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Comparative study of erythrocyte morphology and size in relation to ecophysiological adaptations in Rodentia species

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ABSTRACT. The size of erythrocytes varies widely across mammals. Previously, deviations from allometric relationships and existence of factors regulating erythrocyte size other than body mass have been shown. The contribution of factors such as habitat and taxonomy are still under discussion. In the present study we examined the morphology of erythrocytes in rodent species and determined their diameter, and for *Ondatra zibethicus* and *Sciurus vulgaris* this was done for the first time. We discovered that erythrocyte diameter of the investigated rodent species ranged from 5.5 to 8.4 μm, varying by a factor of more than 1.5. We analyzed our own data obtained for 10 species as well as data from the literature for 22 species. We found that the size of erythrocytes depended on the phylogenetic position, environmental conditions and body mass.

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Сравнительное исследование морфологии и размеров эритроцитов в экофизиологических адаптациях у видов отряда Rodentia

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РЕЗЮМЕ. Размер эритроцитов широко варьирует среди млекопитающих. Ранее обсуждались некоторые противоречия, касающихся аллометрических зависимостей, а также факторы, регулирующие размер эритроцитов, помимо массы тела. Влияние таких факторов, как среда обитания и таксономическое положение на величину эритроцитов все еще остаются предметом обсуждений. В настоящем исследовании мы изучили морфологию эритроцитов грызунов и определили их диаметр, причем для *Ondatra zibethicus* и *Sciurus vulgaris* это было сделано впервые. Диаметр эритроцитов у исследованных видов грызунов варьирует от 5.5 до 8.4 мкм, что составляет более чем 1.5 раза. Многофакторный

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анализ собственных данных (10 видов) и сведений из литературы (22 вида) о диаметре эритроцитов грызунов показал, что величина клеток зависит от размера животного, образа жизни вида и определяется его таксономическим положением.

КЛЮЧЕВЫЕ СЛОВА: эритроциты, адаптации, грызуны, экологическая физиология, морфометрия.

Introduction

Living in a wide variety of environments (at different depths and elevations, in warm or cold climatic conditions), an organism adapts to the habitat by adjusting its physiological traits, including the oxygen transport system. Mammalian erythrocytes, or red blood cells (RBC), are the most common type of blood cells, which have lost the nucleus and mitochondria in the course of evolution. Being essential for tissue oxygenation, erythrocytes take part in its regulation by changing their count, size, morphology, hematocrit, hemoglobin (Hb) concentration, and oxygen affinity.

The erythrocyte size is a stable species-specific characteristic which was formed evolutionarily (Ruiz *et al*., 2004). Across mammalian species, erythrocytes vary in size and shape, and these parameters can define the architecture of the circulatory system, namely capillary diameter (Starostová *et al.*, 2013). In general, mammalian erythrocytes exhibit morphological homogeneity. Erythrocytes in mammals are typically shaped as radially symmetrical, bulging biconcave disks, deformable, and high in surface to volume ratio, except in Camelidae, who have oval / ellipsoidal cells. This shape allows camelids to adapt during drastic dehydration. The bilaminar oval also helps in hypoxic high altitude habitats (Long, 2007). It has been noted that mammalian species vary in erythrocyte thickness; thus, humans and the European rabbit (*Oryctolagus cuniculus* L., 1758) have thicker erythrocytes while the common vole (*Microtus arvalis* Pallas, 1778) has much thinner cells (Kostelecka-Myrcha, 1966; Campbell, 2004; Turgeon, 2004).

According to previously presented data, mammalian erythrocyte diameter may vary more than five-fold, ranging from 2.1 μm in the Java mouse-deer (*Tragulus javanicus* Osbeck, 1765) to 10.8 μm in the Northern elephant seal (*Mirounga angustirostris* Gill, 1866) (Gregory, 2005). Despite the large amount of data on RBC size in mammalian species, the reasons for such pronounced variation are still being discussed (Promislow, 1991; Benga *et al*, 1992; Gregory, 2000; Unruh, 2018).

Body size-related differences in erythrocyte size in mammals have been widely examined (Kostelecka-Myrcha, 1973, 2002; Promislow, 1991). Common patterns, such as dependence of erythrocyte size on body mass, have been detected for 54 species of mammals belonging to 10 orders and having different weight (Kostelecka-Myrcha, 2002). The information concerning the influence of environmental, behavioral and life history factors on the erythrocyte size seems ambiguous.

McNab (2008) established a relationship between the basal metabolic rate in mammalian species and such factor as characteristics of the diet and habitat. In the study of Marsupial species no significant differences in erythrocyte sizes were detected despite marked differences in diet and habitat (Benga *et al*., 1992). Unruh (2018) speculated on the role of the diet type and the related water intake on erythrocyte diameter. Similar values of the size of erythrocytes were found in hares (Lagomorpha) and Carnivores, and, on the contrary, significant differences were demonstrated between members of Artiodactyla and Primates, many of which are herbivores (Promislow, 1991).

It has been shown that rodents' hematological characteristics may be influenced by ambient temperature, burrowing, elevation or diving hypoxia (Wei & Wei, 2001; Frase, 2002; Thomas & Ono, 2015; Bottaeva *et al*., 2019). Schmidt-Nielsen and Knut (1984) explained the relatively high values of RBC volume in pinnipeds (Pinnipedia) by adaptation to hypoxia during prolonged immersion. At the same time, the volume of erythrocytes in dolphins (Delphinidae) does not significantly differ from that in humans, dogs (*Canis familiaris* L., 1758) or jackals (*C. aureus* L., 1758) (Galantsev, 1977). Mammals adapted to hypoxia at high altitudes or through burrowing differ from diving mammals in blood respiratory characteristics (higher HbO_2 affinity) (Bullard *et al*., 1966). Among burrowers, there is neither a dramatic increase in mean cell volume (MCV) such as occurs in diving mammals, nor a decrease in MCV as exists in certain high-altitude natives (Lechner, 1976).

According to the latest reports, the erythrocyte size is phenotypically plastic and significantly affected by temperature and season (Tarakhtii & Davydova, 2007; Goodman & Heah, 2010). On the other hand, some authors advocate the idea of a genetically predetermined erythrocyte size annual variation, occurring independently of acclimatization, and appearing in response to photoperiodic stimuli (Ruiz *et al*., 2004).

Rodents are the largest group of known mammalian species; they have a wide geographic distribution from the Arctic to deserts. There are many morphological and ecological differences among them, including variations in size, weight and habitat. Studies of rodent erythrocytes are of wide interest in relation to the influence of various environmental factors on the morphology and profile of blood. However, the currently available information about the hematological parameters and the morphological features of erythrocytes in some species of rodents, for example, red squirrel, chinchilla, muskrat, is insufficient and contradictory. Moreover, no comparative analysis of erythrocyte sizes among rodent species has been carried out so far.

We hypothesize that differences in erythrocyte size across rodent species result from a balanced interplay of various environmental and physiological factors. The aims of this study were to: 1) study erythrocyte morphology by light microscopy and determine erythrocyte diameter in 10 rodent species; 2) determine the contribution of environmental, phylogenetic and physiological factors to erythrocyte size variation using all the available information on erythrocyte diameter in rodents (22 species).

Material and methods

All the experiments were conducted according to EU guidelines on the use of animals for biochemical research (86/609/EU) with special permission from the Local Ethics Committee of the Institute of Biology. The research was performed using the equipment of the Core Facility of the Karelian Research Centre of the Russian Academy of Sciences.

Studied animals

We investigated wild (North American beaver *Castor canadensis* Kuhl, 1820, muskrat *Ondatra zibethicus* L., 1766, red squirrel *Sciurus vulgaris* L., 1758, European water vole *Arvicola amphibius* L., 1758, bank vole *Myodes glareolus* Schreber, 1780), farmed (nutria *Myocastor coypus* Molina, 1782 and chinchilla *Chinchilla lanigera* Bennett, 1829), and laboratory (rat *Rattus norvegicus* Berkenhout, 1769, golden hamster *Mesocricetus auratus* Waterhouse, 1839, and mouse *Mus musculus* L., 1758) animals. All the studied animals were adults.

Blood samples were obtained from beavers, muskrats and squirrels taken during the hunting seasons of 2011 and 2015 with permits from the Game Management Directorate of the Republic of Karelia, Russia (permit numbers: 000002-2011, 000001-2011 and 00008-2015). European water voles and bank voles were captured with pitfall traps, after which the animals were sacrificed by displacing vertebrae. The voles captured for this study are not included in the list of licensed and protected species. All the wild animals used in the study inhabit Northern European Russia (Karelia). Blood samples of wild rodents were obtained in August–September. Blood was collected by cardiac puncture at the time the animals were killed (*post mortem*).

The nutrias and chinchillas were of a standard breed and kept at a fur farm in Poland (Permission No. 32/2010, First Local Ethical Committee on Animal Testing at the Jagiellonian University in Krakow). Nutrias were kept in cages with water reservoirs, under conventional feeding consisting of a clover-grass mixture with grain supplement. Chinchillas were fed with pelleted food and provided with clean drinking water *ad libitum*. Nutrias and chinchillas were immobilized; blood samples were taken from the coccygeal vein and claw vessel, respectively. Blood sampling was performed in summer season. Adult and healthy Wistar rats, C57/BL6 mice and golden hamsters were bred in a laboratory under constant temperature and light conditions. The animals were kept in standard cages with food and water provided *ad libitum*. Blood was collected from the animals following decapitation.

Seasonal changes in hematological parameters, including erythrocyte size, should be expected when comparing samples taken in seasons with different temperature and light conditions and availability of food resources. Small and short-lived erythrocytes appear under the trying conditions of the autumn-winter period (Tarakhtii & Davydova, 2007). The fact that blood sampling in wild animals was done when the climatic conditions were relativity mild and constant, while the laboratory and farmed animals were bred under standard conditions is sufficient reason to ignore the seasonal factor.

We also analyzed data on 22 Rodentia species (Cricetidae, Dipodidae, Muridae, Sciuridae, Erethizontidae, Hystricidae, Chinchillildae, Myocastoridae, Castoridae and Caviidae) obtained from various sources (Tab. 1).

Body mass in laboratory and farmed species was measured by authors themselves; data for wild species were obtained from Drożdż *et al*. (1971), Segal (1978), MacArthur (1984), and Wauters (2007). Body mass data for species additionally included in the analysis were obtained from the following sources: Foreman (1956), Kostelecka-Myrcha (1966), Moore (1966), Kostelecka-Myrcha (1967), Bolls & Perfect (1972), Bozinovic & Rosenmann (1988), Sabanova (2010), Lantová *et al*. (2011), Bottaeva (2017) and Bottaeva *et al*. (2019).

Light microscopy and erythrocyte morphometry

Blood smears were made immediately after blood collection. Thin blood smears were allowed to air dry and then stained with May-Grünwald and Romanowski stains (MiniMed, Russia). Each smear was examined for cell morphology with light microscope (Axioscop 40, Zeiss, Germany) under oil immersion with 100× objective lens. We considered the presence/absence and frequency of occurrence of polychromatophilic erythrocytes, normoblasts and Howell-Jolly bodies (H-J bodies). Photomicrographs were obtained, transferred to computer, and captured with the help of image software VideoTest 4.0 (Video Test Inc., Russia). To determine the diameter of erythrocytes, we analyzed 10–20 fields of view (more than 50 cells per field of view) from each blood smear. Only clear fields of view with adequate morphology of erythrocytes were examined. The diameter of erythrocytes was measured automatically by the NCR (nuclear-cell ratio) method using image software.

Statistical analysis

Mean erythrocyte diameters of 10 rodent species were calculated. These data were pooled together with data for 22 species from the literature in Table 1.

Based on McNab's classification (McNab, 2008), the 32 species were analyzed according to their habitat affiliations (desert, freshwater, mesic and xeric) with some modifications.

Table 1. Erythrocyte diameters of rodent species.

Family	Genus	Species	Common name	Body mass, g	Habitat	*n*	Erythrocyte diameter, μm	Source
Cricetidae	*Arvicola*	*Arvicola amphibius*	European water vole	138.5	FW*	4	6.5	our data
					FW*	nd	6.7	Gulliver, 1875
	Ondatra	*Ondatra zibethicus*	Muskrat	902.7	FW*	10	7.3	our data
	Chionomys	*Chionomys gud*	Caucasian snow vole	44.8	M	35	5.8	Bottaeva *et al*., 2019
	Myodes	*Myodes glareolus*	Bank vole	16.7	M	1	5.5	our data
					M	32	5.1	Kostelecka-Myrcha, 1967
	Lagurus	*Lagurus lagurus*	Steppe lemming	17.1	M	42	5.1	Kostelecka-Myrcha, 1966
	Microtus	*Microtus subterraneus*	European pine vole	18.2	M	30	4.9	Kostelecka-Myrcha, 1966
		Microtus arvalis	Common vole	16.3	M	27	5.1	Kostelecka-Myrcha, 1966
		Agricola agrestis	Field vole	30.9	M	30	5.0	Kostelecka-Myrcha, 1966
		Alexandromys oeconomus	Tundra vole	50.5	M	32	5.1	Wołk, 1970
		Microtus daghestanicus	Daghestan pine vole	24.7	M	33	5.1	Bottaeva, 2017
	Peromyscus	*Peromyscus leucopus*	White-footed mouse	19.3	M	13	6.1	Foreman, 1956
	Sigmodon	*Sigmodon hispidus*	Hispid cotton rat	98.6	M/X	8	7.6	Foreman, 1956
	Cricetulus	*Cricetulus griseus*	Chinese hamster	37.5	X	40	6.1	Moore, 1966
	Mesocricetus	*Mesocricetus auratus*	Golden hamster	125.0	X	8	6.6	our data
Dipodidae	*Sicista*	*Sicista betulina*	Northern birch mouse	9.2	M	45	5.6	Wołk, 1985
Muridae	*Rattus*	*Rattus norvegicus*	Laboratory rat	233.3	M	17	6.5	our data
	Sylvaemus	*Sylvaemus flavicollis*	Yellow-necked mouse	8.9	M	nd	5.9	Wołk, 1990
		Sylvaemus sylvaticus	Wood mouse	15.4	M	31	4.6	Sabanova, 2010
	Micromys	*Micromys minutus*	Harvest mouse	5.7	M	28	5.5	Wołk, 1985
	Mus	*Mus musculus*	Laboratory mouse	24.0	M	7	6.2	our data
Sciuridae	*Sciurus*	*Sciurus vulgaris*	Red squirrel	300.2	M	5	6.5	our data
		Sciurus carolinensis	Eastern gray squirrel	500	M	12	6.8	Guthrie, 1966
	Urocitellus	*Urocitellus parryii*	Arctic ground squirrel	800	M	19	7.1	Musacchia, 1955
	Spermophilus	*Urocitellus undulatus*	Long-tailed ground squirrel	384.5	M	nd	6.8	Kalabukhova, 2005
	Tamiasciurus	*Tamiasciurus hudsonicus*	American red squirrel	230	M	5	6.7	Musacchia, 1955

Table 1 (continued)

Family	Genus	Species	Common name	Body mass, g	Habitat	*n*	Erythrocyte diameter, μm	Source
Erethizontidae	*Erethizon*	*Erethizon dorsatum*	North American porcupine	8650	M	5	7.8	Musacchia, 1955
Hystricidae	*Hystrix*	*Hystrix cristata*	Crested porcupine	10000	M	nd	7.5	Hawkey, 1975
Chinchillildae	*Chinchilla*	*Chinchilla lanigera*	Chinchilla	633.6	D	12	6.7	our data
Myocastoridae	*Myocastor*	*Myocastor coypus*	Nutria	5000.0	FW	12	7.9	our data
Castoridae	*Castor*	*Castor canadensis*	North American beaver	21000	FW	3	8.4	our data
					FW	22	7.6	Kitts, 1958
Caviidae	*Hydrochoerus*	*Hydrochoerus hydrochaeris*	Capybara	48500	FW	nd	8.8	Hawkey & Dennet, 1989
	Cavia	*Cavia porcellus*	Guinea pig	950	X	nd	7.5	Lewis, 1992

Notes: Body mass and erythrocyte diameter are presented as mean, *n* — number of animals; nd — no data; habitat: D — desert, FW — freshwater, M — mesic and X — xeric.
*Considering that the European water vole and muskrat are adapted to diving (Sokolov *et al*., 1993; Panteleyev, 2001), contrary to MacNab (2008) we classified these species as aquatic, having "freshwater" as habitat.

Table 2. Results of type III MANOVA testing for erythrocyte diameters of species of the order Rodentia.

Factor	η^2, %	d.f.	Mean square	F-ratio	*P* value
Covariates					
Body mass	24.77	1	18.8681	166.06	0.0000
Habitat	4.00	1	3.04588	26.81	0.0000
Main effects:					
Phylogenetic position	11.15	9	0.943792	8.31	0.0000

Notes: η^2 — factorial influence, d.f. — number of degrees of freedom.

Table 3. Results of type III MANOVA testing for erythrocyte diameters of species of the family Cricetidae (Rodentia).

Factor	η^2, %	d.f.	Mean square	F-ratio	*P* value
Covariates					
Body mass	6.34	1	1.72715	15.96	**0.0003**
Phylogenetic position	–	1	0.0112583	0.10	0.7491
Main effects: Habitat	7.08	2	0.965123	8.92	**0.0008**

Notes: η^2 — factorial influence, d.f. — number of degrees of freedom.

Body mass of Rodentia species ranged from 5.7 to 48500 g. All the species were clustered into 17 groups according to their average body mass. The amplitude of body mass variation among species within a group did not exceed 26%.

To assess the effect of the phylogenetic factor (family/genus) we conducted a type III MANOVA (multiple analysis of variance) without intercept testing of Rodentia erythrocyte size using erythrocyte diameter as the dependent variable, phylogenetic position as the main factor, habitat and body mass as covariates due to correlation with the main factor (Tab. 2). Type III MANOVA testing of Cricetidae (Rodentia) erythrocyte size was performed with erythrocyte diameter as the dependent variable, habitat as the main factor, phylogenetic position and body mass as covariates due to correlation with the main factor (Tab. 3).

The statistical analysis was performed using MS Excel software (Microsoft Corp., Inc., USA) and Stat Graphics Version 5.0 (Statistical Graphics Corp., USA).

Results

Erythrocyte morphology

The erythrocyte morphology of the studied species is shown in Figure 1. In all 10 species, erythrocytes were usually round, anucleated, red cells. The RBC size

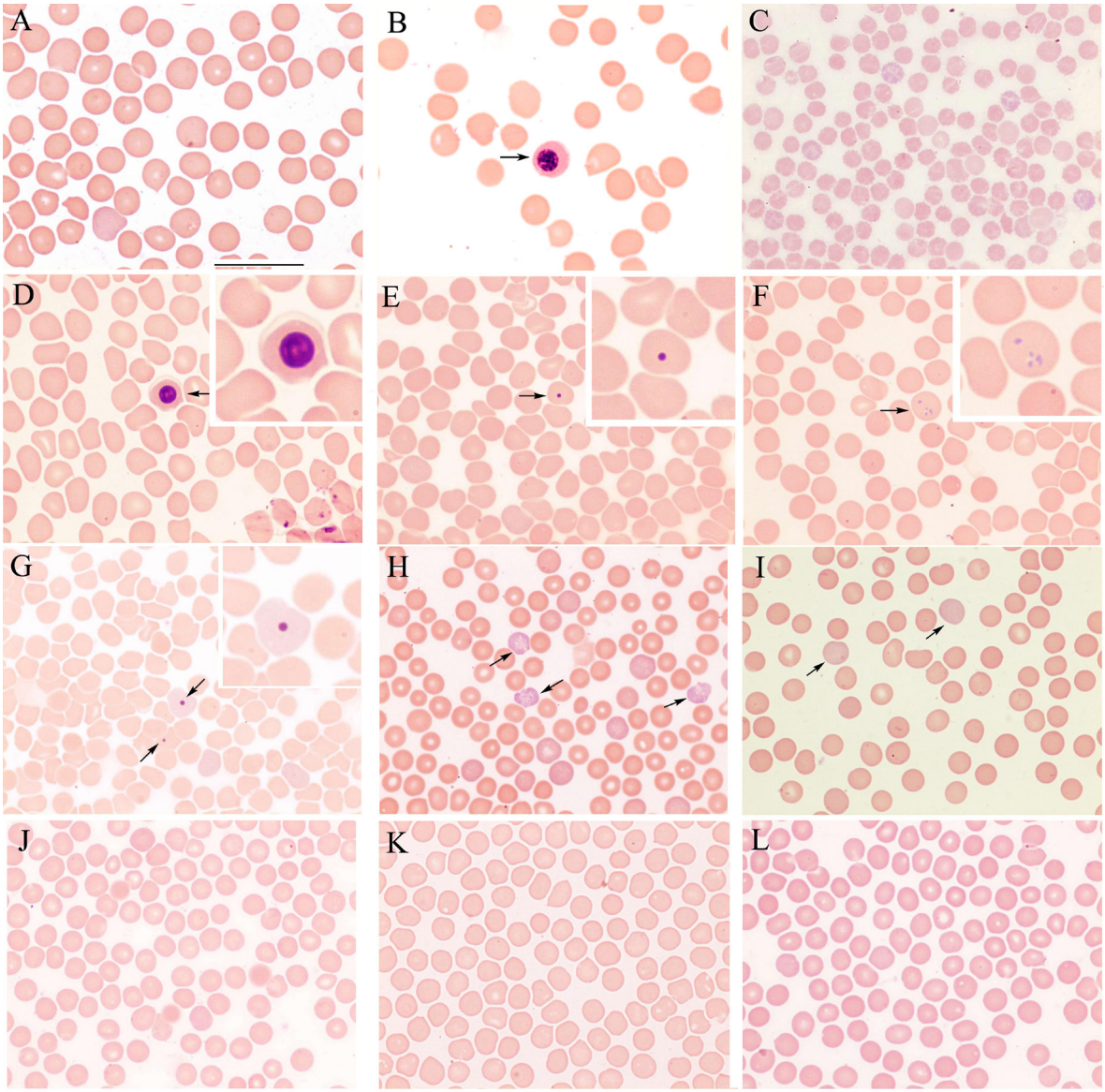

Fig. 1. Erythrocytes in rodents' blood smears. A, B — North American beaver, C — bank vole; D — nutria; E, F — muskrat, G — mouse; H — European water vole, I — rat; J — red squirrel, K — chinchilla, L — hamster. Note: arrow — normoblast in beaver (B) and nutria (D), Howell-Jolly body in muskrat (E) and mouse (G), basophilic stippling in muskrat (F). Bar = 25µm. Stained with May-Grünwald, Romanowsky.

variation and specific features of erythrocyte morphology were seen in peripheral blood smears. The largest erythrocytes were found in the North American beaver (Figs 1A–B). Nutria also had quite large cells. The smallest size of erythrocytes was detected for the bank vole (Fig. 1C). Other species had similar erythrocyte sizes (Figs 1E–K).

Normoblasts or nucleated erythroid cells (Fig. 1D) were occasionally present in the circulating blood of semi-aquatic mammals — beavers, nutria, muskrat, and European water vole. A majority of erythrocytes in diving animals had no central pallor.

H-J bodies are homogeneous, dark purple spherical structures, which are nuclear remnants (Figs 1E–F). H-J bodies were present in the peripheral blood of nutria, muskrat, bank vole, and mouse. H-J bodies in mice were notably larger than in other species (Fig. 1F).

Polychromatophils are young, anucleated erythrocytes. These cells appear gray or bluish-red with May-Grünwald and Romanowski stains. These cells were present in most of the studied species — muskrat, rat, mouse, red squirrel, bank vole, and European water vole (Figs 1G–H). However, polychromatophilic erythrocytes were more common in muskrat, rat, mouse, bank vole, and European water vole than in red squirrel, and were absent in chinchilla and hamster (Figs 1J–K).

Basophilic stippling in erythrocytes appears as multiple fine blue dots in the cytoplasm of erythrocytes. This morphologic feature was sometimes seen in muskrat (Fig. 1L) and bank vole.

Erythrocyte morphometry

The results of the morphometric analysis of erythrocytes in rodent species are illustrated in Table 1. It has been shown through a comparative analysis of our own and previously known data that the largest forms of red blood cells are characteristic of divers with body mass over 4 kg — beaver, nutria, and capybara (*Hydrochoerus hydrochaeris* L., 1766). The smallest surface area and diameter were revealed among *Sylvaemus*, *Microtus,* and *Myodes*.

MANOVA revealed a significant influence of habitat, body mass and phylogeny factors on erythrocyte diameter across the order Rodentia (Tab. 2). The influence of body mass and environment on cell size was confirmed also at a lower taxonomic level (family), although the effect was less pronounced (Tab. 3).

Discussion

Our results showed differences in erythrocyte size and morphology to exist among rodent species. The largest erythrocyte size was observed in such semi-aquatic animals as beavers and nutria. Previous studies demonstrated that the volume of erythrocytes in the Eurasian and North American beavers was almost double that of non-aquatic rodents (Patenaude & Genest, 1977; Girling *et al.*, 2015). Mean erythrocyte diameter in the nutria in the present study (7.84 μm) was similar to the finding of Jelínek (1984) (7.4 μm). Diving marine and semi-aquatic mammals need greater amounts of oxygen. Canfield (1998) reported that erythrocytes in diving mammals may have no central pallor because they are thicker cells, as adaptation for slow release of oxygen. Large erythrocyte size is a useful adaptation allowing a higher concentration of Hb per cell and an increase in blood oxygen-carrying capacity. It has been previously demonstrated that various marine mammals have large erythrocytes — manatee (*Trichechus manatus* L., 1758), dugong (*Dugong dugon* Müller, 1776), beluga whale (*Delphinapterus leucas* Pallas, 1774) (Harvey *et al*., 2009; Reiderson, 2010; Woolford *et al*., 2015). Their erythrocytes are comparable with those in elephants (*Loxodonta africana* Blumenbach, 1797), who have the largest erythrocytes among terrestrial mammals (Harr *et al*., 2010). The meaning of erythrocyte size for diving animals is indicated by the fact that MCV of the Harbor seal (*Phoca vitulina* L., 1758) is positively correlated with the maximum dive duration (Thomas & Ono, 2015).

We found that semi-aquatic rodents were characterized by high occurrence of nucleated erythrocytes. Intensive erythropoiesis might explain the common occurrence of nucleated erythrocytes circulating in the peripheral blood (Harvey *et al*., 2009). We suppose that the presence of nucleated erythrocytes on blood smears in this animal group is a result of active erythropoiesis related to the demand for oxygen reserves in semi-aquatic animals.

This study demonstrated that the European water vole, a small freshwater diver, had the smallest erythrocytes among semi-aquatic rodents. However, the erythrocyte size of the European water vole (body mass ~ 80 g) was comparable to that of non-diving terrestrial mammals — squirrel, chinchilla, and rat (body mass > 300 g). The quite large size of erythrocytes in the European water vole can be considered as one of adaptations to semi-aquatic lifestyle. Another example supporting the role of habitat characteristics on RBC size is the fact that semi-aquatic muskrats have larger erythrocytes (78.43 fl) than terrestrial prairie dogs (*Cynomys ludovicianus* Ord, 1758) (62.00 fl) with similar body mass (Ahlers *et al*., 2011; Donnelly *et al*., 2015). It is obvious that the evolution of the respiratory function of blood in diving species did not follow an increase in the concentration of erythrocytes (Galantsev, 1977). This pattern is more typical of native high-elevation species and provides an enlarged diffusion area per unit volume of blood (Puchalski & Heldmair, 1986). Furthermore, the erythrocyte size is one of the main determinants for the enhancement of platelet adherence (Aarts *et al*., 1983). This fact may be interesting in connection with the increased requirements of diving species for the coagulation system.

Previous studies have shown deviations from allometric relationships and existence of regulating factors other than body mass (Promislow, 1991). The mean diameter of bank vole erythrocytes was the smallest and differed from that of the mouse, whose body mass is similar (~20 g). The erythrocyte size in hamsters is similar to that of rats, chinchillas and squirrels, whose body mass is much greater. Another deviation from the allometric

model is hispid cotton rats (*Sigmodon hispidus* Say et Ord, 1825). Their erythrocyte size (7.6 μm) is larger than would be predicted on the basis of weight. The higher MCV, Hb, and mean corpuscular hemoglobin values in hispid cotton rats compared to closely related species were noted in the study of Katahira & Ohwada (1993). The authors point out that the physiological reason for this difference is not completely understood.

The MCV of erythrocytes in the capybara, the largest rodent, is 132 μm^3, i.e. 32% more than in beavers (Chiacchio *et al*., 2014; Girling *et al*., 2015). The erythrocyte diameter of the crested porcupine (*Hystrix cristata* L., 1758), third largest species among rodents after capybaras and beavers, is 7.5 μm (Hawkey, 1975). Comparing this mean with our results, we see that porcupine's erythrocyte diameter is larger than in most of the rodents, but smaller than in the nutria. Promislow (1991) stated that "body mass is a highly significant predictor of blood physiology at lower taxonomic levels". This assertion can be corroborated by comparing erythrocyte sizes in species from different orders. The erythrocyte diameter of heavy carnivorous species — black bear (*Ursus arctos* L., 1758) and wolf (*Canis lupus* L., 1758) is smaller (6.8 μm) (Gregory, 2000) than in some rodents with smaller body mass. Our analysis has demonstrated that at intermediate taxonomic levels (families within orders) body mass explained 24.77% of erythrocyte size variation. The influence of the body mass factor at a lower taxonomic level (species within family) decreased since the range of values was not so great.

Testing by MANOVA reveals that erythrocyte diameter depends on the family affiliation. However, no size differences were found between species of different genera within a family. A possible explanation is that closely related taxa formed from a common ancestor often have a similar ecology.

Our results revealed that terrestrial rodents' erythrocytes averaged 5.5 to 6.7 μm in diameter, and are consistent with earlier reports (Wyckoff & Frase, 1990; Frase, 2002; Heatley & Harris, 2009). Free-living Murinae and Cricetidae are typically burrowing mammals adapting to hypoxia due to fossorial habit. A common trait for such animals (e.g. black-tailed prairie dog, Middle East blind mole-rat (*Spalax ehrenbergi* Nehring, 1898), Botta's pocket gopher (*Thomomys bottae* Eydoux et Gervais, 1836)) is high erythrocyte counts (MacArthur, 1984). It has been shown that burrowing rodents are characterized by high affinity for O_2 and insensitivity to CO_2 (Hall, 1966; Withers, 1978).

Previous studies indicated that the number of erythrocytes with H-J bodies varied in mammalian species under natural conditions (Zúñiga *et al*., 1996). We noted that mice had a high occurrence of H-J bodies. Similarly, mice are characterized by the highest number of micronuclei in peripheral blood erythrocytes among rodents (Ramírez-Muñoz *et al*., 1999). In clinical and veterinary practice, counting of these fragments of erythrocyte nuclei is used for evaluating splenic function (Corazza *et al*., 1990). The number of erythrocytes containing H-J bodies tends to increase in parallel with nucleated erythrocytes during regenerative anemia. Their occurrence at a high altitude (1800 m) was higher than at medium altitudes (700 m) (12.96% vs. 3.12%) (Yemkuzheva, 2013).

A physiological response to low O_2 availability in high-altitude mammals is to increase the Hb concentration through higher RBC counts (Crait *et al*., 2012). It has been shown that erythrocyte diameter in the house mouse and the Ural field mouse (*Sylvaemus uralensis* Pallas, 1811) was smaller at a high altitude (1000 m) than at medium altitudes (500 and 700 m) (Yemkuzheva, 2013). The chinchilla, who naturally resides at high altitudes, demonstrates smaller erythrocytes than would be predicted on the basis of weight. Smaller but more numerous red blood cells, high Hb values (Ederstrom *et al.*, 1971), and high hemoglobin-oxygen affinities (Ostojic *et al*., 2002) in chinchillas accounted for their greater tolerance to hypoxia. Chinchillas have apparently retained their adaptations to altitude despite almost 100 years of domestication history. Another species native to high altitudes — the guinea pig, features larger erythrocytes and a lower RBC count than chinchillas (Siegel & Walton, 2020). Both laboratory-type and wild-type guinea pigs, as well as chinchillas, have high hemoglobin-oxygen affinities. The cell size differences between chinchilla and guinea pig can be attributed to chinchilla's adaptation to xeric habitats. The increased blood plasma osmolality noted in some desert animals can be the reason that erythrocytes would lose water to the plasma and shrink (Urison & Buffenstein, 1993).

We suppose that differences in degree of the influence body mass on the erythrocyte size depend on other endogenous and exogenous factors (e.g., ecology, blood chemistry, anatomic and physiological adaptations).

Conclusions

In summary, we comparatively analyzed the size of erythrocytes across rodent species. The erythrocyte morphology of two of the studied species — muskrat and red squirrel, is described for the first time. There was a general pattern of erythrocyte size increase with increasing body mass of species. MANOVA testing confirmed the existence of this common for rodent species pattern. Yet, the degree of body mass effect on erythrocyte size differs among taxonomic levels. We suppose there are some deviations from the allometric pattern. Other than body mass influence, the erythrocyte size can be analyzed in connection with ecological factors (habitat, lifestyle, locomotion activity etc.). According to MANOVA results, the largest size of erythrocytes in massive diving animals such as the capybara, beaver, and nutria is a result of the combined effect of semi-aquatic lifestyle and body mass. The relatively large RBCs of the European water vole can be considered as an adaptation to diving. We consider the presence of nucleated erythrocytes in blood of semi-aquatic rodents to be a result of active erythropoiesis related to high requirements for oxygen reserves. Thus, the lifestyle of rodent species affects the morphology and size of red blood cells. Further comparative studies with more species

will expand our knowledge of the role of environmental factors in the respiratory function of blood. The results obtained can also be useful in environmental monitoring when studying the influence of anthropogenic factors on the parameters of the blood system and the state of the organism as a whole in species of the Rodentia order.

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