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Distinctive features of hepatocytes in five small mammal species (insectivores and rodents): taxonomic versus ecological specificity

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Abstract Morphometric parameters of hepatocytes have been studied in five small mammal species with different ecological specificity from two taxonomic groups: a surface-dwelling insectivore (the common shrew, *Sorex araneus*), a subterranean insectivore (the European mole, *Talpa europaea*), surface-dwelling rodents (the bank vole, *Myodes glareolus* and herb field mouse, *Apodemus uralensis*) and a subterranean rodent (the northern mole vole, *Ellobius talpinus*). The results show that the hepatocytes of the European mole differ markedly from those of all other species, being characterized by smaller size and nuclear area, low anisocytosis and anisokaryosis, the almost complete absence of binuclear cells and, on the other hand, high nuclear–cytoplasmic ratio and packing density. The common shrew differs from the European mole containing a relatively high proportion of binuclear hepatocytes, and the size of hepatocytes in this species is smaller than in rodents. Differences in the parameters of these cells between rodents are minimal or absent. The differences observed in the characteristics of hepatocytes between the above species cannot be explained solely by either taxonomic or ecological specificity. Comparative research involving a greater number of closely related species (at the levels of families and orders) is needed to reveal and evaluate the taxonomic and ecological specificity of hepatocytes in greater detail.

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Keywords Liver · Hepatocytes · Bank vole · Herb field mouse · European mole · Northern mole vole

Introduction

The liver is a vitally important multifunctional organ that plays a major role in a variety of metabolic processes and is responsible for the homeostasis of the body as a whole. Analysis of its morphology is routine in practical and experimental medicine, biology and ecology. Although the general structure of the liver has long been known, its specific features in different classes of vertebrates remain of interest to researchers. To date, an evolutionary trend in liver micromorphology has been traced from fishes to mammals (Akiyoshi and Inoue 2004, 2012; Moura et al. 2012; Odokuma and Omokaro 2015). In ecotoxicology, the morphology of the liver is studied as a biomarker of environmental pollution; anatomic pathology deals with liver injury in response to various adverse factors; histochemistry is used to analyze metabolic processes in liver; etc. A number of publications have been devoted to the analysis of liver morphology from a comparative perspective during regeneration (Miyaoka et al. 2012; Popescu et al. 2012). The macro-, micro- and, in certain cases, ultrastructure of the liver tissue have been described in the greatest detail for mammals, including humans and domestic, farm, game and laboratory animals (Schmucker 1990; Sasse et al. 1992; Thoolen et al. 2010). It may be concluded that the liver is one of the best studied organs in terms of morphology.

In this context, it was all the more unexpected to find that only fragmentary data, or no data at all, are available concerning specific features of liver morphology in small rodents widespread in northern Eurasia and widely used as

model species in ecological studies. Occasional publications can be found in which liver micromorphology is described for rare or exotic species (Ikpegbu et al. 2014). In most cases, micromorphological data can be found in studies on animal exposure to a certain factor and qualitative comparison of liver tissue between experimental and control groups (Atlas… 1994; Włostowski et al. 2004; Pereira et al. 2006; Sánchez-Chardi et al. 2009; Jarrar and Taib 2012; Salińska et al. 2012; Tête et al. 2014). However, such studies involve a limited number of species and rarely provide any histomorphometric data, although it is considered that morphometric methods can detect structural changes in organs upon low-level exposure to a given factor when qualitative changes have not yet manifested (Visscher and Stifano 1981; Sorensen 1989; Gaidash and Klimatskaya 2004).

To bridge the information gap concerning "common" small mammal species, we studied the morphology of the liver in the European mole (*Talpa europaea* Linnaeus, 1758), bank vole (*Myodes glareolus* Schreber, 1780) and herb field mouse (*Apodemus uralensis* Pallas, 1811) (Davydova et al. 2015). The study involved a histomorphometric analysis of hepatocytes, the parenchymal cells of the liver that comprise the bulk of the organ (in humans, up to 60% of its total cell population) have exocrine and endocrine functions and are characterized by high metabolic activity and polymorphism (Ham and Cormack 1974). The results showed that the morphometric parameters of hepatocytes differed between the above species, especially between the European mole and the two species of rodents (Davydova et al. 2015).

We hypothesized that the distinctive features of these cells in different species may either be of a taxonomic nature or depend on their ecological characteristics (mode of life and feeding habits). In the former case, these features may reflect phylogenetic relationships between the species and supraspecific taxa; in the latter case, they can be regarded as ecomorphological adaptations.

The purpose of this study was to reveal probable species specificity of morphometric parameters of hepatocytes in different small mammal species and test two alternative hypotheses—"taxonomic" and "ecological"—concerning the nature of this specificity. To this end, we performed a comparative analysis of the liver and hepatocytes in five species with different ecological specificity from two taxonomic groups: a surface-dwelling insectivore (the common shrew), a subterranean insectivore (the European mole), surface-dwelling rodents (the bank vole and herb field mouse) and a subterranean rodent (the northern mole vole) (Fig. 1). Since species specificity of parameters (traits) embraces the entire spectrum of intraspecific variation (population, chorological, chronological), the studied samples of each species were heterogeneous in terms of animal sex, reproductive status, etc. Moreover, morphometric parameters of hepatocytes are important for the diagnosis of histopathologies (Gerlyng et al. 1993; Bezborodkina et al. 2009; Jarrar and Taib 2012), and we considered it necessary to interpret their values from the viewpoint of not only their species specificity but also normal/pathological state of the liver tissue.

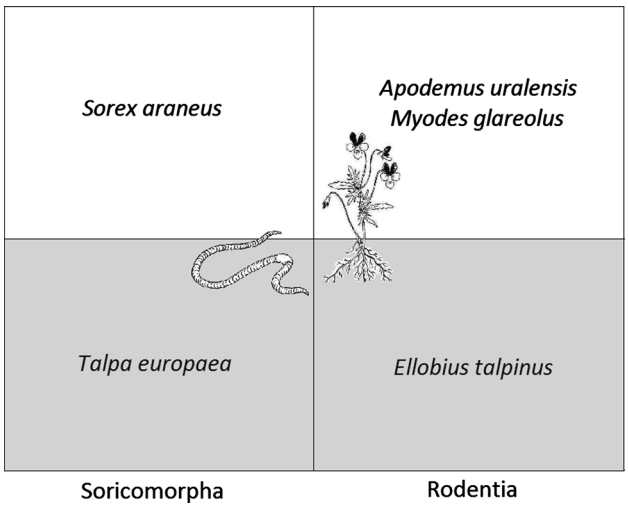

Fig. 1 Scheme illustrating specific ecological features of the five species used in the study

Materials and methods

Animal sampling and study areas

The study was performed on small mammals of two orders, Soricomorpha and Rodentia. The former was represented by the common shrew *Sorex araneus* Linnaeus, 1758 (subfamily Soricinae, family Soricidae) and the European mole *T. europaea* (Talpinae, Talpidae); the latter were represented by the northern mole vole *Ellobius talpinus* Pallas, 1770, the bank vole *M. glareolus* (both of the subfamily Arvicolinae, family Cricetidae) and the herb field mouse *A. uralensis* (Murinae, Muridae) (Wilson and Reeder 2005). Each of these species has specific ecological features depending on the mode of life (surface-dwelling or subterranean) and feeding habit (herbivorous or carnivorous) (Fig. 1).

All these species are common in the ecosystems of the Middle and Southern Urals. Over many years, we have been monitoring their populations in permanent test areas in the Prigorodny (57°28′ N, 59°30′ E) and Nizhnesergin-sky (56°48′ N, 59°23′ E) districts of Sverdlovsk oblast and the Miassky (55°19′ N, 60°13′ E) and Kunashaksky (55°41′ N, 61°27′ E) districts of Chelyabinsk oblast. Samples of the

insectivore and two rodent species (*M. glareolus* and *A. uralensis*) have been collected in fir–spruce forest biotopes, and those of *E. talpinus,* in meadow–steppe biotopes. The animals for this study ($n = 73$) were trapped live to exclude cadaveric autolysis of cells and tissues prior to analysis. We used wooden trap-door traps (modified Sherman traps) for surface-dwelling species and Falkestein–Popov wire traps of different diameters (Deparma 1951) for subterranean species, checking them several times a day. Shrews were studied immediately after trapping; other animals were kept for 1–3 days in a vivarium at room temperature and natural photoperiod. Voles and mice were fed oats and carrots; mole voles received carrots, red beet, and potatoes. Bedding in cages consisted of sawdust and hay. Moles were kept in containers with soil and forest litter and fed earthworms. All the animals had free access to water.

The sample of each species comprised individuals of different sexes and ages. All mole voles in the sample were from different families (Evdokimov and Pozmogova 1998; Evdokimov 2003). Based on a complex of morphological and reproductive traits, the animals were classified into two relative age classes, young and adults. In short-lived surface-dwelling species, the former class comprised animals trapped in the year of birth (immature and mature young of the year); in long-lived subterranean species, it also included animals older than 1-year (Table 1). The animals were anesthetized with diethyl ether, killed by cervical dislocation, and then body and liver weights of each individual (except in *E. talpinus*) were measured with KERN CM 60-2 (Germany) and TANITA 1210 (Japan) digital scales to calculate the hepatosomatic index (Fig. 2).

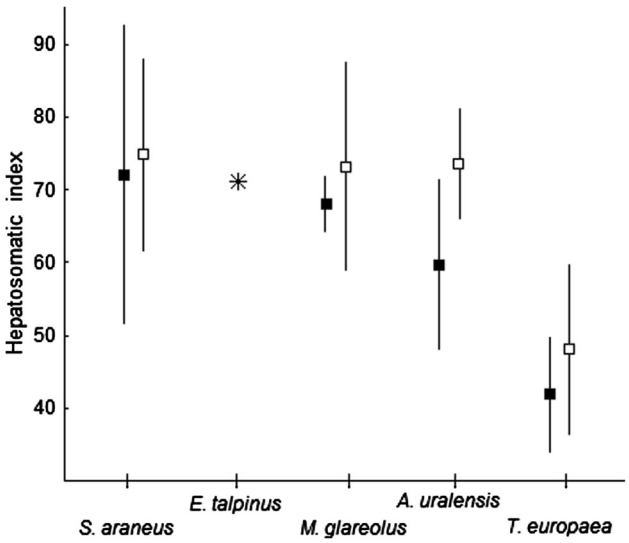

Fig. 2 Hepatosomatic index in males (*solid squares*) and females (*open squares*) of five small mammal species, mean values with confidence intervals. The value for *E. talpinus* (*asterisk*) is from Evdokimov (2002)

Morphometric analysis

Liver samples for micromorphological analysis (fragments of the medial liver lobe not adjacent to the gallbladder) were taken from each animal, fixed in 10% formalin and embedded in paraffin. Histological sections 5–7 μm thick were stained with hematoxylin and eosin and examined under a Leica DM1000 LED microscope with a Leica DFC 295 digital color camera (Leica Microsystems, Germany).

All the samples were analyzed qualitatively to determine whether the liver tissue was in a normal or pathological state (see Table 1). The basic criterion was the

Table 1 Sampling areas and sample structure and sizes

Species	Sampling area	Year	Relative abundance	Age group	Number of animals	
					Male/female	Normal/pathological liver
Sorex araneus	Nizhneserginsky district, the Middle Urals	2014	1.0[a]	Young	3/5	7/1
				Adult	–	–
Talpa europaea	Nizhneserginsky district, the Middle Urals	2008–2010	1.4–11.4[b]	Young	3/4	6/1
				Adult	3/3	2/4
Ellobius talpinus	Kunashaksky district, the Southern Urals	2012	3.5[c]	Young	3/2	0/5
				Adult	2/–	2/0
Myodes glareolus	Prigorodny district, the Middle Urals	2008	5.7[a]	Young	12/5	10/7
				Adult	10/–	7/3
Apodemus uralensis	Miassky district, the Southern Urals	2012–2014	1.8–3.0[a]	Young	10/4	7/7
				Adult	3/1	3/1

[a] *n*/100 trap-days; [b] Number of tunnels per 1 km route (Deparma 1951); [c] Average family size (total number of animals/number of families) (Evdokimov and Pozmogova 1998)

presence of pathologically altered cells with granules, vacuoles, or lipid drops in the cytoplasm and deformations of the nucleus, which were indicative of disturbances in cell metabolism. These alterations were classified as symptoms of parenchymal dystrophy. Since the liver tissue is functionally heterogeneous (Gumucio 1989; Lamers et al. 1989; Gebhardt and Matz-Soja 2014), morphometric analysis of hepatocytes was performed in the central zone of the classic liver lobule in order to standardize measurements. One more reason behind is that hepatocytes around the central vein are relatively poorly supplied oxygen and nutrients (Jungermann and Keitzmann 1996) and, hence, are more sensitive to various factors.

Measurements were made in microscopic images (magnification 630×, 10–20 images per animal) using ImageScope M software (Russia) (http://www.microscop.ru). In mononuclear hepatocytes (100 cells per animal), the maximum (a) and minimum (b) diameters of the nucleus and the area of the cell (S_{cell}) in projection on the image plane were measured and the results were used to calculate the areas of the nucleus ($N = a/2 \times b/2 \times \pi$) and cytoplasm ($C = S_{cell} - N$) and the nuclear–cytoplasmic ratio (N/C). Variation in the nuclear and cell size (in medical terms, anisokaryosis and anisocytosis) was estimated by calculating the interdecile range. The packing density of hepatocytes and the proportion of binuclear cells in the liver parenchyma were evaluated in 10 microscopic fields per sample (one field = 10 000 μm^2) (Table 2).

Statistical analysis

The results were processed statistically with the Statistica v. 8.0 and AtteStat v. 13.1 software packages. The non-parametric Mann–Whitney U test was used to compare the morphometric parameters of normal and altered hepatocytes and the Spearman's rank correlation coefficient, r_S, was used to reveal correlations between different parameters of normal hepatocytes. The dependence of the frequency of liver pathology on animal species, sex, age and reproductive status was evaluated by Pearson's χ^2 contingency tables. Sparse tables (with frequencies of no more than five in some cells) were tested for homogeneity using Simonov–Tsai statistics, with the results being used to determine the validity of approximation by χ^2. In the case of problems with this approximation, the Freeman–Halton extension of Fisher's exact test was applied. Interspecific differences in the morphometric parameters of hepatocytes were evaluated by canonical discriminant analysis.

Results

Hepatosomatic index

This index was the highest in *S. araneus* and the lowest in *T. europaea,* with its values in the three rodent species being intermediate (Fig. 2).

Table 2 Morphometric parameters of normal hepatocytes in small mammals (means with standard errors)

Parameter	*Sorex araneus*	*Talpa europaea*	*Ellobius talpinus*	*Myodes glareolus*	*Apodemus uralensis*
n	7	8	2	17	10
N (μm^2)	23.8 ± 1.53	16.5 ± 1.57	24.7 ± 2.34	27.2 ± 1.62	33.9 ± 2.08
	[19.1–29.9]	[8.3–21.9]		[18.6–40.8]	[26.4–43.6]
Anisocytosis	6.9 ± 0.68	5.6 ± 0.37	8.4 ± 1.30	14.7 ± 1.56	20.1 ± 1.68
	[4.3–9.2]	[4.4–7.5]		[5.1–25.4]	[14.9–32.7]
S_{cell} (μm^2)	168.2 ± 14.91	96.4 ± 5.53*	183.5 ± 4.85	192.6 ± 11.73*	221.5 ± 8.24
	[114.6–232.7]	[74.7–165.4]		[134.3–294.7]	[166.4–264.5]
Anisokaryosis	101.1 ± 14.61	39.2 ± 3.41*	105.3 ± 10.25	124.5 ± 12.12	148.4 ± 10.80
	[46.5–161.5]	[28.7–61.3]		[51.0–207.5]	[109.0–201.0]
N/C	0.18 ± 0.012	0.21 ± 0.019	0.16 ± 0.010	0.17 ± 0.003*	0.19 ± 0.001*
	[0.14–0.24]	[0.13–0.26]		[0.14–0.20]	[0.14–0.24]
Cell packing density, cells/10^5 (μm^2)	301 ± 31.2	501 ± 53.3	292 ± 5.0	312 ± 21.8	260 ± 20.7
	[180–446]	[245–702]		[187–447]	[161–352]
Proportion of binuclear cells (%)	37.2 ± 3.38	0.2 ± 0.08	29.3 ± 0.70	25.5 ± 2.34	31.6 ± 2.82
	[26.0–50.6]	[0.0–0.6]		[7.1–38.7]	[17.5–41.9]

n is the number of animals. Figures in brackets show the range of parameter values. An asterisk indicates values that differ significantly between normal and altered hepatocytes ($p < 0.05$)

Frequency of dystrophic changes

An analysis of the occurrence of liver parenchymal dystrophy showed that its frequency differed depending on animal species: χ^2 (4) = 11.7, $p = 0.02$ (Table 1). The main contribution to these interspecific differences came from *S. araneus*, in which this pathology proved to be significantly less frequent than in *T. europaea* [χ^2 (1) = 6.2, $p = 0.01$], *E. talpinus* [χ^2 (1) = 6.9, $p = 0.008$] and *A. uralensis* [χ^2 (1) = 7.4, $p = 0.006$]. The frequency of dystrophic changes was independent of animal sex in all the five species [*S. araneus,* χ^2 (1) = 0.4, $p = 0.47$; *T. europaea,* χ^2 (1) = 0.6, $p = 0.43$; *E. talpinus,* χ^2 (1) = 1.1, $p = 0.29$; *M. glareolus,* χ^2 (1) = 0.76, $p = 0.38$; *A. uralensis,* χ^2 (1) = 0.76, $p = 0.38$]. This parameter was also independent of animal age in *S. araneus* [χ^2 (1) = 0.7, $p = 0.40$], *T. europaea* [χ^2 (1) = 1.5, $p = 0.22$] and *M. glareolus* [χ^2 (1) = 0.01, $p = 0.08$]; in *E. talpinus* and *A. uralensis*; however, dystrophic changes in the liver were more frequent in young than in adult animals [χ^2 (1) = 7.0, $p = 0.008$ and χ^2 (1) = 3.9, $p = 0.049$, respectively].

Compared to dystrophic cells, normal hepatocytes have a greater nuclear area and nuclear–cytoplasmic ratio but smaller cell area and a lower level of anisokaryosis and anisocytosis. However, the differences are significant in only some of these parameters and not in all species (Table 2).

Correlations between parameters of hepatocytes

Certain parameters of hepatocytes are correlated with each other. Some correlations are common to all the species. For example, the cell area of hepatocytes consistently shows an inverse correlation with their packing density ($r = -0.8$ in *S. araneus* and *T. europaea,* $r = -0.9$ in *E. talpinus* and *M. glareolus* and $r = -0.53$ in *A. uralensis*; $p < 0.05$). The correlations of other parameters differ between the species. On the whole, the parameters of hepatocytes are the least correlated with each other in *T. europaea*, *S. araneus* and *E. talpinus*: statistical significance was confirmed for only 5, 5 and 7 out of a total of 21 pairwise correlations, respectively (compared to 16 correlations in *M. glareolus* and 12 correlations in *A. uralensis*). On the whole (for all the species), the highest levels of correlation with other parameters are characteristics of the nuclear area (a total of 13 pairwise correlations), cell area (16), cell packing density (17) and anisocytosis (18); the lowest level is characteristic of the proportion of binuclear cells (6).

Interspecific differences in the morphometric parameters of hepatocytes

Canonical discriminant analysis revealed significant interspecific differences in the morphometric parameters of hepatocytes, whether normal or altered (Fig. 3; Table 3). The parameters most relevant for discriminating between the species include the proportion of binuclear cells, nuclear area, and anisokaryosis. In particular, the nuclear area, proportion of binuclear cells, and nuclear–cytoplasmic ratio contribute the most to the first canonical variate (CDA 1), which explains 73.5% of the total variance, while anisokaryosis and cell area contribute to the second variate (CDA 2, 20% of the variance).

The hepatocytes of *T. europaea* differ markedly from those of all other species, being characterized by a smaller size and nuclear area, low anisocytosis and anisokaryosis, the almost complete absence of binuclear cells and, on the other hand, a high nuclear–cytoplasmic ratio and packing density. *Sorex araneus* differs from *T. europaea* containing a relatively high proportion of binuclear hepatocytes in the liver and the size of the hepatocytes and anisokaryosis in this species are lower than in rodents. Differences in the parameters of hepatocytes between rodents are minimal and concern only the size and variation of these cells, which are slightly greater in *A. uralensis* than in *M. glareolus.* No differences were observed between the hepatocytes of *E. talpinus* and *M. glareolus* (Table 3).

It should be noted that species identification of the liver tissue is impossible by means of visual examination alone, without histomorphometry, but the qualitative features of

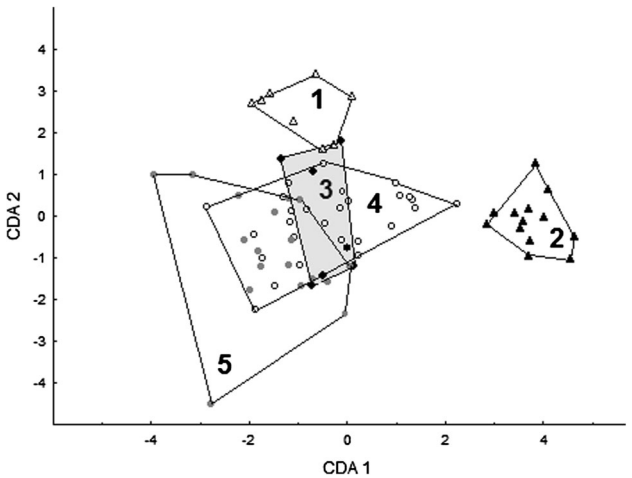

Fig. 3 Ordination of individuals of five small mammal species with respect to morphometric parameters of hepatocytes in the plane of the first two canonical variates: (1) *Sorex araneus,* (2) *Talpa europaea*, (3) *Ellobius talpinus,* (4) *Myodes glareolus,* (5) *Apodemus uralensis*

Table 3 Results of discriminant analysis

Species	*S. araneus* ($n = 8$)	*T. europaea* ($n = 13$)	*E. talpinus* ($n = 7$)	*M. glareolus* ($n = 27$)	*A. uralensis* ($n = 18$)
S. araneus		29.3	9.5	7.8	11.9
T. europaea	18.9***		19.8	17.2	28.8
E. talpinus	4.6**	11.8***		1.5	4.5
M. glareolus	6.2***	19.7***	1.1		2.8
A. uralensis	8.6***	28.4***	3.0*	3.9*	

Note: Figures above and below the diagonal show squared Mahalanobis distances and *F*-test values, respectively; *$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$

hepatocytes are easily distinguished under the microscope (Fig. 4).

Discussion

Each of the morphometric parameters of hepatocytes included in the analysis can characterize the species specificity of normal cells and serve as a diagnostic indicator of the pathological state of liver cells and tissue. Since no preliminary examination of the animals was performed, it was not known in advance whether any pathological changes in the liver would be observed. The need to discriminate the normal and pathological states of the liver tissue became apparent during the initial visual analysis of histological sections, when the symptoms of parenchymal dystrophy were revealed in animals of every species studied (Table 1). However, division into the categories of "normal" and "pathological" in this study was largely arbitrary, because our attention was focused on only one type of hepatic histopathology. The spectrum, severity, and etiology of the observed pathological changes need special study. This is confirmed by the fact that spontaneous lipid dystrophy of hepatocytes with unknown etiology in rodents (namely, *E. talpinus*) has also been observed by other researchers (Manskikh et al. 2015).

The quantitative (morphometric) parameters of cells and tissues are considered to be more objective than their qualitative characteristics. Although histological techniques have been used for centuries, new methods are still being invented and tested, including the development of automated systems for tissue analysis (Liquori et al. 2009; Akiyoshi and Inoue 2012; Ishikawa et al. 2016) and the determination of correction coefficients for morphometric parameters depending on the protocol of histological processing (Kotb 2001). However, only a few studies are available in which the state of liver tissue in small mammals from natural populations has been evaluated not only qualitatively but also by means of histomorphometry. For example, morphometric analysis has provided evidence for the impairment of DNA-synthetic activity against the background of nuclear hypertrophy in the hepatocytes of the root vole (*Microtus oeconomus* Pallas, 1776) from the vicinity of an aluminum smelter (Gaidash and Klimatskaya 2004) and for changes in the size of hepatocytes depending on photoperiod in *M. glareolus* voles under experimental conditions (Bonda-Ostaszewska and Włostowski 2015).

The morphometric parameters analyzed in this study are used as diagnostic criteria in medical practice and physiological experiments (Bezborodkina et al. 2009). For example, the nuclear–cytoplasmic ratio can be used as an indicator to estimate metabolic rate and reveal compensatory responses. In particular, it has been shown that the size of hepatocyte nuclei increases after partial hepatectomy (Popescu et al. 2012). A high nuclear–cytoplasmic ratio may indicate the onset or progression of a pathological process (Herbert 1967). High levels of anisokaryosis and anisocytosis are also regarded as pathological symptoms (Jarrar and Taib 2012). It should be noted that, in health-related fields, the data on variation in the size of hepatocytes and their nuclei are usually presented as a 95% reference range.

The packing density of hepatocytes and the proportion of binuclear cells can be classified as "population parameters" of the liver tissue that characterize its functional activity. Binuclear hepatocytes are often regarded as a cell reserve for regeneration and damaged tissue repair: their number increases in the course of these processes (Gerlyng et al. 1993). Moreover, it has been shown that the proportion of binuclear hepatocytes in laboratory mice increases with age, from 45% in 2-week-olds to 80% in yearlings (Böhm and Noltemeyer 1981). Although the problem of genesis and

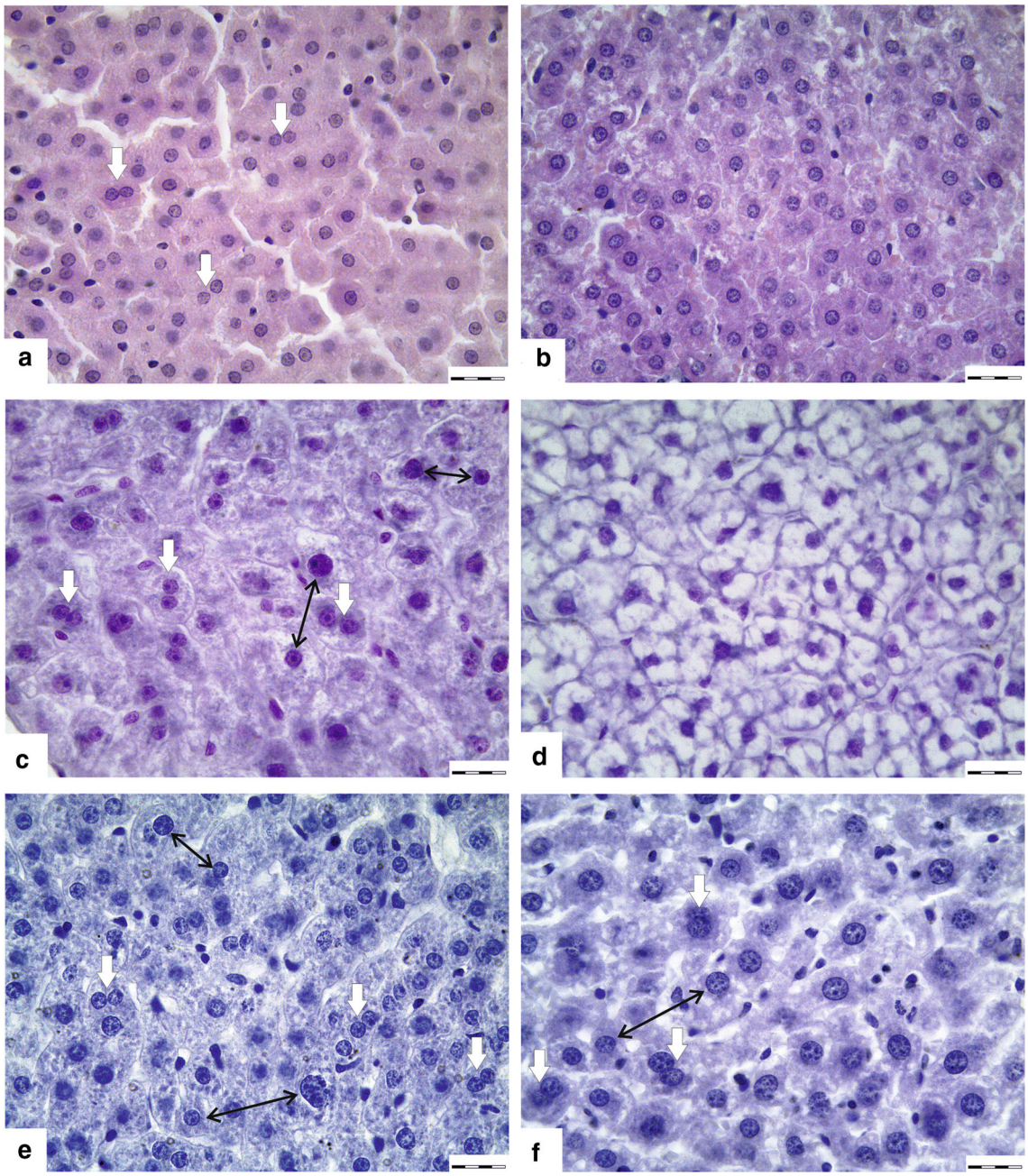

Fig. 4 Histological structure of the liver in **a** *Sorex araneus,* **b** *Talpa europaea,* **c**, **d** *Ellobius talpinus,* **e** *Myodes glareolus,* and **f** *Apodemus uralensis* (hematoxylin–eosin; *scale bar* 20 μm). Examples of pathologically altered hepatocytes: different dystrophic changes (**a, d, f**), binuclear cells (*downwards arrow*), anisokaryosis (*double-headed arrow*)

significance of such cells is still debatable (Tormos et al. 2015), their proportion is one of the parameters most widely used by morphologists. Binuclear hepatocytes in the normal human liver account for up to 20% of the total cell number per unit area, and their proportion in the brown rat (*Rattus norvegicus* Berkenhout, 1769) may reach 30% (Wheatley 1972; Antonova 2009). As for small mammals, the proportion of binuclear cells in the normal liver of the root vole (*Microtus oeconomus* Pallas, 1776) is known to vary from 11.8 to 18.3% (*Atlas*… 1994).

All the parameters included in the analysis characterize the morphofunctional status of hepatocytes and the liver tissue as a whole. However, their use for diagnosing pathological states or evaluating the impact of a certain factor is possible on condition that corresponding reference values are available for the population group of interest

(e.g., age or sex group). The values of these parameters recorded in our study may be regarded as reference values for the five small mammal species included in the analysis. Their comparison with the results published previously shows that the range of variation in the hepatocyte cell area in *M. glareolus* (Table 2) is commensurate with that reported by Bonda-Ostaszewska and Włostowski (2015): 134.3–294.7 vs. 160–280 μm^2, respectively. Unfortunately, no other comparisons could be made because of the absence of relevant published data.

None of the species' ecological characteristics considered in this study (mode of life or feeding habit) can alone be responsible for the differences observed in the parameters of hepatocytes. The historic names of the orders Insectivora and Rodentia (represented by the five species studied) reflect traditional classification criteria used in the systematics of mammals. Together with the structural characters of the limbs, these criteria include specific features of dentition, which are directly related to the type of feeding (Pavlinov 2006). Thus, herbivory or carnivory is not only an ecological characteristic of a given species but also a property of the taxon (order) to which it belongs. This is why interspecific differences in the parameters of hepatocytes cannot be unequivocally explained by taxonomic affiliation or feeding habit.

It is known that metabolic activity is an important factor in the adaptation of animals to their environment (Odokuma and Omokaro 2015), with the liver being directly involved in the corresponding processes. We have assumed that the marked differences in the morphometric parameters of hepatocytes in *T. europaea,* compared to surface-dwelling rodents, reflect specific features of metabolism conditioned by the subterranean mode of life, which implies a stable temperature regime, high digging activity necessary for finding food, and, hence, high energy expenditure that must be replenished within a short time. The diet of *T. europaea* consists of high-calorie and easily digestible animal food consumed in a daily amount equal to 40–50 g (Godfrey and Crowcroft 1960). It has been shown that the stomach of the mole, compared to rodents, has a denser, highly branched vascular system with a high blood flow rate (Galantsev and Rusakov 1967). According to these authors, this is morphological evidence for a high rate of food digestion and metabolism. On the other hand, research evidence is available that the metabolic rate of subterranean mammals (including rodents) is reduced, compared to that in surface-dwelling species (McNab 1966; Goldman et al. 1999).

A popular approach in ecological physiology is to evaluate the relation of cell size and metabolic rate to organ and body weight (Rubner 1883; Darveau et al. 2002; White and Seymour 2003; Savage et al. 2007; Hudson et al. 2013). Interpreting the hepatosomatic index as an indirect ("morphophysiological") indicator of metabolic rate (Fig. 2), we find that this rate is the highest in *S. araneus,* the lowest in *T. europaea,* and intermediate in rodent species. This distribution pattern is in agreement with the accepted concept of correlation between animal size (weight) and metabolic rate. However, the hepatosomatic index and metabolic rate in *E. talpinus* are closer to those in *T. europaea* rather than in *S. araneus.* A probable explanation to the high value of this index in *E. taplinus* is that this subterranean rodent is a winter hibernator and expends especially large amounts of energy during hibernation and upon arousal from torpor (Evdokimov 2002).

Despite the above data, however, the subterranean mode of life cannot be regarded as the sole cause of differences in the parameters of hepatocytes, since such differences have also been revealed between *T. europaea* and *E. talpinus*, another subterranean species. It has become clear that specific features of metabolism in different animal groups should not be analyzed using a mechanistic approach, because these features may be conditioned by a variety of factors (Speakman 1999; Mueller and Diamond 2001; Hoppeler and Weibel 2005).

An argument in favor of the "taxonomic hypothesis" is that the parameters of hepatocytes differ between insectivores and rodents but are similar within the latter group, especially between the taxonomically close species *E. talpinus* and *M. glareolus* (Table 3). However, differences between the hepatocytes of *T. europaea* and *S. araneus* contradict this hypothesis. Unlike rodents, these species do not cluster with each other: *S. araneus* occupies an intermediate position between rodents and *T. europaea* with respect to the dimensional parameters of hepatocytes, but a high proportion of binuclear cells sets it apart from the latter species. This "contradiction" can be explained by a relatively great taxonomic distance between the above two species. According to Faith (1992a, b), this distance is calculated by assigning scores to the lowest ranking taxon that includes both species: score 1 to genus, 2 to family, 3 to order, 4 to subclass, 5 to class, etc. Thus, the distance between *S. araneus* and *T. europaea* receives score 5, compared to score 2 for the distance between *E. talpinus* and *M. glareolus.*

An especially interesting result is that the hepatocytes of *T. europaea* prove to have unique morphometric parameters. However, the small size of these cells, their low variability, high packing density, and almost complete absence of binuclear cells cannot yet be explained by any particular property of this species.

Conclusions

The morphometric parameters of hepatocytes have been studied in five small mammal species with different ecological specificity from two taxonomic groups: a surface-

dwelling insectivore (*S. araneus*), a subterranean insectivore (*T. europaea*), surface-dwelling rodents (*M. glareolus* and *A. uralensis*) and a subterranean rodent (*E. talpinus*). The recorded parameters of hepatocytes can be regarded as reference values for the corresponding species.

The animals in the study samples included individuals with hepatic pathology, but, regardless of its cause, not a single animal was excluded from the analysis. Pathological changes are functional to the same extent as normal changes (e.g., those associated with age-related involution, reproductive cycle, etc.). The results of this study show that the values of parameters characterizing pathological (dystrophic) states of cells and tissues remain within the range characteristic of a given species. Measurements of altered hepatocytes have made it possible to take into account intraspecific variation of test parameters related to pathological changes in cells and tissues.

Our working hypothesis that distinctive features of hepatocytes in these species may be explained by either taxonomic or ecological specificity has not been confirmed or refuted unequivocally. Comparative research involving a greater number of closely related species (at the levels of families and orders) is needed to reveal and evaluate the taxonomic and ecological specificity of hepatocytes in greater detail.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institute of Plant and Animal Ecology, Russian Academy of Sciences (Protocol No. 3 of 18/12/2014).

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