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## Morphometric variation and its relation to the eyes lens weight among three species of wild rodents in Tunisia

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### Abstract

Rodents are important reservoirs for a large number of zoonotic pathogens, and some species are of interest in medical research. Age determination is essential for understanding many aspects of these wild rodents including population growth, dynamics and ecology. We aimed to investigate the intra-specific morphometric variations of three species of Tunisian rodents: *Psammomys (P.) obesus*, *Meriones (M.) shawi* and *Ctenodactylus (C.) gundi*, and to develop a model for relative age-estimation based on eyes lens weight (ELW). Between November 2008 and May 2013, 731 wild rodents were trapped—472 *P. obesus*, 167 *M. shawi* and 92 *C. gundi*—at three study sites for each species. Morphometric description and its variability according to their origin were demonstrated among the three rodent species. Additionally, moderate equations correlating morphometric measurements with ELW were determined for each species of rodents using multivariable logistic regression. Head and body length was identified as the most suitable parameter to estimate the ELW (relative age indicator) in the three rodent species studied. That will be of use in research using these rodent species, negating the need for postmortem examination.

**Keywords:** eye lens weight, morphometric measurements, multivariable logistic regression, Tunisia, wild rodents.

### 1. Introduction

Age determination is a basic requirement when researching ecological events affecting wild animals. Several useful methods for age determination have been reported for small rodents [1]. Since Lord [2] proposed a method of age determination using eye lens weight (ELW), it has become well-known that ELW can be used to determine age in various species of Rodentia order [3-6]. This technique is considered more accurate than others relying on body or skull measurements [1, 7], as ELW increases incrementally with age, and there is little variation within an age cohort. The ELW is a widely used and reliable measure [5, 8, 9] for the reason that it is less variable in different environmental conditions compared with other morphologic measurements [2, 10]; but the rates vary widely between species and calibration needs to be performed for each new species being studied to determine the age cohort.

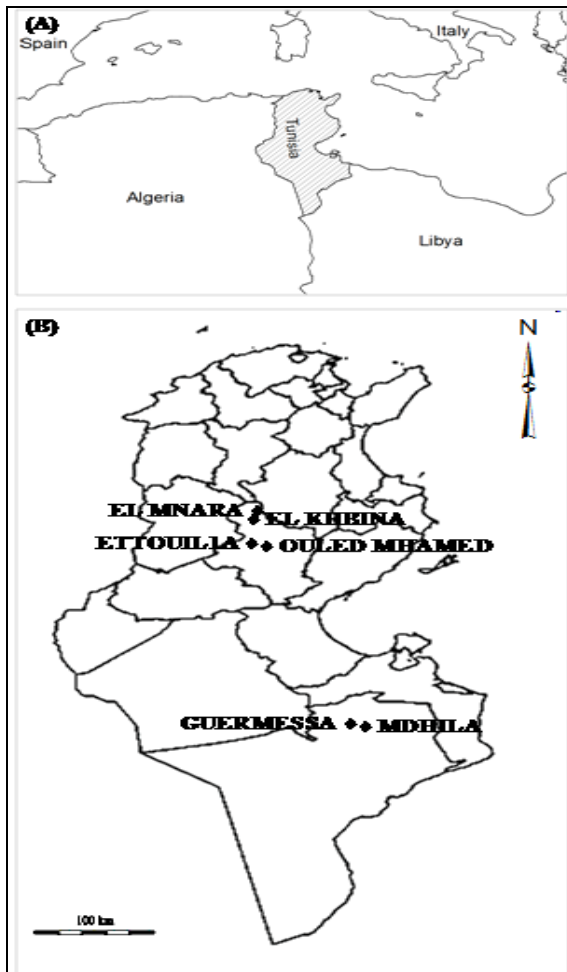
*Psammomys (P.) obesus* (Cretzschmar, 1828) is used in medical research because it has been defined as an important animal model for the study of type 2 diabetes [11-13]. Additionally, *Meriones (M.) shawi* (Duvernoy, 1842) has been recognized as a good animal model in studies of the effect of various substances, such as the studies of hypolipidemic, hypoglycemic and hypocholesterolemic effects of *Coriandrum sativum L.* and argan oil on these species of rodents [14, 15]. In addition to *Ctenodactylus (C.) gundi* (Rothmann, 1776), those rodents were also recognized as reservoir hosts of *Leishmania* parasites in Tunisia [16, 17]. The widespread use of these rodent species in medical and epidemiologic research highlights the importance of knowing the relative age of these rodents captured from their nature biotopes without the need to the autopsy and save the duration of manipulations because no longer need of livestock of these species.

In this study, we provide a morphometric description and investigate the intra-specific morphometric variations among three species of Tunisian wild rodents: *P. obesus*, *M. shawi* and *C. gundi*. Additionally, we correlate ELW to the standard morphometric measurements of each species according to age classes and/or sex, facilitating accurate age estimation and negating the need for postmortem examination.

## 2. Materials and Methods

### 2.1. Study site and rodent trapping

Rodents were trapped from nine study sites (three for each species of rodent) in Sidi Bouzid and Tataouine regions, in central and south Tunisia, respectively. *P. obesus* and *M. shawi* were trapped in previously described zones: Khbina, Mnara, Ouled Mhamed and Ettouila [16]. *C. gundi* were trapped in Khbina (average altitude 231 m; N35 1215.96 E9 42.3824) in Sidi Bouzid Governorate; Guermessa (average altitude 297 m; N32 5931.56 E10 15.138) and Mdhila (average altitude 374 m; N33 0238.46 E10 22.1422) in Tataouine Governorate (Figure 1).



**Fig 1:** Study sites for the three species of rodents. (A) Location of Tunisia in the Mediterranean region. (B) Location of study areas in the Sidi Bouzid and Tataouine governorates

*P. obesus* and *M. shawi* were trapped between November 2008 and March 2010 over 4 days/nights per month at each capture site, respectively, using pincer traps and wire mesh cage traps [16]. *C. gundi* were trapped using pincer traps between May and July 2011, and during April and May 2013. All animal experimentations were in agreement with the guidelines of International Guiding Principles for Biomedical Research Involving Animals.

### 2.2. Morphometric measurements and age estimation

Trapped rodents were collected from the field and transported to the laboratory for examination. In the laboratory, each

rodent was anesthetized with ether and examined. After identification and determination of sex, the animal was weighed and standard morphometric measurements were taken. These included; head and body length (HBL), tail length (TL), ear length (EL) and hind feet length (HFL), using a 1-mm graduated scale in accordance with the previously described method [18, 19]. Following morphological measurements, each rodent was euthanized by cardiac exsanguinations. Both eyes were removed and preserved for at least 2 weeks in 10% formalin, and the lenses extracted, dried for 2 h at 100 °C, and weighed on a pan balance with an accuracy of 0.1 mg [20]. ELW was calculated as the mean value for each individual rodent and was used as the best indirect measure for the age determination [21, 22].

### 2.3. Statistical analyses and modeling

Data are summarized as frequencies and means with 95% confidence intervals (CI). Statistical significance was set at a value of  $P \leq 0.05$ . Between-group comparisons were performed using analysis of variance (ANOVA) or Kruskal–Wallis test for continuous variables, and chi-square test for variables expressed as frequencies. Comparison of morphometric measurements in each capture site were performed using the Dunnett's T3 multiple comparisons test. *P. obesus* and *M. shawi* populations were subdivided into three classes for each species, according to their body weight. The *C. gundi* population was subdivided into two classes according to sex because of the small sample size and the hazardous aspect of the trapping method used for *C. gundi* which did not allow for classification according to the weight. In fact, for *P. obesus*, animals with a body mass of  $\leq 70$  mg were classified as juveniles,  $70 > 150$  mg as sub-adults and  $\geq 150$  mg as adults. For *M. shawi*, animals with a body mass of  $\leq 65$  mg were classified as juveniles,  $60 > 100$  mg as sub-adults and  $\geq 100$ mg as adults [23, 24].

Association between ELW and each of the morphometric variables for the whole population and for each age group was performed using Pearson correlation. Pregnant females were excluded from this study for each species of rodents. Variables were assessed for linearity by graphical examination of the shape of the relationship with the outcome. Screening of variables and their association with ELW was performed using univariable logistic regression analysis, for each species separately. Only variables significantly associated with ELW at the 20% level in the univariable analysis were incorporated in the multivariable logistic regression model. The final models were built using a stepwise backward elimination procedure. The model fit was determined by the estimation of the *R* square and a normal plot of the residuals. All statistical analyses were performed using SPSS software version 17.0 for Windows.

## 3. Results

A total of 731 wild rodents were captured over the five years study period: 472 (64.6%) *P. obesus*; 167 (22.8%) *M. shawi* and 92 (12.6%) *C. gundi*.

### 3.1. Species-specific characteristics

Characteristics and morphometric measurements for each species, at each trapping site are shown in Tables 1–3.

**Table 1:** Sex ratio, mean of the morphometric measurements and eye lens weight (ELW) for each capture zone in *Psammomys obesus*.

Location of capture	Khbina	Mnara	Ouled Mhamed	P value
Sex (%)				
Male	71 (35)	54 (41)	51 (36)	NS <sup>a</sup>
Female	130 (65)	77 (59)	89 (64)	NS
Total	201 (100)	131 (100)	140 (100)	NS
Mean body measurements (CI) <sup>b</sup>				
Weight (g)	109.82 (105.58-114.07)	114.12 (109.11-119.13)	119.15 (114.51-123.79)	0.016
EL (mm)	15.17 (14.97-15.38)	15.34 (15.03-15.66)	15.55 (15.32-15.78)	NS
HBL (mm)	144.59 (142.27-146.91)	145.77 (142.85-148.69)	149.46 (147.23-151.70)	0.018
TL (mm)	118.27 (115.51-121.04)	115.57 (112.25-118.89)	124.49 (122.15-126.83)	< 0.001
HFL (mm)	35.52 (35.24-35.79)	35.02 (34.67-35.38)	36.12 (35.84-36.40)	< 0.001
Mean ELW (CI)				
ELW (g)	41.73 (39.37-44.09)	40.19 (37.64-42.73)	34.90 (33.02-36.77)	< 0.001

<sup>a</sup>: Not significant; <sup>b</sup>: 95% Confidence interval; EL: ear length; HBL: head and body length; TL: tail length; HFL: hind feet length.

**Table 2:** Sex ratio, mean of the morphometric measurements and eye lens weight (ELW) for each capture zone in *Meriones shawi*.

Location of capture	Ettouila	Khbina	Mnara	P value
Sex (%)				
Male	10 (67)	51 (37)	9 (60)	0.031
Female	5 (33)	86 (63)	6 (40)	0.031
Total	15 (100)	137 (100)	15 (100)	0.031
Mean body measurements (CI) <sup>b</sup>				
Weight (g)	69.67 (55.91-83.43)	72.92 (68.54-77.30)	57.07 (38.56-75.57)	NS <sup>a</sup>
EL (mm)	16.53 (15.41-17.66)	16.69 (16.29-17.09)	16.80 (15.73-17.87)	NS
HBL (mm)	123.53 (113.23-133.83)	130.95 (127.73-134.17)	118.13 (104.31-131.96)	0.031
TL (mm)	124.33 (110.69-137.97)	135.63 (132.78-138.48)	138.27 (128.15-148.38)	NS
HFL (mm)	33.00 (31.52-34.48)	33.50 (32.97-34.03)	32.80 (30.73-34.87)	NS
Mean ELW (CI)				
ELW (g)	66.56 (51.39-81.72)	63.91 (60.33-67.49)	49.45 (38.00-60.89)	0.042

<sup>a</sup>: Not significant; <sup>b</sup>: 95% Confidence interval; EL: ear length; HBL: head and body length; TL: tail length; HFL: hind feet length.

**Table 3:** Sex ratio, mean of the morphometric measurements and eye lens weight (ELW) for each capture zone in *Ctenodactylus gundi*.

Location of capture	Guermessa	Khbina	Mdhila	P value
Sex (%)				
Male	9 (43)	9 (45)	27 (53)	NS <sup>a</sup>
Female	12 (57)	11 (55)	24 (47)	NS
Total	21 (100)	20 (100)	51 (100)	NS
Mean body measurements (CI) <sup>b</sup>				
Weight (g)	211.86 (187.33-236.39)	228.75 (206.30-251.20)	189.35 (176.55-202.16)	0.007
EL (mm)	14.76 (13.11-16.41)	17.50 (17.01-17.99)	13.78 (13.01-14.56)	< 0.001
HBL (mm)	198.38 (193.65-203.11)	203.30 (196.27-210.33)	192.88 (189.15-196.62)	0.010
TL (mm)	27.29 (22.99-31.58)	36.35 (32.36-40.34)	30.65 (28.94-32.35)	0.001
HFL (mm)	40.71 (39.33-42.10)	42.80 (41.64-43.96)	39.86 (39.19-40.54)	< 0.001
Mean ELW (CI)				
ELW (g)	141.05 (122.42-159.68)	85.23 (76.10-94.35)	133.72 (122.48-144.96)	< 0.001

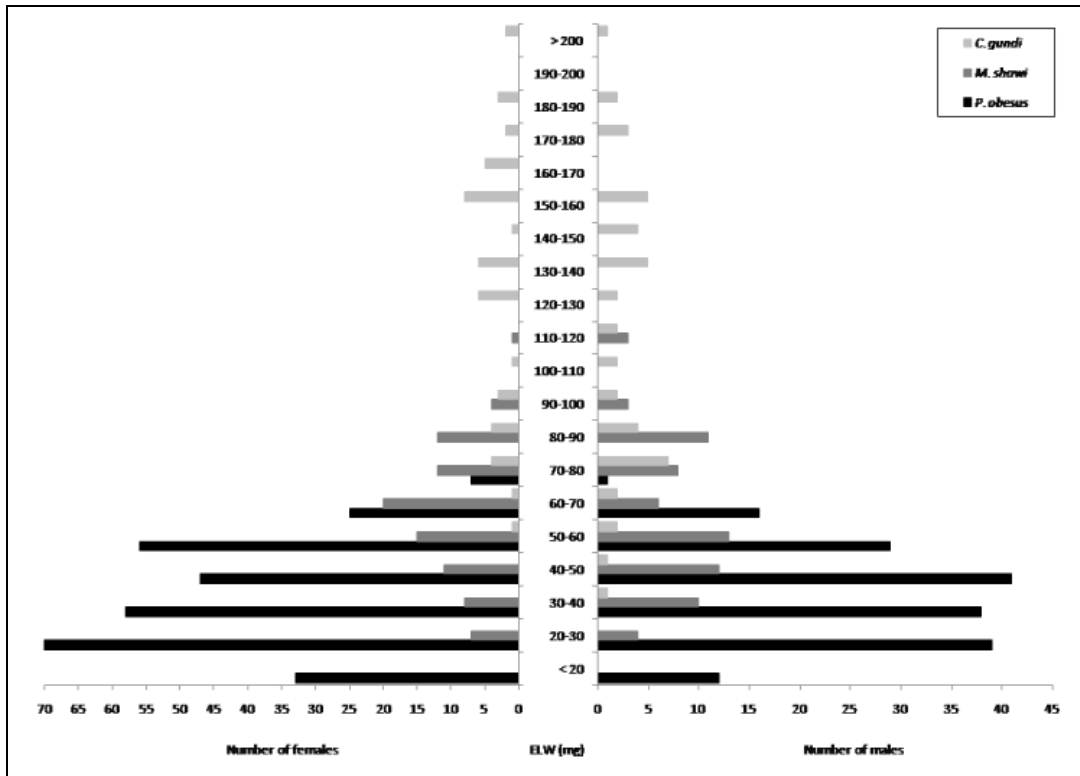
<sup>a</sup>: Not significant; <sup>b</sup>: 95% Confidence interval; EL: ear length; HBL: head and body length; TL: tail length; HFL: hind feet length.

Additionally, pair-wise inter-site morphometric comparisons showed a significant difference between populations from Khbina and Ouled Mhamed for *P. obesus* ( $P < 0.018$ ), and, Khbina and Mdhila for *C. gundi* ( $P < 0.01$ ).

In all sampled areas, TL differed significantly ( $P = 0.001$ ) in *C. gundi*, and both TL and ELW differed significantly ( $P < 0.001$ ) in *P. obesus*. No significant morphometric difference was identified in *M. shawi* according to the sampled areas. No significant difference was detected between sex for all standard morphometric measurements in *M. shawi* and *C. gundi* populations. In the *P. obesus* population, male rodents weighed significantly more than female rodents ( $123.88 \pm$

$29.92$  g compared with  $107.78 \pm 27.58$  g;  $P < 0.001$ ). Additionally, *P. obesus* males were significantly larger than females, with EL measurements of  $15.79 \pm 1.45$  mm compared with  $15.06 \pm 1.55$  mm ( $P < 0.001$ ); HBL  $151.24 \pm 15.10$  mm compared with  $143.47 \pm 15.71$  mm ( $P < 0.001$ ); TL  $122.77 \pm 17.04$  mm compared with  $117.35 \pm 18.95$  mm ( $P = 0.02$ ) and HFL  $35.85 \pm 1.78$  mm compared with  $35.39 \pm 2.04$  mm ( $P = 0.012$ ), respectively.

ELW in *P. obesus* ranged from less than 20 to 80 mg; in *M. shawi* from 20 to 120 mg and in *C. gundi* from 30 to more than 200 mg (Figure 2).



**Fig 2:** Eye lens weight pyramid, stratified according to sex for the three species of rodents; *Psammomys obesus* (*P. obesus*), *Meriones shawi* (*M. shawi*) and *Ctenodactylus gundi* (*C. gundi*)

ELW was significantly heavier in *C. gundi* females compared with males with a mean ELW of  $133.60 \pm 39.82$  mg in females, compared with  $115.72 \pm 42.96$  mg in males ( $P = 0.041$ ). Additionally, ELW was significantly different in each trapping area for the three rodent species with the heaviest ELW: from Khbina in *P. obesus* ( $P < 0.001$ ), from Ettouila in *M. shawi* ( $P < 0.042$ ) and from Guermessa in *C. gundi* ( $P < 0.001$ ).

**3.2. Relative age estimation using predictive models**

Based on the Pearson’s correlation coefficients, univariate analysis between ELW and demographic-morphometric parameters showed a significant linear correlation with: all morphometric measurements in *P. obesus* and *M. shawi* populations; and some morphometric measurements and sex in *C. gundi* population (Table 4).

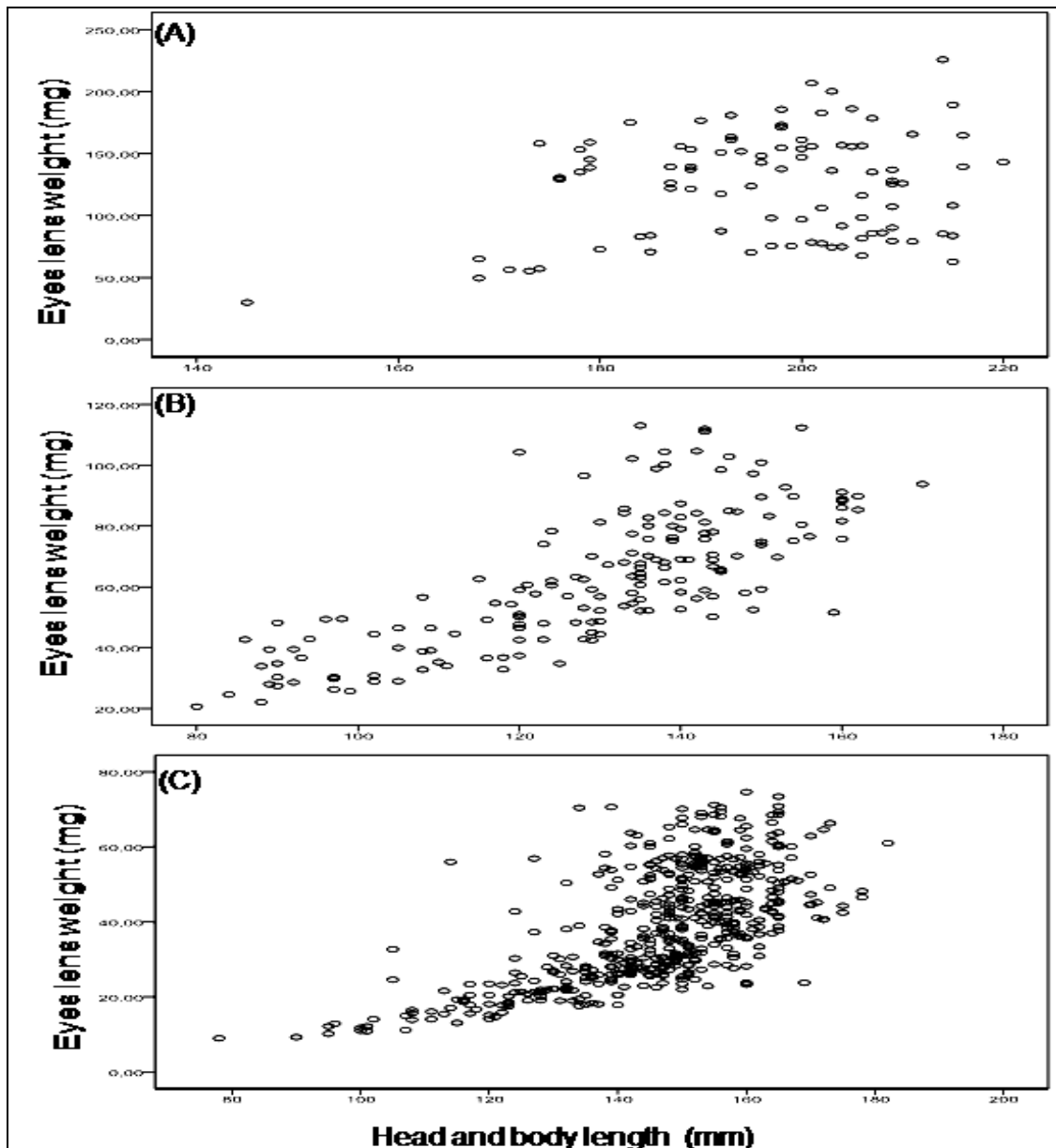
**Table 4:** Morphometric measurements linearly correlated with eye lens weight (ELW) according to Pearson’s coefficient for each species of rodent.

Rodents species	Morphometric Parameters	Pearson’s Coefficient	P
<i>Psammomys obesus</i>	EL	0.44	< 0.001
	HBL	0.65	< 0.001
	HFL	0.44	< 0.001
	TL	0.42	< 0.001
<i>Meriones shawi</i>	HBL	0.75	< 0.001
	TL	0.25	0.001
	HFL	0.50	< 0.001
	EL	0.25	0.001
<i>Ctenodactylus gundi</i>	HFL	-0.53	< 0.001
	EL	-0.57	< 0.001
	HBL	0.22	0.034
	Sex	/	/

EL: ear length; HBL: head and body length; TL: tail length; HFL: hind feet length.

In fact, in *P. obesus* and *M. shawi* the strongest correlation was between HBL and ELW ( $R = 0.65$ ;  $P < 0.001$ ;  $R = 0.75$ ;  $P < 0.001$ , respectively). However, in *C. gundi*, the strongest correlation was between EL and ELW ( $R = - 0.57$ ;  $P <$

$0.001$ ); though this was still only a moderate correlation. In this species, the only positive correlation was between HBL and ELW ( $R = 0.22$ ;  $P = 0.034$ ). Correlations between HBL and ELW for all species are presented in Figure 3.



**Fig 3:** Eye lens weight (ELW) correlation with head and body length (HBL) in the three rodent species. (A): *Ctenodactylus gundi*; (B): *Meriones shawi* and (C): *Psammomys obesus*

Morphometric measurements significantly correlated with ELW were included in the multivariable logistic regression model for the whole population and according to the age group and/or the sex, depending on the rodent species. Pregnant females for each species were excluded from the

study, including 41 *P. obesus*; 9 *M. shawi* and one *C. gundi*. Predictive values were calculated for each age group and/or sex and the total population for each species of rodent (Table 5).

**Table 5:** Predictive model for each age group and/or sex for each species of rodent for eyes lens weight estimation using multivariable analysis. B coefficient, standard error (SE) and confidence interval (CI) were determined for each significant parameter.

Models	Morphometric Parameters	B Coefficient	SE	CI	P
<i>Psammomys obesus</i>					
Total	HBL (mm)	0.61	0.33	[0.54-0.67]	< 0.001
Total males	HBL (mm)	0.77	0.07	[0.62-0.93]	< 0.001
	HFL (mm)	- 1.91	0.66	[(- 3.23)-(- 0.59)]	0.005
Total females	HBL (mm)	0.66	0.04	[0.58-0.75]	< 0.001
Total juveniles	HFL (mm)	1.64	0.41	[0.79-2.48]	< 0.001
Juveniles males	/	/	/	/	/
Juveniles females	HFL (mm)	1.10	0.06	[0.38-1.83]	0.004
	TL (mm)	0.14	0.35	[0.01-0.28]	0.034
Total subadults	HBL (mm)	0.72	0.05	[0.01-0.28]	< 0.001
Subadults males	EL (mm)	2.07	0.98	[0.13-4.02]	0.036

	HBL (mm)	0.66	0.10	[0.45-0.87]	< 0.001
	HFL (mm)	- 1.84	0.82	[(- 3.47)-(- 0.21)]	0.27
Subadults females	HBL (mm)	0.82	0.08	[0.66-0.98]	< 0.001
Total adults	HBL (mm)	0.59	0.21	[0.15-1.02]	0.009
	HFL (mm)	- 4.03	1.31	[(- 6.67)-(- 1.39)]	0.003
Adults males	HBL (mm)	0.59	0.21	[0.16-1.03]	0.009
	HFL (mm)	- 4.78	1.30	[(- 7.43)-(- 2.14)]	0.001
Adults females	/	/	/	/	/
<i>Meriones shawi</i>					
Total	HBL (mm)	0.97	0.08	[0.80-1.14]	< 0.001
	HFL (mm)	- 1.16	0.53	[(- 2.22)-(- 0.10)]	0.032
Total males	HBL (mm)	0.77	0.07	[0.62-0.91]	< 0.001
Total females	HBL (mm)	0.93	0.08	[0.76-1.10]	< 0.001
Total juveniles	HBL (mm)	0.71	0.10	[0.51-0.92]	< 0.001
Juveniles males	EL (mm)	1.81	0.77	[0.22-3.40]	0.027
	TL (mm)	0.24	0.08	[0.07-0.41]	0.006
	HFL (mm)	1.13	0.36	[0.37-1.88]	0.005
Juveniles females	HBL (mm)	0.91	0.15	[0.60-1.21]	< 0.001
Total subadults	HBL (mm)	1.12	0.25	[0.16-1.63]	< 0.001
Subadults males	/	/	/	/	/
Subadults females	HBL (mm)	1.43	0.29	[0.84-2.01]	< 0.001
Total adults	/	/	/	/	/
Adults males	/	/	/	/	/
Adults females	/	/	/	/	/
<i>Ctenodactylus gundi</i>					
Total	EL (mm)	- 8.83	1.06	[(- 10.94)-(- 6.71)]	< 0.001
	HBL (mm)	1.14	0.24	[0.66-1.62]	< 0.001
	Sex	17.17	6.36	[4.52-29.82]	0.008
Total males	EL (mm)	- 9.62	1.50	[(- 12.66)-(- 6.57)]	< 0.001
	HBL (mm)	1.14	0.30	[0.52-1.75]	0.001
Total females	HFL (mm)	- 10.92	2.10	[(- 15.16)-(- 6.68)]	< 0.001
	HBL (mm)	1.66	0.44	[0.76-2.56]	0.001

EL: ear length; HBL: head and body length; HFL: hind feet length; TL: tail length.

In *P. obesus*, the overall correlation coefficient between HBL and ELW was 0.61 ( $P < 0.001$ ). In *M. shawi*, the overall correlation coefficient between HBL and ELW was 0.97 ( $P < 0.001$ ), and between HFL and ELW it was - 1.16 ( $P = 0.032$ ). In *C. gundi*, the overall correlation coefficient between EL and ELW was - 8.83 ( $P < 0.001$ ), between HBL and ELW it was 1.14 ( $P < 0.001$ ) and between sex and ELW it was 17.17 ( $P = 0.008$ ). Those variables allow us to establish the following final equations for the total population of each

species of rodents:

ELW = 0.61 HBL - 51.09 for *P. obesus*,  
 ELW = 0.97 HBL - 1.16 HFL - 24.48 for *M. shawi*, and  
 ELW = 17.17 Sex + 1.14 HBL - 8.83 EL + 4.87 for *C. gundi* population.

Additionally, established equations for each age group and/or sex according to the rodent species are presented in Table 6.

**Table 6:** Predictive equations for each species of rodent according to the age category and the sex. R square was determined for each significant equation.

Model (n)	Equations	R square
<i>Psammomys obesus</i>		
Total (431)	ELW = 0.61 HBL - 51.09	0.45
Total males (176)	ELW = 0.77 HBL - 1.91 HFL - 9.32	0.45
Total females (255)	ELW = 0.66 HBL - 57.28	0.48
Total juveniles (46)	ELW = 1.64 HFL - 34.76	0.25
Juveniles males (11)	/	/
Juveniles females (35)	ELW = 0.14 TL + 1.10 HFL - 31.18	0.61
Total subadults (336)	ELW = 0.72 HBL - 67.00	0.31
Subadults males (127)	ELW = 0.66 HBL + 2.07 EL - 1.84 HFL - 27.84	0.38
Subadults females (209)	ELW = 0.82 HBL - 80.07	0.33
Total adults (49)	ELW = 0.59 HBL - 4.03 HFL + 101.82	0.19
Adults males (38)	ELW = 0.59 HBL - 4.78 HFL + 129.46	0.29
Adults females (11)	/	/
<i>Meriones shawi</i>		
Total (158)	ELW = 0.97 HBL - 1.16 HFL - 24.48	0.59
Total males (70)	ELW = 0.77 HBL - 40.20	0.61

Total females (88)	ELW = 0.93 HBL - 55.87	0.58
Total juveniles (65)	ELW = 0.71 HBL - 33.47	0.43
Juveniles males (29)	ELW = 1.81 EL + 0.24 TL + 1.13 HFL - 51.59	0.59
Juveniles females (38)	ELW = 0.91 HBL - 52.85	0.52
Total subadults (69)	ELW = 1.12 HBL - 85.11	0.22
Subadults males (20)	/	/
Subadults females (49)	ELW = 1.43 HBL - 124.26	0.34
Total adults (24)	/	/
Adults males (21)	/	/
Adults females (3)	/	/
<i>Ctenodactylus gundi</i>		
Total (91)	ELW = 17.17 Sex + 1.14 HBL - 8.83 EL + 4.87	0.49
Total males (45)	ELW = 1.14 HBL - 9.62 EL + 34.60	0.55
Total females (46)	ELW = 1.66 HBL - 10.92 HFL + 249.39	0.38

EL: ear length; HBL: head and body length; HFL: hind feet length; TL: tail length.

#### 4. Discussion

The aim of this study was to identify intra-specific morphometric variation and correlate standard morphometric measurement to the relative age using ELW in three species of Tunisian wild rodents (*P. obesus*, *M. shawi* and *C. gundi*).

*M. shawi* morphometric measurements described in this study were smaller than those reported by Darvish <sup>[25]</sup>, which can be related to the diversity of North African origin of specimens used in its study. However, body measurements were similar to those previously reported <sup>[26]</sup>, who trapped their study population of *P. obesus* in the same governorate than the present study. Similarly, *C. gundi* measurements in the present study were comparable with those previously described <sup>[27]</sup>.

Our study shown that the geographical distribution plays an important role in the morphometric variability observed in *P. obesus* and *C. gundi* but not in *M. shawi*. This could be related to bioclimatic zones in Tunisia. *C. gundi* captured in the south were in arid Saharan areas, whereas those captured in central areas were in semi-arid regions. The homogeneity observed in *M. shawi* could be a reflection of the small number of rodents trapped at some study sites.

Variation in body weight and length measurements in male *P. obesus* could be explained in part by the hazardous nature of the trapping method used in this study which does not consider gender activity among this species of rodents. Variation in morphometric measurements of the three species of rodents in body weight, head-body and tail lengths could be explained by the variation in age proportions in naturally coexisting populations of rodents, which vary over time. Additionally, a continual change in rodent species modifies the morphology and physiology of individual rodents in each population, as a response to both the physical environment and the interactions with other organisms such as pathogens <sup>[28]</sup>. As previously described, two species (*P. obesus* and *M. shawi*) are recognized as reservoirs of *Leishmania major* (Yakimoff and Schokhor, 1914) parasites; this infection can impact the preferential site of infection, particularly the ears and tails, allowing the partial or total destruction of these organs <sup>[16]</sup> which modifies measurements of these two organs. That can explain the removal of these morphometric measurements from the final model for these two rodent species.

Age structure of the study sample based on ELW suggests that *C. gundi* has a greater longevity than *M. shawi* and *P. obesus*. The lowest ELW class (< 20 mg) includes few *P.*

*obesus* and no *M. shawi* and *C. gundi* species. However, ELW might be influenced by rodent weight differences and/or the effect of trapping method used; it is possible that small rodents escaped from the unabated pincer traps. Additionally, the high ELW variability, ranging from > 20 mg to 225 mg, could affect choice of age class and consequently have an important influence on age estimation. ELW varied significantly, depending on the trapping site for all three rodent species. That could be because of genetic differences in eye lens growth rates in sub-species within the same species, as previously described for *Rattus rattus* (Linnaeus, 1758) <sup>[29]</sup> or, more simply, by differences in the population structure for each species at the different sites.

This preliminary study suggests that HBL is a reliable age indicator for the three rodent species studied. The regression analysis established an equation for each species of rodents to relatively estimate age from HBL. In *P. obesus*, HBL was the best predictor for ELW; however in *M. shawi* it was associated with HFL and in *C. gundi* with EL and sex. Interestingly, in *M. shawi* population, a negative correlation between HFL and ELW was detected (Table 5), which could be explained by the fact that this species of rodent has specific leg morphology enabling it to run fast, but not to jump <sup>[30]</sup>. Similarly, a negative correlation was identified between EL and ELW in *C. gundi*. This could be explained by the specific ears morphology; which are shortness, with a large opening allowing a greater width, and have an oval aspect <sup>[31]</sup>.

HBL correlation with ELW decreased with age group, from 1.64 in juveniles to 0.59 in adults *P. obesus*, while it increased from 0.71 in juveniles to 1.43 in subadults *M. shawi* (Table 5). This surprising result can be a species characteristic and/or specificity.

Sex differences were identified for the whole population and in each age category. Besides, stronger correlations were identified in males, for HBL as well as other morphometric measurements such as HFL, EL and TL; and in females for HBL and HFL (in two age category). That may reflect females morphometric homogeneity compared to males. Unfortunately, because of the small number of rodents in each age category, it was not possible to calculate some equations. Additionally, for some equations, the correlation, indicated by the *R* square value, only identified a slight or fair agreement; therefore these results should be interpreted with caution. Furthermore, we could not find similar investigations on those species of rodent species and there age category in the international literature, which precludes a comparison.

## 5. Conclusion

The ability to estimate age from ELW according to the morphometric measurements in these three species of wild rodents will be useful in laboratory practice and medical research as it eliminates the need for postmortem examination.

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