae Scientiarum Bohemoslovacae
600	- Brno 13, 1–46.
601	Burda, H., Bruns, V., Müller, M., 1990. Sensory adaptations in subterranean mammals. In:
602	Nevo E, Reig OA (eds) Evolution of subterranean mammals at the organismal and molecular
603	levels. Alan R. Liss, New York, pp 269–293.
604	Campbell, P., Pasch, B., Warren, A.L., Phelps, S.M., 2014. Vocal ontogeny in neotropical
605	singing mice (Scotinomys). PLoS ONE 9(12):e113628 doi:10.1371/journal.pone.0113628.
606	Conley, K.E., Lindstedt, S.L., 1996. Minimal cost per twitch in rattlesnake tail muscle.
607	Nature 383, 71–72.
608	de Fanis, E., Jones, G., 1995. Post-natal growth, mother-infant interactions and
609	development of vocalizations in the vespertilionid bat Plecotus auritus. J. Zool., 235, 85-97.

Dryden, G.L., 1968. Growth and development of *Suncus murinus* in captivity on Guam. J.
Mammal. 49, 51–62.

- Dubey, S., Salamin, N., Ruedi, M., Barrière, P., Colyn, M., Vogel, P., 2008. Biogeographic
 origin and radiation of the Old World crocidurine shrews (Mammalia: Soricidae) inferred from
 mitochondrial and nuclear genes. Mol. Phylogenet. Evol. 48, 953–963.
- 615 Dubrovskij, V.Y., Bragin, M.A., Bulichev, V.P., Ilchenko, O.G., Lazovskaja, M.V.,
- 616 Lazovskij, A.R., Mir-Kasimova, S.A., Osipov, V.P., Fedorovich, V.V. 2011. Some aspects of
- 617 piebald shrew (Diplomeodon pulchellum Licht. 1823) biology in the Volga-Ural sands. Bulletin
- of Moscow Society of Nature Explorers, Dept. of Biology 116, 24–31. [in Russian]
- 619 Efremova, K.O., Volodin, I.A., Volodina, E.V., Frey, R., Lapshina, E.N., Soldatova, N.V.,
- 620 2011. Developmental changes of nasal and oral calls in the goitred gazelle *Gazella subgutturosa*,
- a nonhuman mammal with a sexually dimorphic and descended larynx. Naturwissenschaften 98,919–931.
- $022 \quad j1j-j31.$
- Elemans, C.P.H., Mead, A.F., Jakobson, L., Ratcliffe, J.M. 2011. Superfast muscles set
 maximum call rate in echolocating bats. Science 333, 1885–1888.
- Elemans, C.P.H., Mead, A.F., Rome, L.C., Goller, F., 2008. Superfast vocal muscles
 control song production in songbirds. PLoS ONE 3(7):e2581 doi:10.1371/journal.pone.0002581.
- 627 Ey, E., Pfefferle, D., Fischer, J., 2007. Do age- and sex-related variations reliably reflect
- body size in non-human primate vocalizations? A review. Primates 48, 253–267.
- Fitch, W.T., Giedd, J., 1999. Morphology and development of the human vocal tract: a
 study using magnetic resonance imaging. J. Acoust. Soc. Am. 106, 1511–1522.
- Fitch, W.T., Hauser, M.D., 2002. Unpacking "honesty": vertebrate vocal production and
 the evolution of acoustic signals. In: Simmons A, Fay RR, Popper AN (eds) Acoustic
 communication, Springer handbook of auditory research. Springer, New York, pp 65–137.
- Fleischer, G., 1978. Evolutionary principles of the mammalian middle ear. Adv. Anat.
 Embryol. Cell Biol. 55, 1–70.

- 636 Forsman, K.A., Malmquist, M.G., 1988. Evidence for echolocation in the common shrew,
- 637 Sorex araneus. J. Zool. 216, 655–662.
- Funakoshi, K., Arai, A., Inoue, T., 2013. Development of sounds during postnatal growth
 of the eastern bent-winged bat *Miniopterus fuliginosus*. Mammal Study 38, 49–56.
- Gaillard, J.M., Pontier, D., Allaine, D., Loison, A., Herve, J.-C., Heizmann, A., 1997.
 Variation in growth form and precocity at birth in eutherian mammals. Proc. R. Soc. Lond. B
 264, 859-868.
- 643 Girgenrath, M., Marsh, R.L., 1997. *In vivo* performance of trunk muscles in tree frogs
 644 during calling. J. Exp. Biol. 200, 3101–3108.
- 645 Gould, E., 1965. Evidence for echolocation in the Tenrecidae of Madagascar. Proc. Am.
- 646 Philosoph. Soc, 109, 352–360.
- 647 Gould, E., 1969. Communication in three genera of shrews (Soricidae): *Suncus*, *Blarina*648 and *Cryptotis*. Comm. Behav. Biol. Part A 3, 11–31.
- Gould, E., Negus, N.C., Novick, A., 1964. Evidence for echolocation in shrews. J. Exp.
 Zool. 156, 19–38.
- Hammerschmidt, K., Newman, J.D., Champoux, M., Suomi, S.J., 2000. Changes in rhesus
 macaque 'coo' vocalizations during early development. Ethology 106, 873–886.
- 653 Hammerschmidt, K., Freudenstein, T., Jurgens, U., 2001. Vocal development in squirrel
- 654 monkeys. Behaviour 138, 1179–1204.
- Heptner, V.G., 1939. The Turkestan desert shrew, its biology and adaptive peculiarities. J.
- 656 Mammal. 20, 139–149.
- Heth, G., Frankenberg, E., Raz, A., Nevo, E., 1987. Vibrational communication in
 subterranean mole rats (*Spalax ehrenbergi*). Behav. Ecol. Sociobiol. 21, 31–33.
- Hetherington, T.E., 1992. Behavioural use of seismic cues by the sandswimming lizard *Scincus scincus*. Ethol. Ecol. & Evol. 4, 5–14.

Hiryu, S., Riquimaroux, H., 2011 Developmental changes in ultrasonic vocalizations by
infant Japanese echolocating bats, *Pipistrellus abramus*. J. Acoust. Soc. Am. 130, EL147–
EL153.

Hutterer, R., Vogel, P., Frey, H., Genoud, M., 1979. Vocalization of the shrews *Suncus etruscus* and *Crocidura russula* during normothermia and torpor. Acta Theriol. 24, 267–271.

666 Ilchenko, O.G., Vakhrusheva, G.V., Tupikin, A.A., Lukyanova, I.V., 2011. Sexual

667 behaviour of piebald shrews (Dipolomesodon pulchellum) in captivity. In: Rozhnov VV (ed)

668 Teriofauna of Russia and Neighboring Countries. KMK, Moscow, p. 187. [in Russian]

Inoue, M., 1988. Age gradation in vocalization and body weight in Japanese monkeys
(*Macaca fuscata*). Folia Primatol. 51, 76–86.

Jin, L., Lin, A., Sun, K., Liu, Y., Feng, J., 2011. Postnatal development of morphological
features and vocalization in the pomona leaf-nosed bat *Hipposideros pomona*. Acta Theriol. 56,
13–22.

Jin, L., Wang, J., Zhang, Z., Sun, K., Kanwal, J.S., Feng, J., 2012. Postnatal development
of morphological and vocal features in Asian particolored bat, *Vespertilio sinensis*. Mammal.
Biol. 77, 339–344.

Jones, G., Hughes, P.M., Rayner, J.M.V., 1991. The development of vocalizations in *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during post-natal growth and the
maintenance of individual vocal signatures. J. Zool. 225, 71–84.

Kahane, J.C., 1978. A morphological study of the human prepubertal and pubertal larynx.
Am. J. Anat. 151, 11–19.

Kahane, J.C., 1982. Growth of the human prepubertal and pubertal larynx. J. Speech Hear.
Res. 25, 446–455.

Kimchi, T., Reshef, M., Terkel, J., 2005. Evidence for the use of reflected self-generated
seismic waves for spatial orientation in a blind subterranean mammal. J. Exp. Biol. 208, 647–
686 659.

687	Klauer, G., Burda, H., Nevo, E., 1997. Adaptive differentiations of the skin of the head in a
688	subterranean rodent, Spalax ehrenbergi. J. Morphol. 233, 53-66.
689	Konstantinov, A.I., Movchan, V.N., 1985. Sounds in the life of animals. Leningrad Univ
690	Press, Leningrad. [in Russian]

Konstantinov, A.I., Movchan, V.N., Shibkov, A.A., 1987. Functional properties of the
auditory system and acoustic signalling in insectivores. J. Evol. Biochem. Physiol. 23, 321–328.
[in Russian]

Lee, S., Potamianos, A., Narayanan, S. 1999. Acoustics of children's speech:
Developmental changes of temporal and spectral parameters. J. Acoust. Soc. Am. 105, 1455–
1468.

Lemasson, A., Mikus, M.-A., Blois-Heulin, C., Lode, T., 2014. Vocal repertoire, individual
acoustic distinctiveness, and social networks in a group of captive Asian small-clawed otters
(*Aonyx cinerea*). J. Mammal. 95, 128–139.

Lewis, E.R., Narins, P.M., Jarvis, J.U.M., Bronner, G., Mason, M.J., 2006. Preliminary
evidence for the use of microseismic cues for navigation by the Namib golden mole. J. Acoust.
Soc. Am. 119, 1260–1268.

Liu, Y., Feng, J., Jiang, Y.-L., Wu, L., Sun, K.-P., 2007. Vocalization development of
greater horseshoe bat, *Rhinolophus ferrumequinum* (Rhinolophidae, Chiroptera). Folia Zool. 56,
126–136.

Madsen, P.T., Surlykke, A., 2013. Functional convergence in bat and toothed whale
biosonars. Physiology 28, 276–283.

Mason, M.J., 2003a. Morphology of the middle ear of golden moles (Chrysochloridae). J.
Zool. 260, 391–403.

710 Mason, M.J., 2003b. Bone conduction and seismic sensitivity in golden moles
711 (Chrysochloridae). J. Zool. 260, 405–413.

- Mason, M.J., 2006. Evolution of the middle ear apparatus in talpid moles. J. Morphol. 267,
 678–695.
- Mason, M.J., 2013. Of mice, moles and guinea-pigs: functional morphology of the middle
 ear in living mammals. Hear. Res. 301, 4–18.
- Mason, M.J., 2015. Functional morphology of rodent middle ears. In: Cox PG, Hautier L
 (eds) Evolution of the rodents: Advances in phylogeny, functional morphology and development.
- 718 Cambridge Univ Press, Cambridge, in press.
- Mason, M.J., Farr, M.R.B., 2013. Flexibility within the middle ears of vertebrates. J.
 Laryngol. Otol. 127, 2–14.
- Mason, M.J., Lai, F.W.S., Li, J.-G., Nevo, E., 2010. Middle ear structure and bone
 conduction in *Spalax*, *Eospalax* and *Tachyoryctes* mole-rats (Rodentia: Spalacidae). J. Morphol.
 271, 462–472.
- Mason, M.J., Narins, P.M., 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. Kerala, Transworld Research Network, pp 121–139.
- Matrosova, V.A., Pivanova, S.V., Savinetskaya, L.E., Volodin, I.A., Volodina, E.V.,
 Shekarova, O.N., 2012. The between-population variation of the alarm call in the speckled
- 729 ground squirrel (Spermophilus suslicus, Rodentia, Sciuridae): Effects of sex, age and body mass.
- 730 Zool. Zhurnal. 91, 453–463. [in Russian]
- Matrosova, V.A., Volodin, I.A., Volodina, E.V., Babitsky, A.F., 2007. Pups crying bass:
 vocal adaptation for avoidance of age-dependent predation risk in ground squirrels? Behav. Ecol.
 Sociobiol. 62, 181–191.
- McDowell, S.B., 1958. The greater Antillean insectivores. Bull Amer Museum Nat History
 115, 113–214.
- McShane, L.J., Estes, J.A., Riedman, M.L., Staedler, M.M., 1995. Repertoire, structure,
 and individual variation of vocalizations in the sea otter. J. Mammal. 76, 414–427.

738	Michalak, I., 1987. Growth and postnatal development of the European water shrew. Acta
739	Theriol. 32, 261–288.
740	Monroy, J.A., Carter, M.E., Miller, K.E., Covey, E., 2011. Development of echolocation
741	and communication vocalizations in the big brown bat, Eptesicus fuscus. J. Comp. Physiol. A
742	197, 459–467.
743	Morton, E.S., 1977. On the occurrence and significance of motivation-structural rules in
744	some bird and mammal sounds. Am. Nat. 111, 855–869.
745	Moss, C.F., Redish, D., Gounden, C., Kunz, T.H., 1997. Ontogeny of vocal signals in the
746	little brown bat, Myotis lucifugus. Anim. Behav. 54, 131–141.
747	Movchan, V.N., Shibkov, A.A., 1982. Structural patterns of acoustic signals in shrews
748	(Soricidae). Zool. Zhurnal 61, 1695–1705. [in Russian]
749	Movchan, V.N., Shibkov, A.A., 1983. Development of acoustic signals in shrews and
750	hedgehogs in ontogeny. In: Sokolov VE (ed) Behaviour of animals in communities, Proceedings
751	of Soviet Union conference on animal behaviour. Nauka, Moscow, pp 28-30. [in Russian]
752	Narins, P.M., Lewis, E.R., Jarvis, J.U.M., O'Riain, J., 1997. The use of seismic signals by
753	fossorial southern African mammals: a neuroethological gold mine. Brain Res. Bull. 44, 641-
754	646.
755	Narins, P.M., Reichman, O.J., Jarvis, J.U.M., Lewis, E.R., 1992. Seismic signal
756	transmission between burrows of the Cape mole-rat, Georychus capensis. J. Comp. Physiol. A
757	170, 13–21.
758	Nesterova, N.L., 1996. Age-dependent alarm behavior and response to alarm call in bobac
759	marmots (Marmota bobac Mull.). In: Le Berre M, Ramousse R, Le Guelte L (eds) Biodiversity
760	in Marmots. International Network on Marmots, Moscow-Lyon, pp 181–186.
761	Nevo, E., Heth, G., Pratt, H., 1991. Seismic communication in a blind subterranean

762 mammal: a major somatosensory mechanism in adaptive evolution underground. PNAS 88,
763 1256–1260.

- Novick, A., Griffin, D.R., 1961. Laryngeal mechanisms in bats for the production of
 orientation sounds. J. Exp. Zool. 148, 125–145.
- Owings, D.H., Loughry, W.J., 1985. Variation in snake-elicited jump-yipping by blacktailed prairie dogs: ontogeny and snake specificity. Z. Tierpsychol. 70, 177–200.

Pistorio, A.L., Vintch, B., Wang, X., 2006. Acoustic analysis of vocal development in a
New World primate, the common marmoset (*Callithrix jacchus*). J. Acoust. Soc. Am. 120,
1655–1670.

- Rado, R., Himelfarb, M., Arensburg, B., Terkel, J., Wolberg, Z., 1989. Are seismic
 communication signals transmitted by bone conduction in the blind mole rat? Hear. Res. 41, 23–
 30.
- Rado, R., Levi, N., Hauser, H., Witcher, J., Adler, N., Intrator, N., Wollberg, Z., Terkel, J.,
 1987. Seismic signalling as a means of communication in a subterranean mammal. Anim. Behav.
 35, 1249–1251.

Rado, R., Terkel, J., Wollberg, Z., 1998. Seismic communication signals in the blind molerat (*Spalax ehrenbergi*): electrophysiological and behavioral evidence for their processing by the
auditory system. J. Comp. Physiol. A 183, 503–511.

- Randall, J.A., 2010. Drummers and stompers: vibrational communication in mammals. In:
 O'Connell-Rodwell CE (ed) The use of vibrations in communication: Properties, mechanisms
 and function across taxa. Kerala, Transworld Research Network, pp 99–120.
- Ratcliffe, J.M., Elemans, C.P.H., Jakobsen, L., Surlykke, A., 2013. How the bat got its
 buzz. Biol. Lett. 9, 20121031 http://dx.doi.org/10.1098/rsbl.2012.1031.
- Rome, L.C., Syme, D.A., Hollingworth, S., Lindstedt, S.L., Baylor, S.M., 1996. The
 whistle and the rattle: The design of sound producing muscles. PNAS 93, 8095–8100.
- Schneiderová, I., 2014. Vocal repertoire ontogeny of the captive Asian house shrew *Suncus murinus* suggests that the male courtship call develops from the caravanning call of the young.
 Acta Theriol., 59, 149–164.

790	Stoeger-Horwath, A.S., Stoeger, S., Schwammer, H.M., Kratochvil, H., 2007. Call
791	repertoire of infant African elephants: First insights into the early vocal ontogeny. J. Acoust.
792	Soc. Am. 121, 3922–3931.

- Suthers, R.A., Fattu, J.M., 1973. Mechanisms of sound production by echolocating bats.
 Amer. Zool. 13, 1215–1226.
- Swan, D.C., Hare, J.F., 2008. Signaler and receiver ages do not affect responses to
 Richardson's ground squirrel alarm calls. J. Mammal. 89, 889–894.
- Thomas, J.A., Jalili, M.S., 2004. Echolocation in insectivores and rodents. In: Thomas JA,
 Moss CF, Vater M (eds) Echolocation in bats and dolphins. Univ Chicago Press, Chicago, pp
 547–564.
- 800 Titze, I.R., 1994. Principles of voice production. Prentice-Hall, Englewood Cliffs, NJ.
- 801 Tomasi, T.E., 1979. Echolocation by the short-tailed shrew *Blarina brevicauda*. J.
 802 Mammal. 60, 751–759.
- 803 Torriani, M.V.G., Vannoni, E., McElligott, A.G., 2006. Mother-young recognition in an 804 ungulate hider species: a unidirectional process. Amer. Nat. 168, 412–420.
- Tsuji, K., Ishikawa, T., 1984. Some observations of the caravaning behaviour in the house
 musk shrew *Suncus murinus*. Behaviour 90, 167–183.
- 807 Vakhrusheva, G.V., Ilchenko, O.G., 1995. Maintaining and breeding the piebald shrew in
 808 captivity. Inter Zoo News 42, 89–93.
- Vakhrusheva, G.V., Ilchenko, O.G., 2010. Keeping and breeding the piebald shrew
 (*Diplomesodon pulchellum*) at the Moscow Zoo. In: Spitsin VV (ed) The maintenance and
 cultivation of rare species of mammals in zoos and breeding stations. Moscow Zoo, Moscow, pp
 36–44. [in Russian]
- 813 Vlasák, P., 1972. The biology of reproduction and post-natal development of *Crocidura*814 *suaveolens* Pallas, 1811 under laboratory conditions. Acta Univ. Carol. Biol. 1970(3), 207–292.

- 815 Volodin, I.A., Matrosova, V.A., Volodina, E.V., Garcia, A.J., Gallego, L., Márquez, R.,
- Llusia, D., Beltrán, J.F., Landete-Castillejos, T., 2015a. Sex and age-class differences in calls of
 Iberian red deer during rut: reversed sex dimorphism of pitch and contrasting roars from farmed
 and wild stags. Acta Ethol. 18, 19–29.
- 819 Volodin, I.A., Sibiryakova, O.V., Kokshunova, L.E., Frey, R., Volodina, E.V., 2014. Nasal
 820 and oral calls in mother and young trunk-nosed saiga antelopes, *Saiga tatarica*. Bioacoustics 23,
 821 79–98.
- Volodin, I.A., Zaytseva, A.S., Ilchenko, O.G., Volodina, E.V., 2015b. Small mammals ignore common rules: A comparison of vocal repertoires and the acoustics between pup and adult piebald shrews *Diplomesodon pulchellum*. Ethology 121, 103–115.
- Volodin, I.A., Zaytseva, A.S., Ilchenko, O.G., Volodina E.V., Chebotareva, A.L., 2012.
 Measuring airborne components of seismic body vibrations in a Middle-Asian sand-dwelling
 Insectivora species, the piebald shrew (*Diplomesodon pulchellum*). J. Exp. Biol. 215, 2849–
 2852.
- Volodina, E.V., Matrosova, V.A., Volodin, I.A., 2010. An unusual effect of maturation on
 the alarm call fundamental frequency in two species of ground squirrels. Bioacoustics 20, 87–98.
 Wang, L., Lin, A., Xiao, Y., Jiang, T., Feng, J., 2014. Postnatal development in the bigfooted bat, *Myotis macrodactylus*: wing morphology, echolocation calls, and flight. Acta Theriol.
 59, 435–441.
- Wells, K.D., 2007. The ecology and behaviour of amphibians. The University of ChicagoPress, Chicago.
- Willi, U.B., Bronner, G.N., Narins, P.M., 2006a. Middle ear dynamics in response to
 seismic stimuli in the Cape golden mole (*Chrysochloris asiatica*). J. Exp. Biol. 209, 302–313.
- Willi, U.B., Bronner, G.N., Narins, P.M., 2006b. Ossicular differentiation of airborne and
 seismic stimuli in the Cape golden mole (*Chrysochloris asiatica*). J. Comp. Physiol. A 192, 267–
 277.

- 841 Zaytseva, A.S., Vakhrusheva, G.V., Ilchenko, O.G., Volodin, I.A., 2013. Postnatal
- 842 development of piebald shrews (*Diplomesodon pulchellum*, Insectivora, Soricidae) in captivity.
- 843 Zool. Zhurnal 92, 1463–1474. [in Russian]
- 844 Zhang, L., Jones, G., Parsons, S., Liang, B., Zhang, S., 2005. Development of 845 vocalizations in the flat-headed bats, *Tylonycteris pachypus* and *T. robustula* (Chiriptera:
- 846 Vespertilionidae). Acta Chiropter. 7, 91–99.

Table 1. Results of two-factor GLMM (with age as a fixed factor and the identity of a litter as the random factor) and Pearson correlation coefficients for age effects on variables of four call types, during the ontogeny of piebald shrews. Key: f0max = maximum fundamental frequency; duration = call duration; fpeak = maximum amplitude frequency. Significant differences (for Pearson correlations after Bonferroni correction at *p* < 0.016) are shown in bold.

853

Call type	f0max		Duration		fpeak	
	GLMM	Correlation	GLMM	Correlation	GLMM	Correlation
Short LF	$F_{10,82} = 1.91$	<i>r</i> = -0.196	$F_{10,82} = 1.93$	<i>r</i> = -0.243	$F_{10,82} = 2.37$	<i>r</i> = 0.262
squeak	<i>p</i> = 0.06	<i>p</i> = 0.047	<i>p</i> = 0.052	<i>p</i> = 0.013	<i>p</i> < 0.05	<i>p</i> = 0.008
Modulated	$F_{9,55} = 1.70$	<i>r</i> = 0.298	$F_{9,55} = 2.05$	<i>r</i> = 0.026	$F_{9,55} = 2.68$	<i>r</i> = 0.424
HF squeak	<i>p</i> = 0.11	<i>p</i> = 0.010	<i>p</i> = 0.051	<i>p</i> = 0.83	<i>p</i> < 0.05	<i>p</i> < 0.001
Screech	$F_{9,41} = 11.92$	r = 0.727	$F_{9,41} = 2.50$	<i>r</i> = -0.366	F _{9,41} =5.18	<i>r</i> = 0.620
	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.05	<i>p</i> = 0.004	<i>p</i> < 0.001	<i>p</i> < 0.001
Click	$F_{10,34} = 1.30$	r = 0.306	$F_{10,34} = 3.80$	r = 0.077	$F_{10,34} = 1.55$	<i>r</i> = 0.466
	<i>p</i> = 0.27	<i>p</i> = 0.023	<i>p</i> < 0.01	<i>p</i> = 0.58	<i>p</i> = 0.16	<i>p</i> < 0.001

854

Table 2. Pearson's correlation coefficients (r) for log body mass, head length and variables857of four call types of piebald shrews. N – number of mean values of call variables per litter per858age-group. Correlations considered significant after Bonferroni correction at p < 0.016 (0.05/3)859are shown in bold.

Call type N		f0max		duration		fpeak	
		body mass	head length	body mass	head length	body mass	head length
Short LF	103	<i>r</i> = -0.174	<i>r</i> = -0.172	<i>r</i> = -0.155	<i>r</i> = -0.165	<i>r</i> = 0.267	<i>r</i> = 0.315
squeak		<i>p</i> = 0.08	<i>p</i> = 0.08	<i>p</i> = 0.12	<i>p</i> = 0.10	<i>p</i> = 0.006	<i>p</i> = 0.001
Modulated	75	<i>r</i> = 0.256	<i>r</i> = 0.295	<i>r</i> = 0.155	r = 0.086	r = 0.402	<i>r</i> = 0.447
HF squeak		<i>p</i> = 0.026	<i>p</i> = 0.010	<i>p</i> = 0.18	<i>p</i> = 0.46	<i>p</i> < 0.001	<i>p</i> < 0.001
Screech	61	<i>r</i> = 0.713	<i>r</i> = 0.749	<i>r</i> = -0.402	<i>r</i> = -0.397	<i>r</i> = 0.662	<i>r</i> = 0.706
		<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> = 0.001	<i>p</i> = 0.002	<i>p</i> < 0.001	<i>p</i> < 0.001
Click	55	<i>r</i> = 0.175	<i>r</i> = 0.229	<i>r</i> = 0.086	<i>r</i> = 0.092	<i>r</i> = 0.439	<i>r</i> = 0.430
		<i>p</i> = 0.20	<i>p</i> = 0.09	<i>p</i> = 0.53	<i>p</i> = 0.50	<i>p</i> < 0.001	<i>p</i> = 0.001

863

Figure legends

864

Fig. 1. Spectrogram illustrating eight call types of piebald shrew pups at 5 days of age. The
spectrograms were created with a Hamming window, 48 kHz sampling rate, FFT 1024 points,
frame 50% and overlap 87.5%. The audio files with these calls are provided in Supplementary
Material 2.

869

Fig. 2. The measured acoustic variables in (left) power spectrum and spectrogram with waveform (right). Designations: duration – call duration, modulation period – period of the deep sinusoidal frequency modulation, then used for calculating "pulse rate" of screeches and short screeches; f0max – maximum frequency of f0, f0min – minimum frequency of f0, fpeak – maximum amplitude frequency. The spectrogram was created with a Hamming window, 48 kHz sampling rate, FFT 1024 points, frame 50% and overlap 96.87%.

876

Fig. 3. Percent of recording trials containing the given call type (n = 105 recording trials in total across all ages).

879

Fig. 4. Percent of recording trials containing the given call type in each age: (A) four types
of squeaks; (B) screeches, short screeches, clicks and whimpers.

882

Fig. 5. Age-related changes in (A) body mass and (B) head length (n = 8 for 1-2 days, n = 10 for 3-4 days, and n = 11 for all other ages). Central points show means, whiskers show *SD*.

885

Fig. 6. Age-related changes in the maximum fundamental frequency (f0max), call duration
(duration) and peak frequency (fpeak) of four call types (short LF squeak, modulated HF squeak,
screech and click) during the ontogeny of piebald shrews. Central points show means of

individual litter means, whiskers show *SD*. Note that click durations are nearly ten times shorterthan those in the other call types.

891

Fig. 7. Age-related changes in the rate of the deep sinusoidal frequency modulation ("pulse rate") of screeches. Central points show means of individual litter means, whiskers show *SD*. Representative spectrograms (with time on *x*-axis and frequency on *y*-axis) for four selected ages are presented above the graph.

896

897 Fig. 8. Reconstructions of left middle and inner ear structures of three species of shrews: 898 Diplomesodon pulchellum (subadult female; upper row), Crocidura suaveolens (middle row) and 899 Sorex araneus (bottom row). On the left are ventral views of the middle ear ossicles, 900 ectotympanic and bony labyrinth of each species; rostral is to the left. In the middle are lateral 901 views of the same. On the right are the middle ear structures only, as seen from within the 902 tympanic cavity. Scale bar 3 mm. Key: 1 = anterior semicircular canal; 2 = posterior semicircular 903 canal; 3 =lateral semicircular canal; 4 =cochlea; 5 =incus; 6 =malleus; 7 =anterior process of 904 malleus; 8 = orbicular apophysis of malleus; 9 = stapes; 10 = ectotympanic bone. The 905 ectotympanic bone is shown in translucent red, the malleus in green, the stapes in yellow and the 906 bony labyrinth in white. The incus is shown in blue except in Sorex, in which it is 907 indistinguishably fused with the malleus.

908

910

Supplementary Materials

911

912 Supplementary Material 1. Descriptive statistics of acoustic variables for seven call types
913 during the ontogeny of piebald shrews.

914

915 Supplementary Material 2. Audio. Eight call types of piebald shrew pups at 5 days of 916 age: three short LF squeaks, one long LF squeak, two modulated HF squeaks, two flat HF 917 squeaks, two screeches, one short screech, three clicks, one whimper.







Call types

Figure 4 Click here to download high resolution image



Figure 5 Click here to download high resolution image









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