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# Age and sex skull variation in a model population of the common shrew (*Sorex araneus*)

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**Abstract.** Sexual and age variation of the common shrew was assessed in 173 individuals captured in the Novohradské hory mountain range in South Bohemia, Czech Republic. Age variation was found in seven of the fourteen measurements examined. In six of them (height of mandible, height of mandible measured below the first molar, length of mandible, cranial width, condylobasal length, length of neurocranium), values in adults were higher than those in juveniles, while the opposite was found for the length of the lower incisor. Evidence of sex differences was found only in three measurements: height of mandible measured below the first molar, cranial width and length of the lower incisor. Our results suggest the need for separating age and sex groups in studies of skull variation in *Sorex* shrews.

**Key words:** morphometry, age variation, sexual dimorphism, Soricidae

## Introduction

There are many studies concerned with morphological variation of the common shrew (*Sorex araneus*). Most of them focused on the Dehnel's phenomenon, i.e. the winter reduction of some internal organs and several body and skull measurements (e.g. Dehnel 1949, Pucek 1955, 1963, 1970), or morphological differences between chromosomal races (e.g. Wójcik et al. 2000, Stefen 2013). Relatively detailed information is available about skull development during nidal life of the common shrew (Vogel 1973). Unfortunately, much less attention has been paid to skull variation in shrews during the spring-autumn period. In general, it was assumed that there are no or only negligible sex differences in skull measurements (e.g. Schubarth 1958, Homolka 1980, Poroshin et al. 2010). As to the differences in skull measurements between young in the calendar year of their birth and overwintered individuals, previous studies dealt mostly with changes in braincase height or condylobasal length (e.g. Kubik 1951, Pucek 1955, 1970), with a few exceptions (Homolka 1980, Spitzenberger & Bauer 2001). In fact, detailed analyses of sex and age differences in skull measurements within a common shrew population, based on a large enough sample, are very scarce. We used classic morphometry (linear measurements), as we wanted to retain information

about size variation in the population. In addition, this method gives values that are commonly used in the mammalogical literature. It should be stressed that without such analysis it is impossible to distinguish geographical variation due to environmental variables from intrapopulation variation due to sex and age differences. Therefore, the aim of our study was to fill this gap in the literature and make a detailed comparison of skull measurements in two age groups of shrews with the aim of determining if there is sexual dimorphism, especially in adults, which have been rarely studied to date.

## Material and Methods

### Material

The material used in this study consisted of skulls of common shrews snap-trapped between 1972 and 1976 in the locality Žofín situated in the Novohradské hory mountain range, South Bohemia, Czech Republic. Shrews were collected mostly along streams flowing through a wet meadow, while a smaller part of the material was collected in a nearby beech-spruce forest, at an elevation of ca 750 m. For further details about the locality, see Vohralík et al. (1972).

All captured animals were processed by standard mammalogical methods, i.e. measured, dissected and conserved in 4% formaldehyde. Later, skulls were

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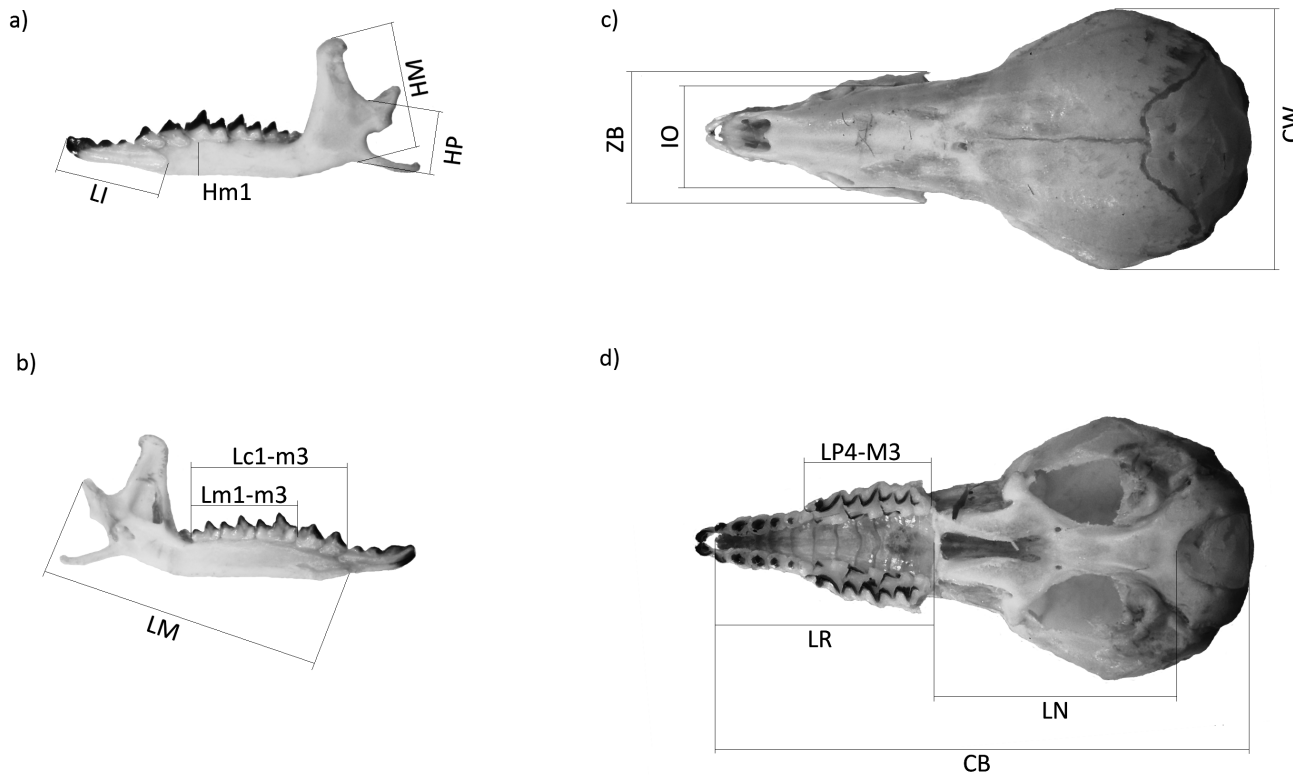


Fig. 1. Mode of taking of the skull measurements. Mandible: a) buccal view, b) lingual view; cranium: c) dorsal view, d) ventral view.

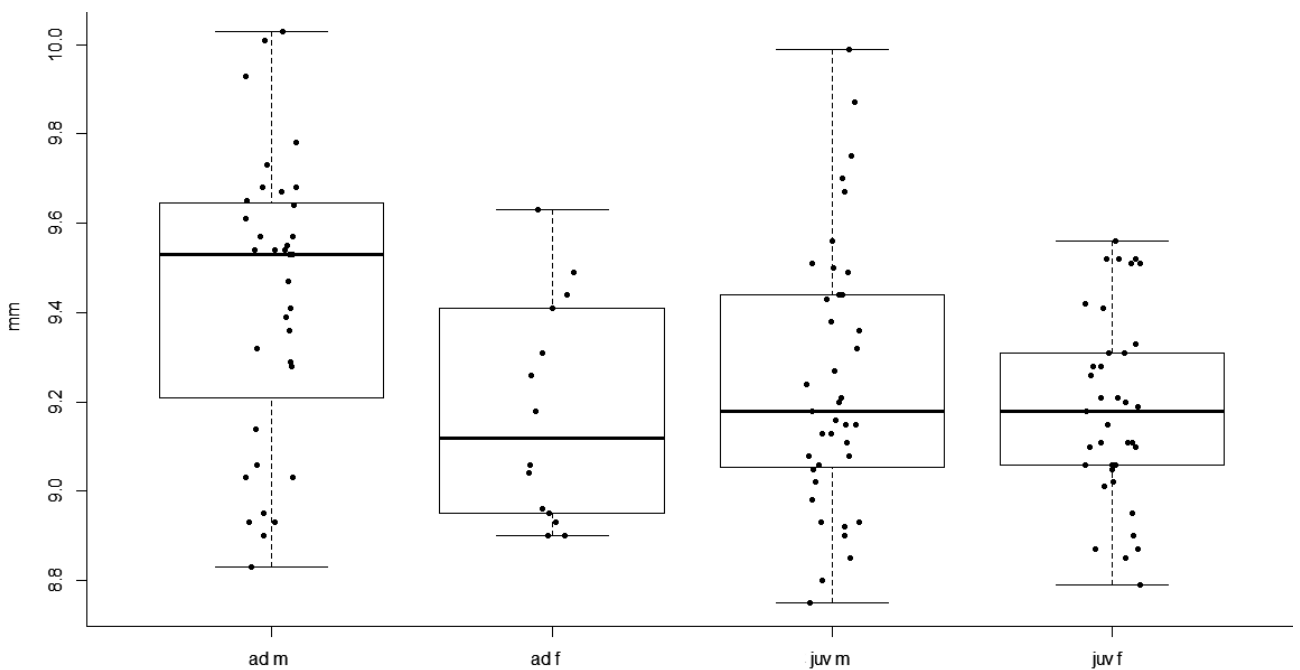


Fig. 2. Variation of the cranial width (CW). Abbreviations: ad m (adult males), ad f (adult females), juv m (juvenile males), juv f (juvenile females). Boxplots show the interquartile range with median for each group. Dots represent individual values, outliers are shown as circles.

extracted and cleaned by *Dermestes maculatus* beetles. We divided the animals ( $n = 173$ ) into four groups – juvenile males (52 specimens), juvenile females (47 specimens), adult males (53 specimens), and adult females (21 specimens). The juvenile categories

include only immature individuals trapped between September and November of the year they were born in. They were identified based on the size of testes in males and absence of embryos and signs of previous parturition in females. In addition, juveniles exhibited

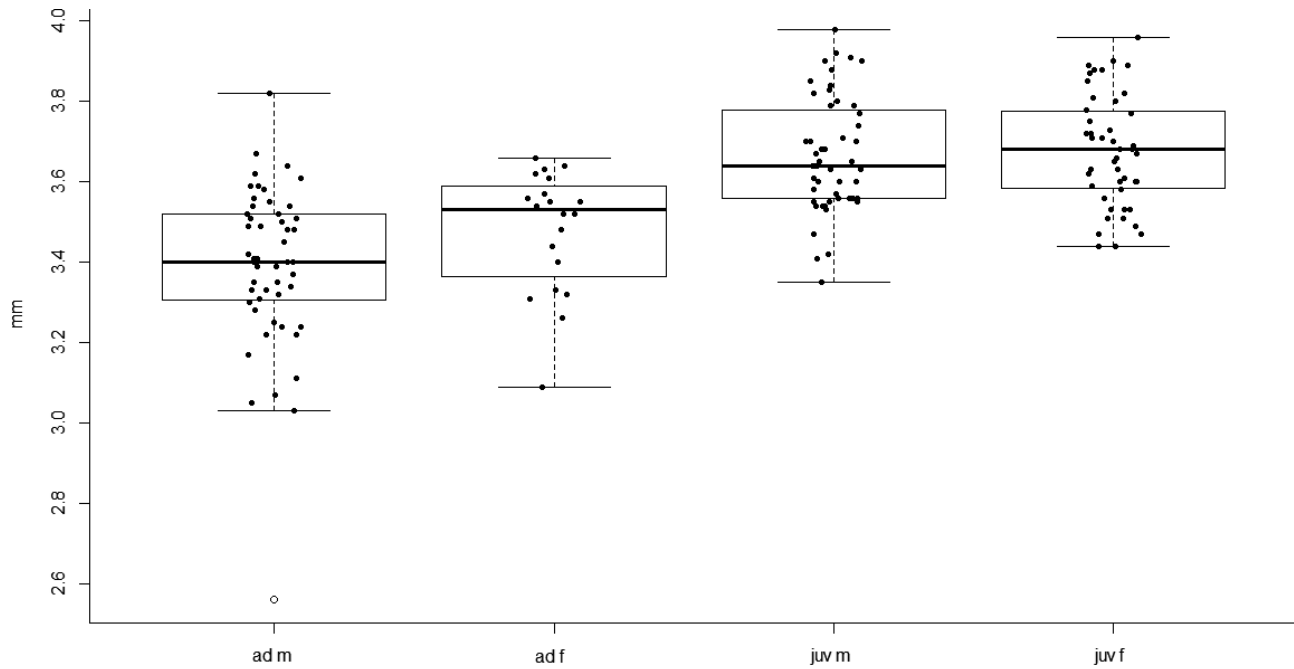


Fig. 3. Variation of the length of the lower first incisor (LI). For description see Fig. 2 legend.

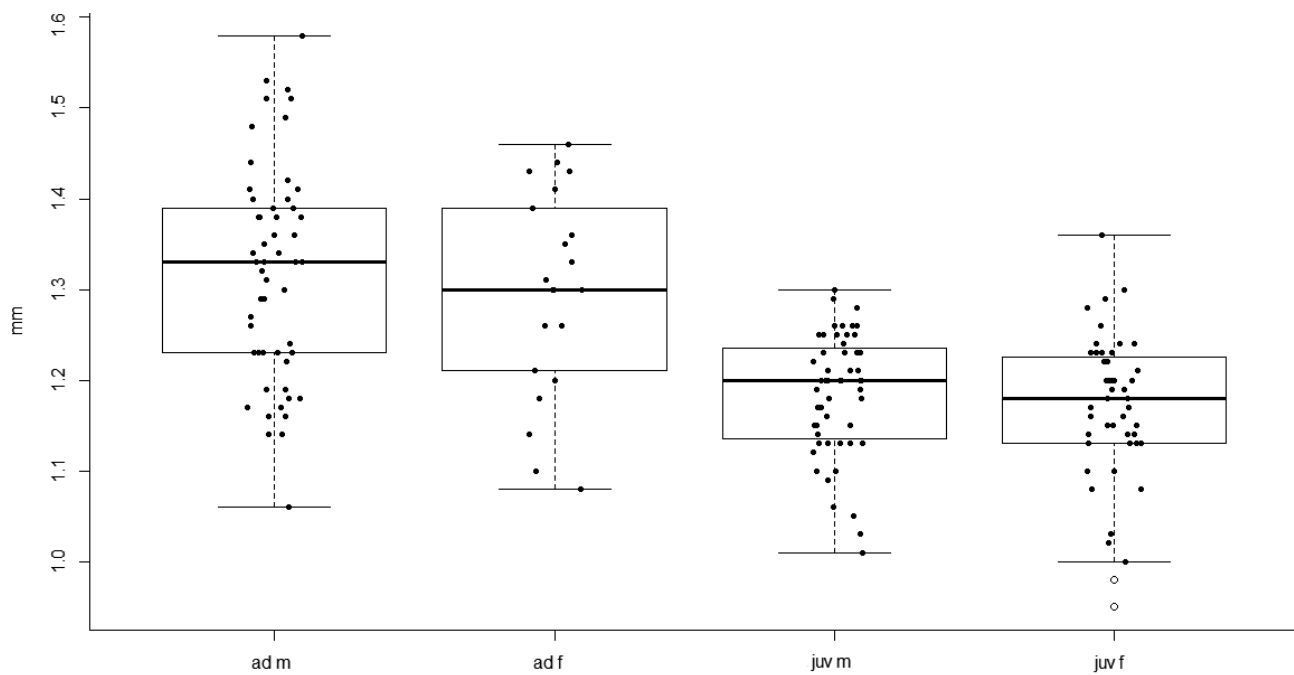


Fig. 4. Variation of the height of mandible measured below the first molar (Hm1). For description see Fig. 2 legend.

visibly more prominent hairs on the tip of their tail and different pelage colour. Teeth of juveniles clearly differ from those of adults by less abrasion. Adults include overwintered individuals trapped between April and November.

#### Measuring and statistics

Skulls were magnified under an Olympus SZX 12 stereomicroscope and high-resolution photos were

taken with an Olympus DP70 camera. Pictures of crania from dorsal and ventral views and left mandibles from buccal and lingual views were taken after placing them on a horizontal surface without any correction of their position. All measurements were recorded from the images in the tpsDig 2 software (Rohlf 2016) to the nearest 0.01 mm. We took seven cranial and seven mandibular measurements mostly according Vesmanis (1976), see Fig. 1. On the buccal

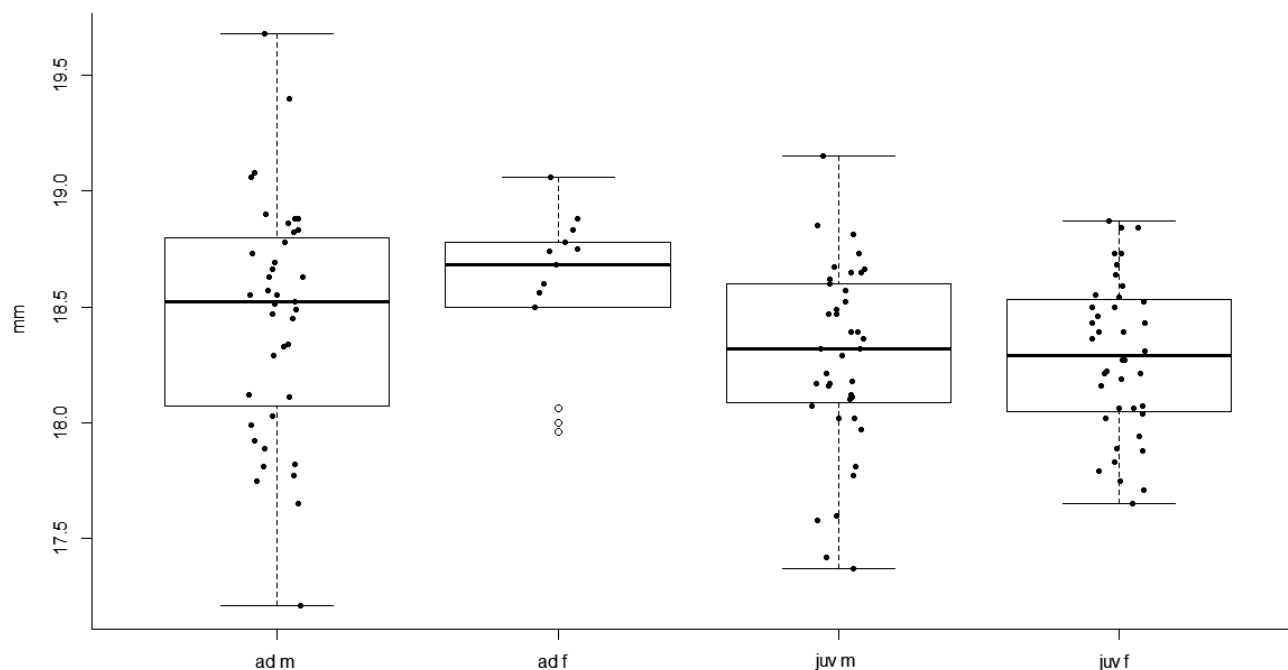


Fig. 5. Variation of the condylobasal length (CB). For description see Fig. 2 legend.

side of the mandible, we measured height of mandible (HM) and postcoronoid height (HP), both taken at the least vertical distance, height of mandible below the first molar (Hm1), measured at the aboral margin of *foramen mentale*, and length of incisor (LI), measured at the greatest length of the visible part of the incisor, without the root. On the lingual side, we measured length of mandible (LM), length of tooth row (Lc1-m3), and length of molar row (Lm1-m3). From the dorsal view of the skull, we measured cranial width (CW), zygomatic width (ZB), and interorbital width (IO). Condylobasal length (CB), length of rostrum (LR), length of neurocranium (LN), and length of upper molariform tooth row (LP4-M3) were measured from the ventral view of the skull. All teeth measurements were taken across crowns, with the exception of LI. One person took all the pictures and performed the measurements.

All variables were normally distributed (Shapiro-Wilk test). The effect of age and sex was tested by two-way ANOVA. Mutual differences between all four groups (juvenile males, juvenile females, adult males, adult females) were tested by independent samples t-test. Using a general linear model (MANOVA), we revealed a significant effect of age and sex on all measurements (age:  $F = 11.62$ ,  $p < 0.001$ ; sex:  $F = 2.60$ ,  $p = 0.003$ ). Principal component analysis (PCA) showed correlations between our measurements (see Supplementary material, Table S1). Additionally, we tested the effect of age and sex on the first four principal

components (two-way ANOVA, all results in Table S2) with eigenvalues higher than 1.0 (Table S3). Statistical significance was evaluated at  $\alpha$  level of 0.05.

Descriptive statistics, two-way ANOVA and t-tests were performed in the PAST software (Hammer 2016), general linear models and PCA in Statistica 7 software (StatSoft, Inc. 2004). All plots were generated in R version 3.3.3 (R Core Team 2017).

## Results

### Age variation

Age variation was discovered in seven of the fourteen measurements examined (Tables 1, 2, Figs. 2-5). In six of them, the values in adults were higher than those in juveniles, mostly in both sexes (HM, Hm1, LM, CB, and LN), but only in males in the case of CW (Fig. 2; males:  $t_{74} = 2.52$ ,  $p = 0.014$ ; females:  $t_{50} = 0.15$ ,  $p = 0.878$ ). Conversely, length of the lower first incisor (LI) was much shorter in adults than in juveniles (Fig. 3;  $F = 100.03$ ,  $p < 0.001$ ). In seven measurements (HP, Lc1-m3, Lm1-m3, ZB, IO, LR, LP4-M3), we did not find any differences between adults and juveniles. Age has a significant effect on all the tested principal components (PC1:  $F = 9.07$ ,  $p = 0.003$ ; PC2:  $F = 27.97$ ,  $p < 0.001$ ; PC3:  $F = 36.93$ ,  $p < 0.001$ ; PC4:  $F = 6.84$ ,  $p = 0.010$ ).

### Sexual dimorphism

Influence of sex as an important variable was found only in three of the fourteen measurements examined

**Table 1.** Summary statistics. N (sample size), arithmetic mean (mean), SD (standard deviation), min (minimum value), max (maximum value). All measured values are in millimetres. For measurement abbreviations see Material and Methods.

	adults									
	males					females				
	N	mean	SD	min	max	N	mean	SD	min	max
HM	52	<b>4.48</b>	0.15	4.22	4.98	21	<b>4.49</b>	0.11	4.30	4.71
HP	53	<b>2.14</b>	0.10	1.94	2.40	21	<b>2.16</b>	0.10	2.00	2.38
Hm1	53	<b>1.32</b>	0.12	1.06	1.58	21	<b>1.30</b>	0.11	1.08	1.46
LI	51	<b>3.39</b>	0.20	2.56	3.82	20	<b>3.48</b>	0.15	3.09	3.66
LM	52	<b>9.73</b>	0.27	9.11	10.44	20	<b>9.76</b>	0.19	9.45	10.36
Lc1-m3	51	<b>5.30</b>	0.15	4.88	5.67	20	<b>5.33</b>	0.12	5.03	5.56
Lm1-m3	52	<b>3.68</b>	0.11	3.46	3.90	21	<b>3.71</b>	0.08	3.52	3.87
CW	36	<b>9.43</b>	0.32	8.83	10.03	14	<b>9.18</b>	0.25	8.90	9.63
ZB	45	<b>4.81</b>	0.23	4.31	5.31	16	<b>4.82</b>	0.29	4.31	5.39
IO	48	<b>3.46</b>	0.16	3.13	3.74	18	<b>3.48</b>	0.17	3.24	3.87
CB	39	<b>18.45</b>	0.51	17.21	19.68	13	<b>18.57</b>	0.35	17.96	19.06
LR	47	<b>7.86</b>	0.27	7.20	8.47	17	<b>7.90</b>	0.26	7.43	8.36
LN	41	<b>8.12</b>	0.24	7.66	8.69	13	<b>8.17</b>	0.22	7.76	8.41
LP4-M3	52	<b>4.28</b>	0.14	4.00	4.54	19	<b>4.33</b>	0.10	4.15	4.49

	juveniles									
	males					females				
	N	mean	SD	min	max	N	mean	SD	min	max
HM	51	<b>4.45</b>	0.14	4.18	4.69	47	<b>4.42</b>	0.13	4.19	4.69
HP	51	<b>2.16</b>	0.08	1.93	2.33	47	<b>2.15</b>	0.10	1.93	2.36
Hm1	52	<b>1.18</b>	0.07	1.01	1.30	47	<b>1.17</b>	0.08	0.95	1.36
LI	51	<b>3.67</b>	0.14	3.35	3.98	47	<b>3.68</b>	0.14	3.44	3.96
LM	50	<b>9.63</b>	0.24	8.99	10.15	47	<b>9.64</b>	0.21	9.23	10.01
Lc1-m3	52	<b>5.29</b>	0.11	5.07	5.54	47	<b>5.32</b>	0.10	5.08	5.48
Lm1-m3	52	<b>3.67</b>	0.08	3.42	3.82	47	<b>3.68</b>	0.07	3.51	3.83
CW	39	<b>9.25</b>	0.30	8.75	9.99	37	<b>9.19</b>	0.21	8.79	9.56
ZB	44	<b>4.76</b>	0.20	4.33	5.25	43	<b>4.75</b>	0.16	4.36	5.12
IO	45	<b>3.43</b>	0.13	3.18	3.74	47	<b>3.44</b>	0.10	3.23	3.69
CB	40	<b>18.29</b>	0.40	17.37	19.15	40	<b>18.29</b>	0.33	17.65	18.87
LR	47	<b>7.85</b>	0.22	7.33	8.23	47	<b>7.87</b>	0.21	7.31	8.27
LN	40	<b>7.96</b>	0.21	7.67	8.42	41	<b>7.91</b>	0.24	7.49	8.47
LP4-M3	49	<b>4.28</b>	0.11	4.00	4.56	47	<b>4.30</b>	0.10	4.07	4.59

(Table 2). The height of mandible measured below the first molar (Hm1) was significantly greater in males than in females ( $t_{172} = 2.47, p = 0.015$ ); confirmed by two-way ANOVA ( $F = 8.80, p = 0.004$ ; Fig. 4). The cranial width (CW) in adult males was considerably greater than in adult females (Fig. 2;  $t_{49} = 2.66, p = 0.011$ ). In juveniles, no sexual dimorphism in CW was found ( $t_{75} = 1.04, p = 0.303$ ). However, it is apparent that in both age categories males attained much higher maximum values than females. Two-way ANOVA revealed a significant main effect

of sex on the length of the lower incisor (LI) ( $F = 12.09, p < 0.001$ ), LI was also the only measurement showing significant interaction between age and sex ( $F = 6.21, p = 0.014$ ). Although means and medians were higher in females than in males (Fig. 3, Table 1), t-test was insignificant both when comparing adult ( $t_{70} = 1.77, p = 0.081$ ) and juvenile shrews ( $t_{98} = 0.48, p = 0.634$ ). In all other dental measurements (length of lower tooth row, length of lower molar tooth row and length of upper molariform tooth row), means were slightly higher in females of both age groups, but the

**Table 2.** Results of two-way ANOVA test. *P*-values (*p*) of significant effects of age and/or sex and interaction between them are highlighted in bold. For measurement abbreviations see Material and Methods.

	Age		Sex		Age × Sex Interactions	
	F value	<i>p</i>	F value	<i>p</i>	F value	<i>p</i>
HM	4.732	<b>0.031</b>	1.352	0.247	0.972	0.326
HP	0.218	0.641	0.026	0.873	0.583	0.446
Hm1	85.190	<b>&lt; 0.001</b>	8.798	<b>0.004</b>	3.262	0.073
LI	100.030	<b>&lt; 0.001</b>	12.090	<b>&lt; 0.001</b>	6.207	<b>0.014</b>
LM	8.268	<b>0.005</b>	0.064	0.801	0.453	0.502
Lc1-m3	0.025	0.875	2.624	0.107	0.119	0.730
Lm1-m3	0.928	0.337	2.374	0.125	0.692	0.407
CW	7.622	<b>0.007</b>	9.047	<b>0.003</b>	3.705	0.057
ZB	2.980	0.086	0.372	0.543	0.308	0.580
IO	1.526	0.219	0.053	0.818	0.189	0.664
CB	7.029	<b>0.009</b>	0.023	0.880	0.958	0.330
LR	0.144	0.705	0.240	0.625	0.066	0.797
LN	23.200	<b>&lt; 0.001</b>	3.256	0.073	2.932	0.089
LP4-M3	0.024	0.876	2.320	0.130	0.417	0.519

differences were not statistically significant (Table 2). Other measurements showed no sex differences. Sex has a significant effect only on the second principal component ( $F = 9.78$ ,  $p = 0.002$ ).

## Discussion

### Age variation

In his review about seasonal and age changes in shrews, Pucek (1970) states that the only cranial dimensions that change throughout the postnatal life of the common shrew are the depth and the breadth of the braincase. This statement can be attributed to the fact that previous studies focused mostly on seasonal changes of the braincase in relation to the Dehnel's phenomenon. Later, Homolka (1980) assessed fourteen skull measurements in shrews and found that four of them change during the postnatal life. Total length of the skull and length of the upper tooth row were significantly shorter in overwintered shrews compared with those in their first calendar year. As both measurements included the first upper incisor, the difference can be explained by continuous abrasion of this tooth during the individual's life. Length of the nasal bones was also found to decrease with age. Height of the braincase changed during the year in agreement with the Dehnel's phenomenon. There is an obvious discrepancy with our observations, although the measurements taken by Homolka are not always identical with those used in our work. We found significant age variation in seven of the fourteen measurements examined. Adults attained higher values than juveniles in six of these

measurements, while the opposite was true only for the length of the lower first incisor. The shortening of the lower incisor with advancing age in our sample is undoubtedly due to tooth wear. This conclusion is supported by the findings of Pankakoski (1989), who observed that in *Sorex araneus* and *S. minutus*, tooth wear is almost twice as fast in overwintered adults than in juveniles. Similarly, Stefen (2013) found that the length of the first lower incisor and length of the mandibular tooth row (including the first incisor) differ significantly in subadult and adult individuals.

The most frequently studied skull measurement is the condylobasal length (CB). There is no definitive consensus about its postnatal changes. The majority of studies (e.g. Dehnel 1949, Pucek 1955, Schubarth 1958, Hürka 1986, Spitzenberger & Bauer 2001) did not find any significant differences between overwintered individuals and individuals in their first calendar year. On the other hand, Kubik (1951) found that overwintered individuals attain lower values of CB than young before overwintering. Homolka (1980) studied two populations of *S. araneus* living at markedly different elevations. He reported significantly higher CB in overwintered (adults trapped between April and November, in the second year of their life) than in juvenile shrews from the High Tatra Mountains, while in the lowland south Moravian population no such difference was present. Unfortunately, detailed studies of cranial intrapopulation variation in the common shrew are still very rare. As our analysis revealed statistically

significant differences in size and shape between the two age groups, age should be considered in future studies of *S. araneus* morphometric variation.

### *Sexual dimorphism*

Most authors studying morphological variation of the *S. araneus* skull mentioned sexual dimorphism only briefly or did not discuss it at all (e.g. Churchfield 1990, Hausser et al. 1990, Churchfield & Searle 2008). Despite considerable sexual dimorphism in the postcranial skeleton (Dolgov 1961, 1985, Brown & Twigg 1970), it is generally accepted that the skull does not exhibit any dimorphism.

Early studies about morphological variation of the common shrew skull did not take sex into account (e.g. Dehnel 1949, Kubik 1951). Pucek (1955) found that overwintered females attain lower values in the height of the braincase than overwintered males. He assumed that this difference was caused by a later onset of reproductive activity in overwintered females, their gravidity, and consequent effect on their morphology. Schubarth (1958) confirmed Pucek's findings and also suggested that the condylobasal length attains somewhat higher values in males than in females. Surprisingly, even more recent studies did not group the specimens by sex (Homolka 1980) or found only negligible sex differences (Hůrka 1986, Yudin 1989, Spitzenberger & Bauer 2001, Mishta 2007, Poroshin et al. 2010, Zidarova 2015).

Significant sexual dimorphism was found in three of the fourteen measurements evaluated, and there was a significant effect of sex on the second principal component representing the shape. Higher values of the height of mandible measured below the first molar (Hm1) were revealed in males, which corresponds with the results of Poroshin et al. (2010). Although Poroshin et al. (2010) took this measurement in a slightly different manner, i.e. below the second molar, they found a statistically significant difference as well. The cranial width (CW) is well studied because of the changes it undergoes during winter (Dehnel's phenomenon). Greater values in overwintered individuals were found by Dehnel (1949), Kubik (1951), and Schubarth (1958). On the other hand, Pucek (1955) and Homolka (1980) did not detect any difference between the age groups. Our results (Table 1, Fig. 2) suggest that the matter is more complex. We found that only overwintered males have considerably broader CW than juveniles of both sexes, while overwintered females do not differ from either juvenile males or juvenile females. The discrepancy

between these results could be explained by the fact that other authors did not divide their material by sex. The mean values of CW published by Spitzenberger & Bauer (2001), who divided their material into sex and age groups, agree with our results.

We found longer lower incisor (LI) in females than in males, where the difference was more pronounced in adults than in juveniles. Tooth wear is the principal cause of the gradual decrease of LI over the individual's life. Therefore, there are two possible explanations for the observed sex difference: different hardness of the teeth or different diet composition in males and females. As sex differences in the hardness of the *S. araneus* tooth enamel have not been found (Adamczewska-Andrzejewska 1966), we believe the effect of different diet in males and females is a more plausible explanation. White & Searle (2009) found a correlation between the mechanical potential of the mandible and climate factors in *S. araneus* females, but not in males. If the differences between males and females are contingent on climate conditions, diet, as a proxy for climate (e.g. Rudge 1968), can be a relevant explanation for our findings. Unfortunately, no information is currently available about potential sex differences in diet of the common shrew.

It should be noted that our results do not always agree with those reported in other studies of *S. araneus* populations from various parts of the species' range. Therefore, we hypothesise that age variation and sexual dimorphism in cranial morphology of the common shrew can be expressed to various degrees in different populations, depending on the environmental factors in different parts of its range.

### **Conclusions**

Here, we show significant sex and age differences in several skull measurements in the studied South-Bohemian population of the common shrew. We investigated fourteen skull measurements and demonstrated changes in seven of them during the postnatal life of the individual. We found sexual dimorphism in three measurements. These facts should be considered in future studies about *Sorex* shrews.

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## Supplementary online material

**Table S1.** Results of PCA. Factor loadings of the variables.

**Table S2.** Results of two-way ANOVA test on the first four principal components (PC). *P*-values (*p*) associated with significant effects of age and/or sex and interaction between them are highlighted in bold.

**Table S3.** Results of PCA. Eigenvalues of the correlation matrix ([http://www.ivb.cz/folia\\_zoologica/supplementarymaterials/novakova\\_vohralik\\_tables\\_s1\\_s2\\_s3.docx](http://www.ivb.cz/folia_zoologica/supplementarymaterials/novakova_vohralik_tables_s1_s2_s3.docx)).