

SEXUAL DIMORPHISM IN MORPHOMETRIC AND HEMATOLOGICAL INDICES OF SYMPATRIC *RATTUS NORVEGICUS* AND *RATTUS RATTUS* POPULATIONS IN AGROECOLOGICAL ZONES OF CENTRAL PUNJAB, PAKISTAN

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Abstract

Morphometric and hematological characteristics are important indicators of physiological condition, biological variation, and adaptation in wild rodents. However, these traits may vary according to species, sex, and environmental conditions. The present study evaluated sex-associated morphometric and hematological variation in sympatric populations of brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) from agroecological areas of Sargodha, Punjab, Pakistan. A total of 65 wild rodents were collected between April and July 2024, comprising 35 brown rats and 30 black rats. Species identification was performed using a dichotomous identification key. Morphometric characteristics, including head-and-body length, head length, tail length, body weight, hind-foot length, ear length, head width, body width, body girth, eye diameter, and front-foot length, were recorded using standardized measurements. Blood samples were collected by cardiac puncture into EDTA-containing tubes, and complete blood count parameters were assessed. Differences in hematological parameters were evaluated using a two-sample t-test. Marked interspecific and sex-associated variation was observed in body size. Male brown rats had the highest mean body weight (125.9 g), followed by female brown rats (106.2 g), whereas female black rats had the lowest mean body weight (75.30 g). Brown rats generally exhibited greater body dimensions than black rats. Hematological profiles also differed between species, particularly in leukocyte distribution. Brown rats showed higher mean WBC counts ($8.60 \times 10^3/\mu\text{L}$) than black rats ($6.77 \times 10^3/\mu\text{L}$), while black rats had a higher mean lymphocyte proportion (61.0%) than brown rats (54.4%). Monocytes were markedly higher in brown rats (34.5%) than black rats (12.9%; $p < 0.001$). In black rats, males had significantly higher WBC counts, monocyte percentages, and eosinophil percentages than females,

whereas females showed higher MCV. In brown rats, MCH differed significantly between sexes ($p = 0.02$), while most erythrocytic and platelet parameters showed no significant sex-related differences. The findings demonstrate substantial morphometric and hematological variation between sympatric brown and black rats and indicate that sex contributes to variation in selected blood parameters. The observed differences provide useful baseline information for understanding biological variation and physiological adaptation of free-ranging rodent populations in agroecological environments of Central Punjab.

Introduction

Rodents are one of the most widespread and ecologically versatile classes of mammals, which are important in terrestrial ecosystems as consumers, prey, seed dispersers, and food-web components. They are also valuable in a veterinary, agricultural, and public-health context, given their proximity to human settlements and agricultural areas. The two rat species that are most successful are commensal rodents, such as the brown rat, *Rattus norvegicus*, and the black rat, *Rattus rattus*, which have been found to be extremely tolerant of their environment and have been able to use a wide range of food and shelter sources. Both species often overlap in range and can be found on agricultural land where environmental factors, food, and anthropogenic activity can affect their morphology and physiology (Islam et al., 2021; Buchholz et al., 2019). These rodents are frequently found in agricultural and peri-domestic habitats in Pakistan and therefore biological parameters are of relevance for understanding its adaptation to local agroecological conditions.

Sexual dimorphism, which is the occurrence of consistent differences between the morphology or physiology of the sexes of the same species is common among mammals. Variations in body size, body weight, head size, tail size, and other morphometric traits can result from various sources of variation in growth, reproductive investment, energy consumption and endocrine regulation. However, there may be some physiological and metabolic differences between men and women which can cause somatic differences in body composition and characteristics (Maggi & Della Torre, 2018; Mauvais-Jarvis, 2024). Morphometric assessment can thus be helpful in discerning

sex-associated patterns and examining the body condition of sympatric rodent populations. It is also worth to note that the morphometric variations can be measured between *R. norvegicus* and *R. rattus*, and between them and other rodent species, thus suggesting that morphometric traits can be useful for comparative studies among wild rodent populations (Rizzoli et al., 2011).

The blood parameters are another sensitive and useful measure of physiology in mammals. Complete blood count parameters such as red blood cell (RBC) count, hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, differential leukocyte counts and platelet count can be indicators of oxygen transport, immune activity, stress responses, nutritional status and general health. There are a number of factors that can affect hematological values, such as species, sex, age, environmental factors, nutrition, disease, and physiological stress (McClure, 1999; Pilny, 2008; Siegel & Walton, 2020). Thus, sex differences in hematology may offer clues to sex differences in physiology under similar environmental conditions.

Leukocyte differentials could also be sex-dependent indicators of the differences in immune regulation, stress response and physiological state (Davis et al., 2008; Rosales, 2020; Li et al., 2019). The primary importance is attributed to leukocytes, as their distribution and number might change in the presence of immune activation, inflammation, environmental stress, and processes of disease (Chmielewski & Strzelec, 2018). Platelet count is also an important part of haematology assessment as platelets respond to physiological challenges and tissue injury, in addition to

contributing to hemostasis (Van der Meijden & Heemskerk, 2019).

Morphological and hematological traits are also subject to further modulation by environmental factors, which are potentially different for females and males. Wild rodents are subject to changing conditions with respect to temperature, food resources, water, habitat quality, pathogen exposure, and other ecological pressures that may impact their physiological status. Spatial differences in environmental exposures could confer interpopulation variation in immune defense and physiological traits in wildlife (Becker et al., 2020). Therefore, the present research was aimed to compare the sexual dimorphism of morphometric and hematological indices in *Rattus norvegicus* and *Rattus rattus* in agroecology zones of Central Punjab, Pakistan in sympatric condition. The aim of the study is to describe sex-associated differences in body size and blood parameters of these two sympatric species, and to serve as reference data for physiological adaptation and biological variation of naturally occurring rodent populations.

Materials and Methods

Material

Materials used at various stages of this study include: wooden box, surgical gloves, EDTA tubes, dissection box, Vernier caliper, digital weight balance, insulin syringes, ethanol, dropper, beakers, cotton, and tap water.

Sample collection

65 samples of rodents were collected from April to July, 2024. Among them 35 were brown rats and 30 were black rats. The rats were collected from different areas of Sargodha, Punjab, Pakistan. A small amount of bait, such as chunky peanut butter or mutton fat was placed on the snap trap in areas where mice and rats had been observed. In basements, outbuildings and similar places where there had been no regular activity by humans, their nesting materials, urine and feces, food eaten or gnaw marks were noted. Once they were captured they were taken to Zoology laboratory, The University of Lahore (Sargodha Campus) and maintained by giving enough food and drinking water.

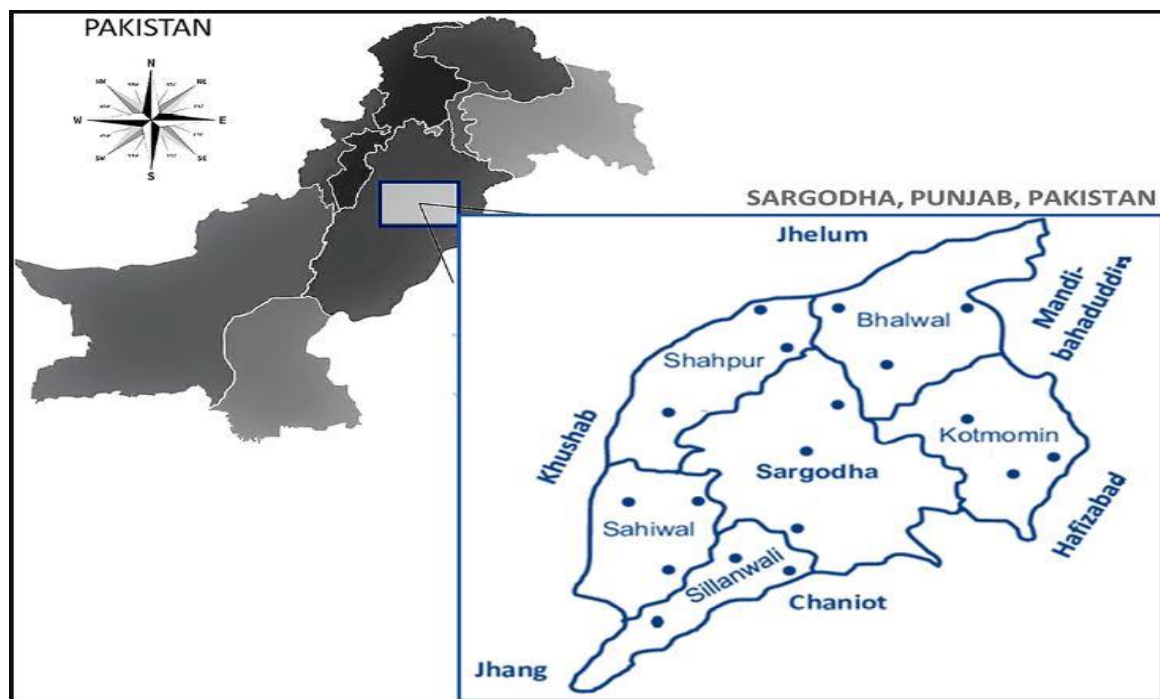


Figure 1: Area of Samples

Species Identification

Available key (dichotomous key) was used in the identification of collected samples. Two species of Rodents, black rat (*Rattus rattus*), brown rat (*Rattus norvegicus*) were identified.

Identification key for brown rat, *Rattus norvegicus*

One of the most widely distributed specie of rat is the brown rat (*Rattus norvegicus*). They are weighing between 140 and 500 g (5 and 17+3.4

oz.). One of the largest muroids which is brown to brownish-grey. The brown rat's nose is pointed, ears are large with no fur, and its tail is long and scaly. Compared to male rats, female brown rats are slightly smaller and less in weight. The link for the identification of *Rattus norvegicus* is given below(<https://animalia.bio/index.php/brown-rat>)



Figure 2: Male brown rat



Figure 3: Female brown rat

Identification key for black rat (*Rattus rattus*)

A *Rattus rattus* has a long, long tail. Black rats have lighter, black to light brown, under bellies. Furthermore, they have slightly shorter fur of a blackish-gray color and are a bit smaller than the Brown rat. In addition, they are located in

agricultural areas such as barns and crop fields. They like to nest in the upper floors of buildings in towns and cities, and are often found in false ceilings and in wall cavities. The link for the identification of *Rattus rattus* given below; (<https://animalia.bio/index.php/black-rat>)



Figure 4: Male black rat



Figure 5: Female black rat

Morphometry of samples

Initially, Vernier caliper was used to perform the Morphometry of all the samples. The morphometry involves of head length, tail

length, snout length, eye length, head width, hind foot length, ear length, body length, body width, body girth, and front foot length.

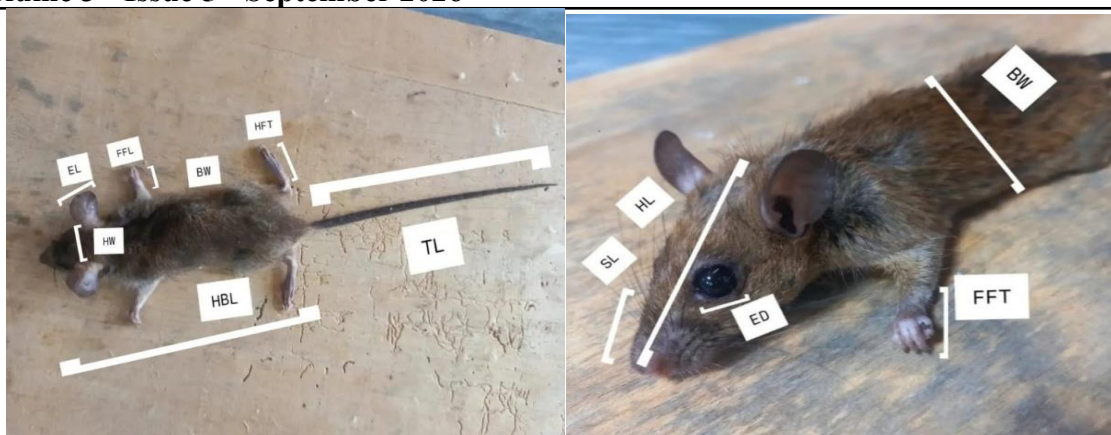


Figure 6: (HBL) Head Body Length, (HL) Head Length, (TL) Tail Length, (SL) Snout length, (EL) Eye diameter, (HL) Head length, (HW) Head width, (HFT) Hind foot length, (BW) Body weight, (BG) Body girth, (FRL) Front foot length.

Blood Collection

With the help of insulin syringe, blood was collected from the heart puncture. The complete blood count (CBC) involved in this analysis required an anticoagulant, so blood was transferred into tubes that contain 0.5 ethylene diamine tetra acetic acid (EDTA).

CBC Blood Test

A Complete blood count is a blood test that will measure: RBC count, WBC count, hemoglobin, hematocrit/PCV, MCV, MCH, MCHC, differential leukocyte count, and platelet count. The sex differences are as follows: Red blood cell counts and hemoglobin levels are higher in males, whereas the white blood cell counts are often higher in females, indicating higher immune response levels. These differences may have implications for disease susceptibility, such as infection, anemia and hematological cancers, and highlight the need to include sex in studies of rodent health.

Morphological analysis

The morphometric parameters of the wild rats were measured, taking into account gender differences, such as body weight, body length, organ size. Both male and female rats were taken from the habitat, and their physical parameters were measured with standardized calipers and digital scale. The sex size dimorphism of the animals was compared and special emphasis was given to the differences in size of the whole body and organs.

Statistical Analysis

The use of a T test was performed to analyze the data using Minitab.

Results

Variation in the morphology of the brown rat, *Rattus norvegicus*.

The morphometric features of Brown rats *Rattus norvegicus* females and males are shown in Tables 1 and 2 respectively. In general, sex differences were found in measurements, especially body length and the body weight. Head and body length (HBL) of female Brown rats varied from 14.1 to 21.5 cm (18.5 cm on average), while males varied from 13.6 to 23.1 cm (18.3 cm on average). The average head length (HL) for females was 4.4 in and for males was 4.3 in. Tail length (TL) had an average of 16.5 and 17.2 for females and males, respectively. The difference between males and females was relatively large for body weight (BW). Maximum, minimum and average Body Weight for female Brown rats was 168.8, 40.5, and 106.2 respectively, while it was 233.1, 88.0, and 125.9 for males. The average values of hind-foot length (HFL) were fairly similar for both sexes (3.08 and 3.06, respectively). The average ear length (EL) was 2.30 for both sexes. The average HW is 2.08 in females and 2.20 in males, and the average value of the trait BW is 3.40 in females and 3.65 in males.

The average body girth (BG) was 12.0 in females compared with 12.2 in males. Eye diameter

(ED) was essentially the same in both sexes, with the means of 0.61 and 0.60 respectively. The average FF value was 1.50 for both the sexes. The mean TL final was found to be 35.2 in females and 35.6 in males. The average values of body dimensions, tail length, body weight, head width, body girth and TL were higher in the males compared to the females, on the whole. Some body dimensions showed similar overall mean values for both sexes but other measurements were generally higher in males.

Morphometric Variation in Black Rat (*Rattus rattus*)

The morphometric data of the female Black rats (*Rattus rattus*) is presented in Tab. 3. HBL ranged from 11.4 to 19.1, with an average of 15.28. The mean values for HL and TL were 3.91 and 16.00, respectively. The average body weight of the Brown rats ranged from 27.5 to 101.2, with the average being of 75.30, which is less than the average body weight of both female and male Brown rats. The average HFL of female Black rats was 2.90, and the averages for EL and HW were 2.10 and 1.90, respectively. The mean value of the character chosen as BW was 3.10. The mean values of body girth and eye diameter were 10.80 and 0.61 respectively. The mean value of the FF measurement was 1.19 and the mean value of the final TL measurement was 31.80. As a whole, the

morphometric profile of the female Black rat suggested that the body dimensions and body weights of the female Black rats were generally smaller than those of the female Brown rats in Tables 1 and 2.

Comparative Morphometric Pattern among the Rat Groups

The overall body size was clearly different between the 3 groups when compared to each other. Male Brown rats had the highest mean body weight (125.9), female Brown rats had the second highest mean body weight (106.2) and female Black rats had the lowest mean body weight (75.30). The average HBL of the Brown rats was also greater compared to the Black rats of each sex (18.5 for female and 18.3 for male rats). There were similar trends for average body girth, which was 12.0, 12.2 and 10.80 in female Brown rats, male Brown rats and female Black rats, respectively. The measurements made on the tail were fairly comparable for both sexes of Brown rats, with lower values in the female Black rats. The average final TL of female Brown rat group was 35.2 and male Brown rat group 35.6, whereas the female Black rats' average final TL was 31.80. The overall results of these comparisons showed that the Brown rats were larger in general than the female Black rats examined in the present study, the most obvious difference being in body weight.

Table 1: Morphometric parameters of Female Brown Rat (*Rattus norvegicus*)

Morphometry	Maximum	Minimum	Average
HBL	21.5	14.1	18.5
HL	5.1	3.7	4.4
TL	20.3	14.2	16.5
BW	168.8	40.5	106.2
HFL	3.5	2.8	3.08
EL	2.3	2.0	2.30
HW	2.5	1.7	2.08
SL	1.0	1.0	0.92
BW*	5.2	2.4	3.40
BG	14.9	8.5	12.0
ED	0.7	0.5	0.61
FF	1.8	1.1	1.50
TL*	42.1	27.8	35.2

Table 2: Morphometric parameters of Male Brown Rat (*Rattus norvegicus*)

Morphometric trait	Maximum	Minimum	Average
HBL	23.1	13.6	18.3
HL	5.3	4.4	4.3
TL	22.3	12.6	17.2
BW	233.1	88.0	125.9
HFL	3.1	3.0	3.06
EL	2.5	2.4	2.30
HW	2.7	1.8	2.20
SL	0.6	1.2	0.90
BW	5.3	2.2	3.65
BG	15.0	11.3	12.2
ED	0.5	0.5	0.60
FF	1.7	2.1	1.50
TL	45.1	26.2	35.6

Table 3: Morphometric parameters of Female Black Rat (*Rattus rattus*)

Morphometric trait	Maximum	Minimum	Average
HBL	19.1	11.4	15.28
HL	4.1	3.7	3.91
TL	20.1	14.0	16.00
BW	101.2	27.5	75.30
HFL	3.4	2.8	2.90
EL	1.8	2.0	2.10
HW	2.2	1.7	1.90
SL	1.2	0.7	0.90
BW	3.9	1.9	3.10
BG	13.1	6.2	10.80
ED	0.8	0.6	0.61
FF	1.1	1.1	1.19
TL	39.2	25.5	31.80

Morphometric Parameters of Male Black Rats

The morphometrics of the male black rats (*Rattus rattus*) are shown in Table 4. The mean head-and-body length (HBL) was 14.9 cm having a maximum of 17.3 cm and minimum of 12.1 cm. The average head length (HL) was 3.6 cm and the tail length (TL) was 16.5 cm. The average weight of the bodies was 97 g with a range of 85 to 102 g. The mean length of hind-foot was 2.65 cm, whereas the mean lengths of ear and head were 2.09 and 2.10 cm, respectively. The average snout length (SL) was 0.60 cm and the mean body width (BW) was 2.90 cm. The average body girth (BG) was 10.50 cm, the average eye diameter (ED) and the average front-foot length (FF) were 0.64 and 1.30 respectively. The average total length of male black rats was 31.2 cm.

Hematological Parameters of Male and Female Black Rats

Table 5 shows the comparative values of hematological parameters of male and female black rats (*Rattus rattus*). Male black rats had a significantly higher mean WBC count ($8.71 \pm 0.74 \times 10^3/\mu\text{L}$) than females ($5.28 \pm 0.83 \times 10^3/\mu\text{L}$; $p = 0.005$). The mean percentage of neutrophils was higher in males than in females ($53.4 \pm 4.5\%$ vs. $41.4 \pm 4.6\%$) but this difference was not statistically significant ($p = 0.07$). Lymphocyte percentage was also higher in males ($63.5 \pm 5.1\%$) than in females ($59.1 \pm 4.6\%$) but the difference was not significant ($p = 0.5$). There was a significant difference in monocytes percentage ($21.3 \pm 5.3\%$ vs. $6.45 \pm 0.8\%$ males vs. females, $p = 0.01$). Eosinophil percentage was also significantly higher in

males ($0.95 \pm 0.29\%$) compared with females ($0.14 \pm 0.04\%$; $p = 0.01$). The percentage of basophils was slightly higher in males ($1.22 \pm 0.22\%$) than females ($0.80 \pm 0.17\%$) but this difference was not significant ($p = 0.1$). The mean RBC count was $6.08 \pm 0.33 \times 10^6/\mu\text{L}$ in males and $5.78 \pm 0.23 \times 10^6/\mu\text{L}$ in females, with no significant difference ($p = 0.4$). In addition, males had a higher concentration of hemoglobin (11.29 ± 0.54 g/dL) than females (10.76 ± 0.45 g/dL), although this difference was not significant ($p = 0.4$). However, MCV

was significantly different between sexes, females had a higher mean value ($57.62 \pm 1.4 \mu\text{m}^3$) than males ($51.0 \pm 2.9 \mu\text{m}^3$; $p = 0.05$). The mean MCH was 21.94 ± 1.1 pg in females and 19.05 ± 1.1 pg in males, with no significant difference ($p = 0.07$). MCHC values were also comparable between females (33.18 ± 1.1 g/dL) and males (32.75 ± 1.2 g/dL; $p = 0.7$).

There was no significant difference between female and male counts ($412 \pm 39 \times 10^3/\mu\text{L}$ versus $429 \pm 39 \times 10^3/\mu\text{L}$, respectively; $p = 0.7$).

Table 4: Morphometric parameters of Male black rat

Morphometric parameter	Maximum	Minimum	Average
HBL	17.3	12.1	14.9
HL	4.2	3.5	3.6
TL	18.5	14.0	16.5
BW (g)	102	85	97
HFL	3.1	2.9	2.65
EL	2.9	2.2	2.09
HW	1.9	2.5	2.10
SL	0.8	0.7	0.60
BW	4.1	3.1	2.90
BG	8.9	10.5	10.50
ED	0.5	0.7	0.64
FF	1.5	1.2	1.30
Total Length (TL)	35.8	26.1	31.2

Table 5: Comparison of studied complete blood count parameters between Male and female black rat from Sargodha, Punjab, Pakistan. Data is expressed as Mean $\pm$ Standard Error of Mean. P-value indicates the result of 2-sample t-test calculated for each studied parameter.

Parameters	Female Black rat	Male Black rat	P-value
White blood cells ($\times 10^3/\mu\text{L}^{-1}$)	5.28 ± 0.83	8.71 ± 0.74	0.005**
Neutrophil %	41.4 ± 4.6	53.4 ± 4.5	0.07
Lymphocytes %	59.1 ± 4.6	63.5 ± 5.1	0.5
Monocyte %	6.45 ± 0.8	21.3 ± 5.3	0.01**
Eosinophil %	0.14 ± 0.04	0.95 ± 0.29	0.01**
Basophil %	0.80 ± 0.17	1.22 ± 0.22	0.1
Red Blood Cells ($\times 10^6/\mu\text{L}^{-1}$)	5.78 ± 0.23	6.08 ± 0.33	0.4
Hemoglobin x g/dL	10.76 ± 0.45	11.29 ± 0.54	0.4
Mean corpuscular volume (μm^3)	57.62 ± 1.4	51.0 ± 2.9	0.05*
Mean corpuscular hemoglobin (pg)	21.94 ± 1.1	19.05 ± 1.1	0.07
Mean corpuscular hemoglobin concentration (gdl ⁻¹)	33.18 ± 1.1	32.75 ± 1.2	0.7
Platelets ($\times 10^3/\mu\text{L}^{-1}$)	412 ± 39	429 ± 39	0.7

Hematological Parameters of Brown and Black Rats**Leukocyte Parameters**

Leukocyte parameters of Brown rats (*Rattus norvegicus*) and Black rats (*Rattus rattus*) are shown in Figure 1. The mean WBC count was higher in Brown rats ($8.60 \times 10^3/\mu\text{L}$) than in Black rats ($6.77 \times 10^3/\mu\text{L}$). Brown rats had a higher percentage of neutrophils (46.8%) than Black rats (30.0%). Black rats had a significantly greater mean percentage of lymphocytes (61.0%) than did Brown rats (54.4%). The differences were significant for the percentage of monocytes which was significantly high in Brown rats (34.5%) compared to Black rats (12.9%). This difference was statistically significant ($p < 0.001$). The eosinophils percentages of Brown rats (0.52%) and of Black rats (0.49%) were amazingly close and basophil percentages were also very similar, with mean percentages of 0.93% and 0.98%, respectively. In general, the most variation among the leukocytes was seen with the monocytes, followed by the neutrophils and lymphocytes Figure 7a.

Blood parameters of erythrocytes and platelets:

The erythrocytic and platelet parameters of Brown and Black rats are presented in Figure 7b. The mean RBC counts for Brown rats were $5.88 \times 10^6/\mu\text{L}$ as compared to Black rats which has a mean count of $5.91 \times 10^6/\mu\text{L}$. Likewise, there was a small difference in hemoglobin concentration with mean values of 10.87g/dl in Brown rats and 10.99g/dl in Black rats. The mean corpuscular volume (MCV) was slightly elevated in Black rats ($54.75 \mu\text{m}^3$) compared to the Brown rats ($53.5 \mu\text{m}^3$) while mean corpuscular hemoglobin (MCH) was elevated in Black rats (20.69 pg) than in Brown rats (19.92 pg). The MCHC in Brown rats (33.32 g/dL) was slightly raised compared to that of Black rats (32.99 g/dL). None of the erythrocytic parameters reported had a statistically significant difference between the two species based on the reported p-values. The platelet counts were comparatively high in both groups with mean of $455 \times 10^3/\mu\text{L}$ for Brown rats and $420 \times 10^3/\mu\text{L}$ for Black rats. Brown rats had a higher mean platelet count but this was not statistically significant ($p = 0.4$) Figure 7b.

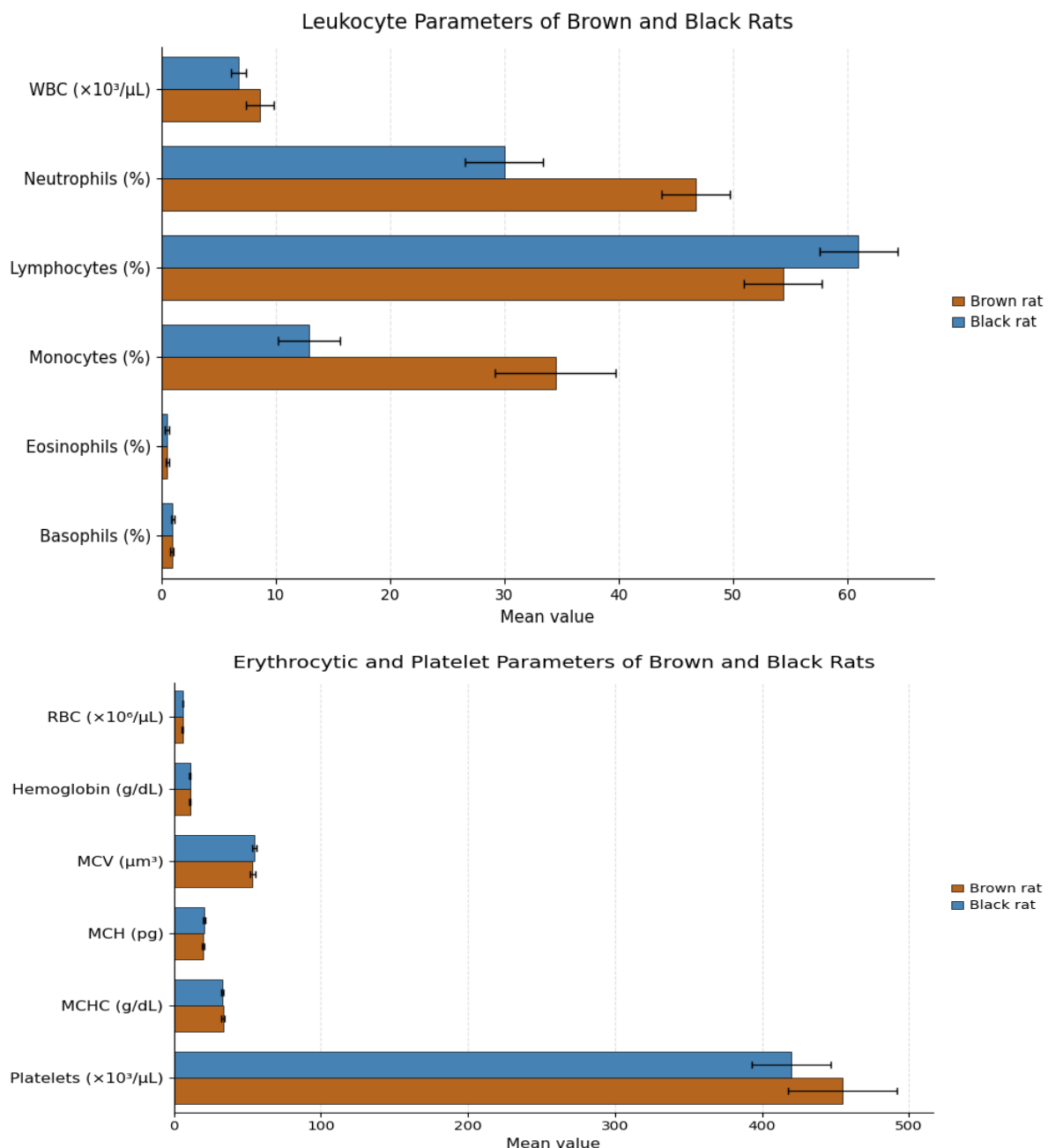


Figure (7a-7b): Leukocyte parameters of Brown and Black rats b) Erythrocytic and platelet parameters of Brown and Black rats

Sex-Specific Hematological Variation in Brown Rats

The figures of the erythrocytic and platelet parameters of the female and male Brown rats are displayed in figure 8a. The mean RBC count ($6.25 \times 10^6/\mu\text{L}$) and hemoglobin concentration (11.53 g/dL) in male Brown rats were marginally higher than those of females ($5.60 \times 10^6/\mu\text{L}$ and 10.30 g/dL, respectively).

There was no significant difference between the male and female in mean values of MCV and MCH. The mean value of MCV was higher in females ($54.8 \mu\text{m}^3$) than in males ($51.8 \mu\text{m}^3$) and the mean value of MCH was higher in males (21.60 pg) than in females (18.62 pg). MCHC was higher in females (34.45 g/dL) than males (31.80 g/dL). Also, males had higher platelet counts ($479 \times 10^3/\mu\text{L}$) than

females ($437 \times 10^3/\mu\text{L}$). Of these parameters, MCH was the only one to demonstrate a statistically significant difference between the sexes ($p = 0.02$); the others demonstrated no significant difference.

Leukocyte Variation between Brown and Black Rats

The comparative leukocyte profile of Brown and Black rats is given in Figure 8b. Brown rats had a higher mean WBC count ($8.60 \times 10^3/\mu\text{L}$) than Black rats ($6.77 \times 10^3/\mu\text{L}$). Brown rats (46.8%) also had more neutrophils than black rats (30.0%). On the other hand, the

percentage of lymphocytes of Black rats was higher (61.0%) compared to Brown rats (54.4%). The most significant difference was seen in the monocyte numbers (34.5% in Brown rats vs 12.9% in Black rats; $p < 0.001$). The numbers of eosinophils and basophils were found to be similar in the two groups of rats, Brown (0.52%) and Black (0.49%). In general, the findings reveal more inter-species differences in leukocytes parameters compared to eosinophils and basophils, with the monocytes, neutrophils and lymphocytes showing the most marked differences.

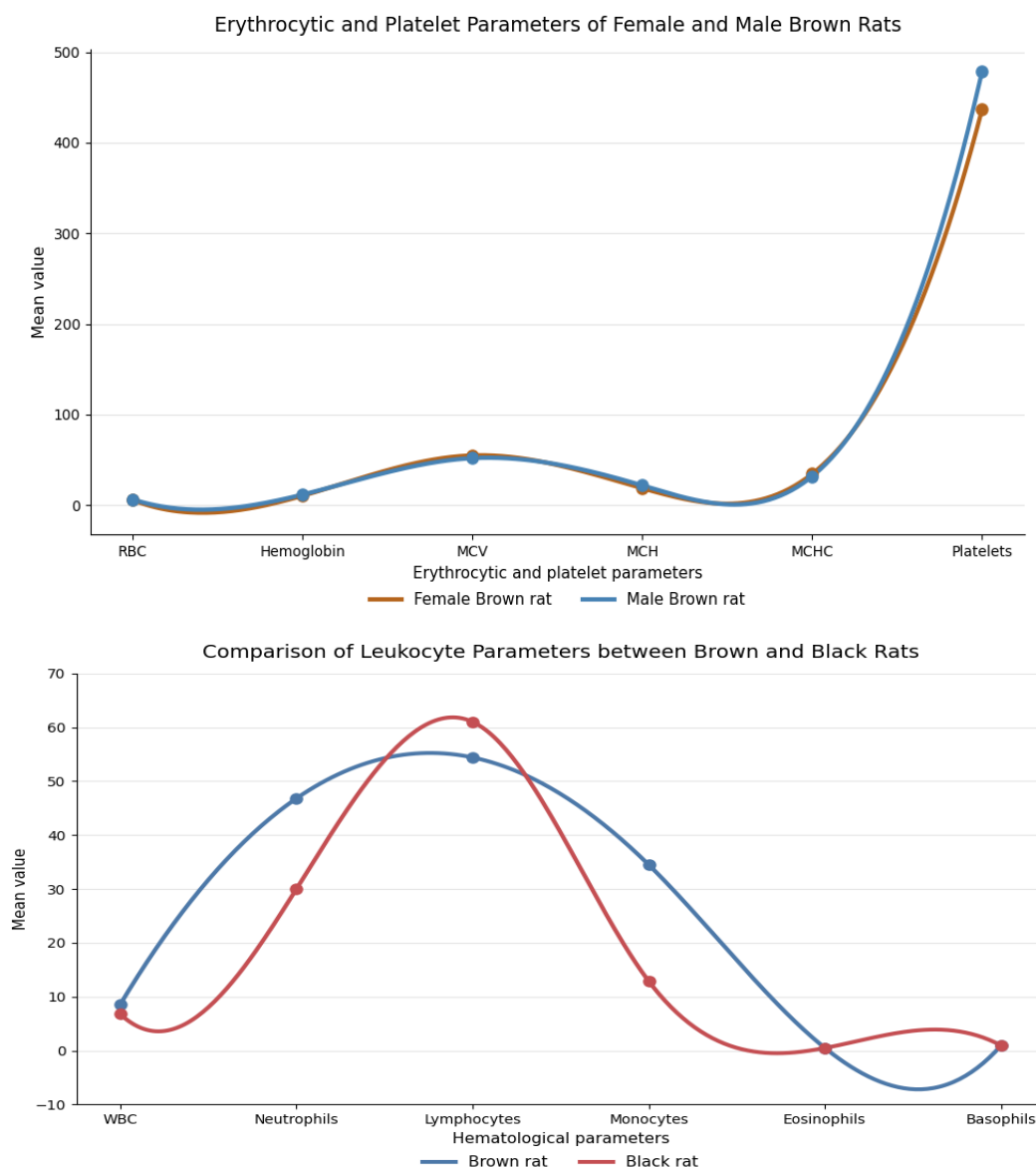


Figure (8a-8b): a) Sex-specific variation in erythrocytic and platelet parameters of Brown rats b) Comparative leukocyte parameters of Brown and Black rats.

Discussion**Morphometric variation and sexual dimorphism**

The morphometric results showed variation in body size among the studied rat groups. Female Brown rats have an average body weight of 106.2 while the male Brown rats have an average body weight of 125.9. The average body weight of female Black rats (75.30) was significantly lower than that of male Black rats. The trend was that *R. norvegicus* in the populations studied tended to be larger than *R. rattus*. The observed interspecific variation is in line with the known morphological differentiation of commensal rodents, where body size and external characters are significant attributes to distinguish sympatric species and populations (Islam et al., 2021; Kusmeirczyk et al., 2020).

The higher body weight recorded in male Brown rats compared with females is indicative of sexual dimorphism in somatic growth. The difference between males and females with regard to body mass was not statistically tested in the morphometric tables presented herein, but the pattern (numerically) is the males have the larger body mass. Body size differences between sexes are ubiquitous in mammals, and can result from differences in growth rate, energy allocation, reproductive effort, or hormonal control. Male-biased body size may be linked to more investment in somatic growth, while females may invest more in their reproduction and maternal functions (Mauvais-Jarvis, 2024; Maggi and Della Torre, 2018). The greater average body weight of the male *R. norvegicus*, then, may be due to sex-related growth and/or to physiological differences.

Additional morphometric comparisons also revealed that the average tail length, head width, body girth, and last measurement on the tail were slightly greater for male Brown rats than for female Brown rats. Several measurements, such as ear length and eye diameter, however, were very close in the sexes. This means that not all morphological traits were sexually dimorphic. Certain features of similar relatively stable structures may be constrained by function and/or development, while other features may be more directly influenced by overall body size and thus show

greater sex-related differences. For the same purpose, morphometric analysis of *Mus musculus*, *Rattus norvegicus* and *Rattus rattus* has been shown to be important to characterize rodent populations and distinguish morphometric variation between species (Michaux et al., 2007; Odigie et al., 2018).

The comparatively large size of the body of *R. norvegicus* over that of *R. rattus* might also hold ecological significance. Both species share some of the same human-associated environments but they differ in their use of habitats, their climbing ability, their use of resources, and their space utilization. Thus, among the differences in body size, niche separation and impact on use of agricultural and peri-domestic environments could be influenced by this variation. Beyond genetic variation, morphological traits may be influenced by food availability, environmental factors, population density, and developmental history, among other factors (Buchholz et al., 2019; Wang, 1998; Cassano & Alcalde, 2026).

Hematological differences between Brown and Black rats

Hematological blood parameters show significant inter-specific differences, with the greatest differences in the leukocyte profile. The mean of WBC count was higher in brown rats compared to black rats, however, this difference was not significant ($8.60 \times 10^3/\mu\text{L}$ in brown rats vs. $6.77 \times 10^3/\mu\text{L}$ in black rats, $p = 0.1$). Pathological conditions are not necessarily indicated by moderate differences in WBC counts between free-ranging rodent populations because physiological condition, age, sex, environmental exposure, infection, stress, and inflammatory status can also influence these counts (McClure, 1999; Pilny, 2008; Siegel and Walton, 2020). The percentage of neutrophils was significantly different between the two species, with the Brown rat having a mean of 46.8% and the Black rat a mean of 30.0%. Neutrophils are an integral part of innate immunity and quickly react to inflammatory stimuli, microbial invasion and tissue injury. The abundance of these can thus be used to infer the immediate immune status of an animal (Jaillon et al., 2013; Rosales, 2020). In the present data set,

however, this difference in neutrophil percentage was not statistically significant ($p = 0.9$), and should not be construed as a species-level difference. Rather, the disparity in numbers may be due to inter-individual biological variation or to variation in environmental exposure.

Black rats had higher numbers of lymphocytes (61.0%) than the Brown rats (54.4%). Lymphocytes play a key role in adaptive immunity, antigen-specific immune responses, immunological memory, and immune regulation (Li et al., 2019). The higher proportion of lymphocytes, and lower proportion of neutrophils, in Black rats suggest a difference in the distribution of leukocytes between the two species. These changes in leukocytes can be caused by a variety of factors including immune history, age structure, stress, infection, or environmental exposures, and may not be attributable to just one, or a single cause (Davis et al., 2008).

Monocytes are important phagocytes that play a role in inflammatory responses, tissue repair, pathogen recognition, and differentiation into macrophages and related cells. This striking difference in monocyte numbers could reflect inter-population differences in inflammatory or immune activation. An increase in leukocytes or monocytes should, however, not be considered disease, as there are a number of physiological and ecological factors that can influence the leukocytes profile (Chmielewski and Strzelec, 2018).

Rodents can be exposed to pesticides, fertilizers, contaminated food and other anthropogenic stressors in agricultural environments. Chemical exposures have been shown to affect hematological parameters in rats under experimental conditions. For instance, pesticide exposure has been reported to alter the hematological indices in experimental rats (Saleem et al., 2025), and Bisphenol A exposure has been reported to alter the hematological and antioxidant biomarkers (F. Mahraan et al., 2025). Likewise, the exposure to mixtures of persistent organic pollutants has been linked to alterations in haematological parameters in mice (Sheaheadah et al., 2017).

The percentage of eosinophils and basophils were fairly equal in both Brown and Black rats. The mean percentage of eosinophils in Brown rats and Black rats were 0.52% and 0.49%, respectively, while the mean percentage of basophils were 0.93% and 0.98%, respectively. There was no significant difference in the proportion of these cell types indicating that significant differences between species were limited to monocytes, neutrophils, and lymphocytes. Eosinophils and basophils typically constitute a smaller percentage of circulating leukocytes, and both can be affected by parasite exposure, allergy, or inflammation and can be affected by environmental factors (Pilny, 2008; Siegel and Walton, 2020).

Erythrocytic indices and sex-related variation in Brown rats

There were relatively small differences between the Brown and the Black rats in their erythrocytic results. Brown rats and Black rats had 10.87 and 10.99 g/dL hemoglobin, respectively, with RBC counts of $5.88 \times 10^6/\mu\text{L}$ and $5.91 \times 10^6/\mu\text{L}$, respectively. These parameters are found to be similar, indicating that the oxygen-carrying capacity was relatively maintained in the two species under the conditions studied. Laboratory and small mammal standard hematological parameters include RBC count, hemoglobin, MCV, MCH and MCHC as complementary parameters of abundance and characteristics of the erythrocytes (Kerr, 2008; Willard & Tvedten, 2011; Latimer, 2011).

Upon sex comparison, Brown rats showed a higher mean value of RBC count ($6.25 \times 10^6/\mu\text{L}$) and hemoglobin concentration (11.53 versus 10.30 g/dL) than female rats. These differences, though not statistically significant, are biologically similar to known sex-associated differences in erythropoiesis. Sex hormones may affect the activity of erythropoiesis and production of red cells and the effects of testosterone on erythrocyte mass and hemoglobin levels in men have been reported (Helman and Rubenstein, 1975; Kaniyas et al., 2016). Another factor contributing to sex differences in hematological and physiological characteristics between males and females may be due to metabolic

regulation differences (Murphy, 2014; Neamah & Lafta, 2026).

The MCV was slightly more in female Brown rats than males while MCH was higher in male rats than in females. The sex-related differences were significantly different for MCV ($p = 0.02$) but not for the other parameters. The finding indicates that, although both sexes had similar RBC and hemoglobin parameters, there may be differences in the red-cell parameters between the sexes. Hematozoological comparisons between sexes have shown that blood parameters and properties of red blood cells can also vary significantly between males and females and might be influenced by hormonal status (Grau et al., 2018; Murphy, 2014).

Female Brown rats had higher MCHC than males but this was not statistically significant. The average hemoglobin concentration in packed erythrocytes is reflected by MCHC so that variation in this index may occur independently of changes in the total number of RBCs. The overall pattern thus suggests that sex differences in individual indices of erythrocytes were more pronounced than in total red-cell numbers (Hawkey, 2017; Grau et al., 2018). Laboratory-generated reference data may not be directly applicable to free-ranging or agroecological populations due to differences in dietary and environmental conditions, climate, and exposure to infectious diseases (Foufopoulos et al., 2022; Macdonald & Feber, 2015).

Platelet variation

There was no significant difference between the Platelet Count of male Brown rat and female Brown rat (p value 0.5). Similarly, the mean platelet count of Brown rats was $455 \times 10^3/\mu\text{L}$ while that of Black rats was $420 \times 10^3/\mu\text{L}$ with no significant difference between the two species ($p = 0.4$). These findings suggest that platelet numbers were not as variable as some of the other leukocyte populations. In tissue injuries, platelets play a crucial role in hemostasis, maintaining the integrity of the blood vessel walls, in inflammatory responses, and in communicating with immune cells (Golebiewska & Poole, 2015; Scridon, 2022). Recent studies also highlight platelets' role in

intricate signaling pathways and their contribution to various physiological and pathological functions in addition to their traditional function in blood coagulation (Tian et al., 2025). Thus, even if the differences between the rodents at this time were not statistically significant, the evaluation of these differences adds to the understanding of hematological variation among the groups of rodents studied.

Sexual Dimorphism in *Rattus rattus*

The morphometric traits of male *Rattus rattus* are listed in Table 4. The average body weight, total length, tail length, and head-and-body length were 97 g, 14.9 cm, 16.5 cm, and 31.2 cm, respectively. The tail's length in relation to the head and body length is in line with *R. rattus*'s distinctive body proportions. Table 5 demonstrated several sex-associated differences in the hematological profile of *R. rattus*. Male rats had significantly higher WBC counts than females as well as higher monocyte ($p = 0.01$) and eosinophil ($p = 0.01$) percentages. Sex is an important biological determinant of immune-cell composition and function, with differences arising through interactions between sex hormones, sex chromosomes, and immune regulatory pathways (Dunn et al., 2024; Liao et al., 2026). Previous research in laboratory rats has also demonstrated sex-related differences in peripheral blood leukocyte profiles, supporting the presence of sex-associated variation in leukocyte parameters (Stefanski and Grüner, 2006). Recent studies underline that sex differences in immune responses are complex and may change depending to the immune cell type and physiological setting (Dunn et al., 2024; Liao et al., 2026). In contrast, MCV was higher in females than males. According to Kong et al. (2014), sex-related variations in rats' haematological and iron-related characteristics, such as erythrocytic indices, may be caused by variations in hepcidin regulation and iron metabolism. The current study did not test iron status or hepcidin concentrations, hence this process cannot be directly verified even though it offers a potential explanation for the observed MCV difference. Neutrophils, lymphocytes, basophils, RBC count, haemoglobin, MCH, MCHC, and platelet

count did not significantly change between male and female black rats ($p > 0.05$). These results show that biological sex can affect immunological and haematological traits, but they also highlight the possibility that environmental and physiological factors could alter these differences (Stefanski and Grüner, 2006; Dunn et al., 2024; Liao et al., 2026).

Comparison with previous experimental and comparative studies

The results obtained here are generally consistent with those reported previously which showed that hematological traits are species specific, sex specific, environmentally responsive and physiologically regulated. Comparative studies of rodents highlight the importance of species-specific hematological profiles and reference ranges and not assuming the same baseline values for all species (Kusmeirczyk et al., 2020; Siegel and Walton, 2020). The differences found between Brown and Black rats with respect to the distribution of leukocytes thus may reflect biological differentiation between sympatric species.

Experimental studies also show that environmental chemicals do affect characteristics of hematology and biochemistry. Exposure to pesticides in rats has been reported to cause blood parameter changes (Saleem et al., 2025), and exposure to endocrine-disrupting chemicals like dicyclohexyl phthalate and di-n-hexyl phthalate has been linked to changes in blood parameters, biochemical alterations, and changes at the tissue level in rats (Aydemir et al., 2023). Likewise, the exposure to BPA has been shown to alter hematological and antioxidant biomarker in experimental animals (F. Mahraan et al., 2025).

However, the present results should be considered with regard to the environmental acclimatization. Physiological responses to environmental conditions can vary between species, such as differences in hematological and metabolic changes in response to hypoxia (Arias-Reyes et al., 2021). In the present study, hypoxia was not a key factor investigated in the environment, however, this evidence further supports the general rule that closely related rodents respond differently to environmental

stressors. Recent studies on Wistar rats have shown that the parameter of erythrocytes, hemoglobin and hematocrit are influenced by the improvement of welfare conditions (Amriani et al., 2026).

Conclusion

The present study demonstrates that sympatric populations of *Rattus norvegicus* and *Rattus rattus* exhibit distinct morphometric and hematological profiles, with variation occurring both between species and between sexes. Brown rats were generally larger than black rats, with male *R. norvegicus* showing the highest mean body weight and greater values for several external body measurements. Hematological comparisons revealed that interspecific variation was more evident in leukocyte profiles than in erythrocytic and platelet parameters, particularly for monocytes, lymphocytes, and neutrophils. Sex-related differences were also evident, especially in the WBC, monocyte, eosinophil, and MCV values of black rats and in MCH among brown rats. In contrast, several erythrocytic indices and platelet counts remained relatively stable between sexes or species. Overall, these findings indicate that species identity and sex are important sources of biological variation in wild rats, while environmental and physiological factors may additionally contribute to the observed patterns. The study provides useful baseline morphometric and hematological information for free-ranging *Rattus* populations in the agroecological environment of Central Punjab and may serve as a reference for future ecological, physiological, veterinary, and wildlife-health investigations.

Declarations

Ethics approval and consent to participate

This study did not involve any human participants. All procedures involving animals were conducted in accordance with institutional and national guidelines for the care and use of animals. Ethical approval was obtained from the relevant departmental/institutional committee of the University of Sargodha, Sargodha, Pakistan.

Consent for publication

The collection sites are properties of the Government of Punjab, Pakistan. Permission for sample collection was obtained from the Department of Botany, University of Sargodha, Sargodha, Pakistan (A Government governed body).

Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

I.U., conducted the experiments, collected plant samples, and wrote the original draft of the manuscript. A.B.G., supervised the study and designed the experiments. A.A.S., performed data analysis. T.R., contributed to manuscript review, editing, and revision. T.A., and T.A., assisted in revision and prepared figures and tables. All authors read and approved the final manuscript.

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