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Original Study

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Assessment of craniometric sexual dimorphism and ontogenetic variation in invasive *Rattus norvegicus* and *R. rattus* from urban and peri-urban areas of Gauteng Province, South Africa

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Abstract: We evaluated craniometric sexual dimorphism and ontogenetic (age) variation in invasive *Rattus norvegicus* and *R. rattus* from urban and peri-urban areas of Gauteng Province, South Africa, using univariate and multivariate analyses. Two-way analysis of variance (ANOVA), percent contribution of the sum of squares (%SSQs) of each source of variation, principal components analysis (PCA) and unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis showed no sexual dimorphism in both species, however in both species, significant age variation between five age classes based on maxillary molar toothrow cusp eruption and wear was found and the age classes were pooled into juveniles (i.e., individuals of tooth-wear class I), sub-adults (II–III), and adults (IV–V). Few variables showed statistically significant sex-age interaction. The largest %SSQs to the total variance were due to *error* (i.e., *residual*), suggesting that apart from sex, age, and their interaction, there were other components that are responsible for the variation. Our approach may be useful for partitioning the effect of sexual dimorphism and ontogenetic variation in other studies, such as our stable isotope analysis-based trophic ecological studies of *Rattus* species from urban and peri-urban areas of Gauteng Province, South Africa.

Keywords: craniometrics; invasive; muridae; ontogenesis; synanthropic.

1 Introduction

Three invasive synanthropic murid species, the brown rat (*Rattus norvegicus*), the black rat (*Rattus rattus*), and the Tanezumi rat (*Rattus tanezumi*) have been reported to occur in South Africa (Bastos et al. 2011) where they cause negative health and socio-economic impacts, such as zoonotic diseases and damage to agricultural products and infrastructure (Kay and Hoekstra 2008). *R. norvegicus* is considered to have originated from eastern Siberia and northern China (Long 2003) and was first recorded in South Africa in 1832 after the arrival of the first Europeans (Avery 1982). *R. rattus* is considered to have originated from India and southern Asia, and spread to almost all the continents, except Antarctica (Aplin et al. 2003). The native range of *R. tanezumi* is considered to stretch from Afghanistan to the islands of Japan (Long 2003) and was only recorded to occur in South Africa (and Africa) ~15 years ago following routine genetic profiling of rodents in the country (Bastos et al. 2011). *Rattus rattus* and *R. tanezumi* are morphologically indistinguishable, leading to both species being placed within the *R. rattus* species complex (Musser and Carleton 2005), and may explain why *R. tanezumi* was not detected in South Africa (and Africa) until recently (Bastos et al. 2011).

R. norvegicus was considered to be restricted to the coastal regions of South Africa, however recent species

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distribution models (SDMs) have predicted that it may not only be restricted to the coast but also occurs in inland urban areas (Ringani et al. 2022). *Rattus rattus* was considered to be widely distributed throughout South Africa, except in the drier parts (Skinner and Smithers 1990), and this distribution is also supported by recent SDMs (Ringani et al. 2022). Although the distribution of *R. tanezumi* remains poorly documented (Ramatla et al. 2019), recent SDMs predicted it to mainly occur in most inland urban areas and along the coast (Ringani et al. 2022). All these SDMs showed that all three species of *Rattus* have wide distributions in South Africa that overlap extensively, suggesting that their establishment and spread may be influenced by similar factors such as proximity to urban areas under wet and moderate climatic conditions (Ringani et al. 2022). In Gauteng Province, all three species occur in urban and peri-urban landscapes that are related to general lack of sanitation and municipal services particularly in informal human settlements (Jassat et al. 2013).

A stable isotope analysis (SIA) study was initiated to assess the trophic ecology of the three *Rattus* species in urban and peri-urban areas of Gauteng Province and evaluate how access to available food resources and associated niche complementarity may aid their establishment. However, given that sexual dimorphism and ontogeny can affect patterns of food resource use and trophic inter-relationships among rodents (Kajiura and Rollo 2011), it was necessary to determine the nature and extent of sexual dimorphism and ontogenetic variation within the species based on craniometric data which forms the subject of the current study.

Sexual dimorphism and ontogenetic variation represent critical life history traits of rodents (Schulte-Hofstedde 2007), and together with other factors such as habitat and season can affect the trophic niche utilised by a species (Hobson and Quirk 2014). While pelage colouration and body mass have been used to age small mammals, these are considered inappropriate, as they may be influenced by factors such as the concentration of humic substances in the soil that are created during the decomposition of organic matter (Rios and Álvarez-Castañeda 2007) and the availability and accessibility of quality food (van Rensburg et al. 2004), respectively. Maxillary molar eruption and wear, and their associated craniometric analysis, however, are considered more appropriate for ageing small mammals (Chimimba and Dippenaar 1994). While craniometric analysis of sexual dimorphism and ontogenetic variation based on a range of univariate analyses can be used for ageing small mammals, Leamy (1983) suggested the use of the more computationally simplistic percent contribution of the sum of squares (%SSQ) of each source of variation that can be computed from a two-way analysis of variance (ANOVA) table.

The aim of the present study therefore, was to first independently assess the nature and extent of sexual dimorphism and ontogenetic variation in *R. norvegicus* and *R. rattus*, two of the three invasive synanthropic species that occur in urban and peri-urban landscapes in Gauteng Province in South Africa that had adequate samples for analysis, using two-way ANOVA and %SSQ for comparison, and multivariate analyses based on craniometric data. This was undertaken in order to ascertain: 1) whether the sexes should be pooled or analysed separately; and 2) which samples represent different relative age groups (i.e., juveniles, sub-adults, and adults) within the sampled populations of the two species.

2 Materials and methods

2.1 Study area and sampling

Genetically-identified (through Cyt *b* mtDNA sequencing) *R. norvegicus* and *R. rattus* in this study were from urban Alexandra (−26.1038° S; 28.0962° E) and Tembisa (−26° S; 28.214° E) Townships, and peri-urban University of Pretoria (UP) Experimental Farm (−25.749° S; 28.238° E), Gauteng Province, South Africa (Figure 1; Supplementary Appendices 1–3) and were sourced from previous studies (Bastos et al. 2011; Julius 2013; Lithole 2015; Mostert 2009). These were either live-trapped using Sherman traps (H.B. Sherman Traps Inc. Florida, U.S.A.), obtained opportunistically from members of the public and pest control companies, or through an invasive synanthropic rodent extermination programme by the Johannesburg Metropolitan Municipality, Gauteng Province, South Africa. Specimens were weighed and dissected in a biosecurity level 2 laboratory in the Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa. All specimens are currently housed in the Department of Zoology and Entomology at the University of Pretoria, Pretoria, South Africa and their voucher numbers are provided in Supplementary Appendices 1–3, and will eventually be deposited in the Ditsong Museum of Natural History (formerly the Transvaal Museum), Pretoria, South Africa.

2.2 Skull preparation, ageing, and cranio-dental measurements

Skulls were soaked in a 90 °C detergent/water solution to loosen connective tissue and dissolve adipose tissue (Mairs et al. 2004), and loosened tissue was removed, and dried skulls examined under a Vickers stereomicroscope (Vickers Instruments Ltd., York, England). Skulls were allocated to five relative age classes based on the degree of maxillary molar toothrow cusp eruption and wear (Chimimba and Dippenaar 1994) as exemplified by *R. norvegicus* from Alexandra Township (Figure 2) that had the largest sample of both sexes and all five tooth wear classes (Table 1). As modified from Chimimba and

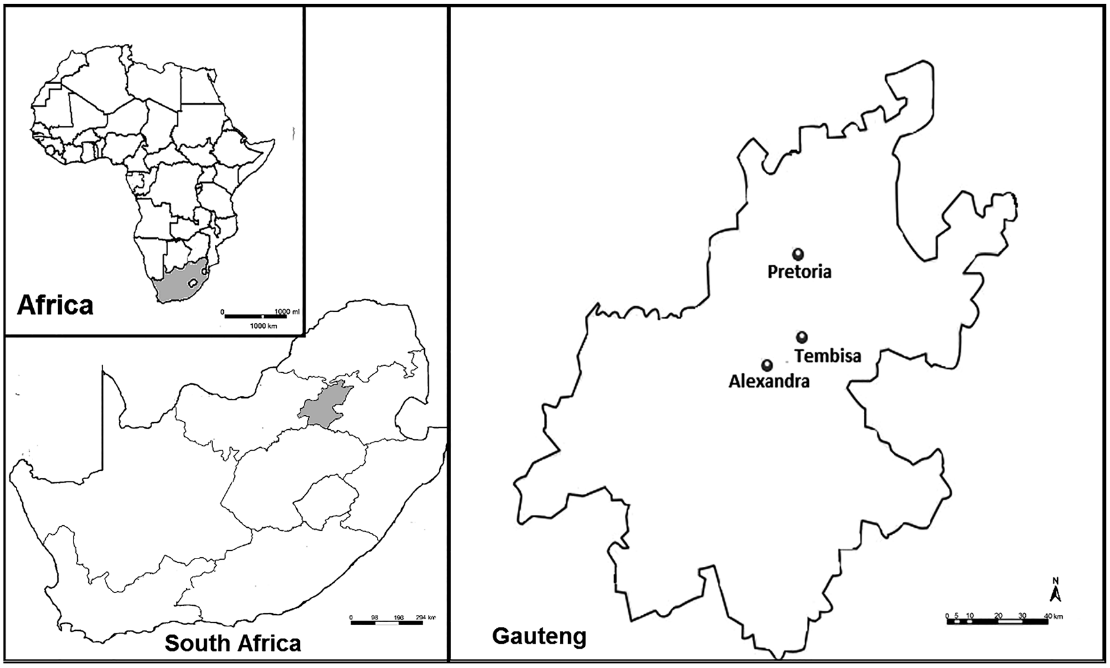


Figure 1: A map (with an insert of Africa and South Africa) showing the sampling sites of *R. norvegicus* and *R. rattus* in Gauteng Province, South Africa.

Dippenaar (1994), and exemplified by *R. norvegicus*, sexual dimorphism and ontogenetic variation were assessed using 13 linear cranio-dental measurements (Figure 3) recorded to the nearest 0.05 mm using a pair of Mitutoyo digital callipers (Mitutoyo American Corporation, Aurora, Illinois, U.S.A.).

2.3 Statistical analysis

Craniometric data were first tested for normality and homoscedasticity, and subjected to a two-way analysis of variance (ANOVA) to test for sexual dimorphism, age variation, and their interaction. Where statistically significant differences were detected, Tukey's *post hoc* tests were used to identify non-significant subsets of relative age classes ($P > 0.05$). The percent sum of squares (%SSQs) from the derived two-way ANOVA table were used to identify sources of variation (Leamy 1983) with reference to sex, relative age, their interaction, and *error* (i.e., *residual*). The %SSQs were computed by dividing the SSQ associated with each source of variation by the total SSQ. The samples however, were characterised by small sample sizes in some sampling localities and age classes, such that not all age classes could be subjected to univariate analysis.

Because of equivocal results of some univariate analyses, multivariate analyses were also used to further evaluate sexual dimorphism and age variation. This included principal components analysis (PCA) of standardized variables based on correlation coefficients among variables (Sneath and Sokal 1973). However, given some relatively small sample sizes, the data for each of the three populations were first subjected to Kaiser–Meyer–Olkin (KMO) tests in order to assess their suitability for PCA (Kaiser 1970). These exploratory analyses revealed that unlike the data for *R. rattus* from UP Experimental Farm (KMO = 0.41), the data for *R. norvegicus* from Alexandra (KMO = 0.82) and Tembisa (KMO = 0.68) Townships were at least suitable for PCA. Additional exploratory PCAs also revealed relatively low levels of variation explained by successive principal components to the total variance (e.g., the first two PCA axes of *R. norvegicus* from Alexandra (60.52%); see Results section).

Consequently, the results of the exploratory analyses and for comparison, the data for the three populations were further subjected to Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis based on Euclidean distances and correlation coefficients among groups (Sneath and Sokal 1973) which are not constrained by the data suitability requirements of PCA. Unlike the univariate analyses, the multivariate analyses also allowed the analysis of a wider range of localities, sexes, and age classes regardless of sample size (see Table 1). All univariate and multivariate analyses were based on the 13 cranio-dental measurements and were based on algorithms in R (R Core Team 2021) and in Paleontological Statistics Software package (PAST) (Hammer et al. 2001).

3 Results

3.1 Univariate assessment

The results of two-way ANOVA and %SSQs of sexual dimorphism and ontogenetic variation of *R. norvegicus* from Alexandra and Tembisa Townships, and *R. rattus* from UP Experimental Farm (Table 2) were broadly similar. The two-way ANOVA showed more cranio-dental measurements with statistically significant *F*-values for age than for sex in all the three samples (Table 2). Age also gave

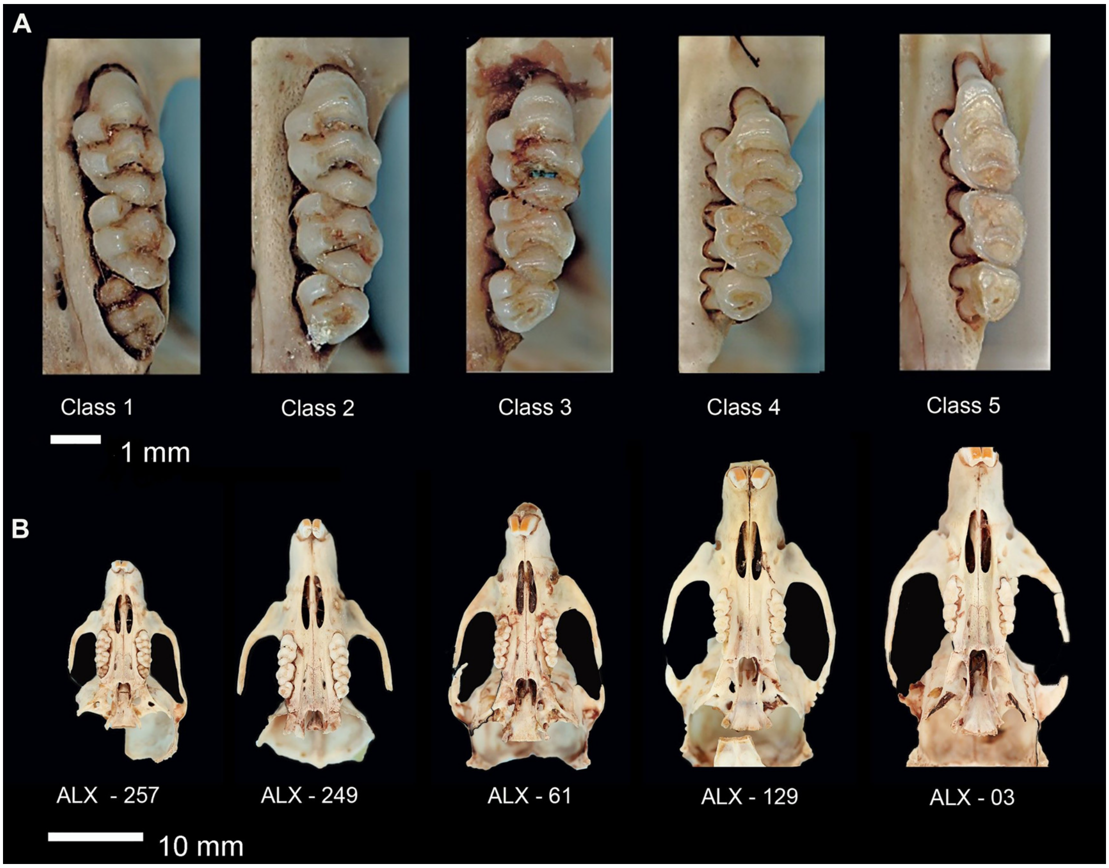


Figure 2: (A) Left maxillary molar toothrow showing the degree of molar eruption and wear and definitions of five tooth-wear classes (I–V) used for relative ageing of *R. norvegicus and R. rattus* from Gauteng Province, South Africa. Age class I: M^3 in the process of erupting, with no erosion of enamel on either M^1 or M^2, and if erosion was apparent, it was slight with no connections between the dentine areas; Age class II: The enamel was lightly worn in some areas and dentine was slightly apparent where erosion of enamel occurred, and dentine areas may have joined cusps together; Age class III: The cusps were connected by dentine areas either longitudinally or transversely where enamel was eroded; Age class IV: The enamel was completely worn, although outlines of the cusps still remained; and Age class V: The enamel was completely worm and only the underlying dentine was visible, with the cusp outlines being barely visible. Scale = 1 mm. (B) Ventral view and specimen voucher number of each representative skull positioned under its corresponding left maxillary molar toothrow. Scale = 10 mm.

Table 1: Sample size (*n*) of sexes and relative age classes of *R. norvegicus* from Alexandra and Tembisa Townships, and *R. rattus* from the University of Pretoria (UP) Experimental Farm, Gauteng Province, South Africa used for craniometric analyses. Age classes are defined and illustrated in Figure 2.

Species	Sampling site	Sex	Age class (*n*)					Total
			I	II	III	IV	V	
R. norvegicus	Alexandra Township	Male	1	2	6	20	1	30
		Female	1	2	8	15	6	32
	Tembisa Township	Male	1	–	2	4	–	7
		Female	–	–	3	8	1	12
R. rattus	UP Experimental Farm	Male	1	1	5	3	–	10
		Female	–	–	1	4	–	5

higher %SSQ values (range %SSQ = 0.76–56.55 for *R. norvegicus* from Alexandra Township; range %SSQ = 3.08–64.96 for *R. norvegicus* from Tembisa Township; range %SSQ = 4.84–66.63 for *R. rattus* from UP Experimental Farm than for sex [range %SSQ = 0.01–6.39 for *R. norvegicus* from Alexandra Township; range %SSQ = 0.18–15.93

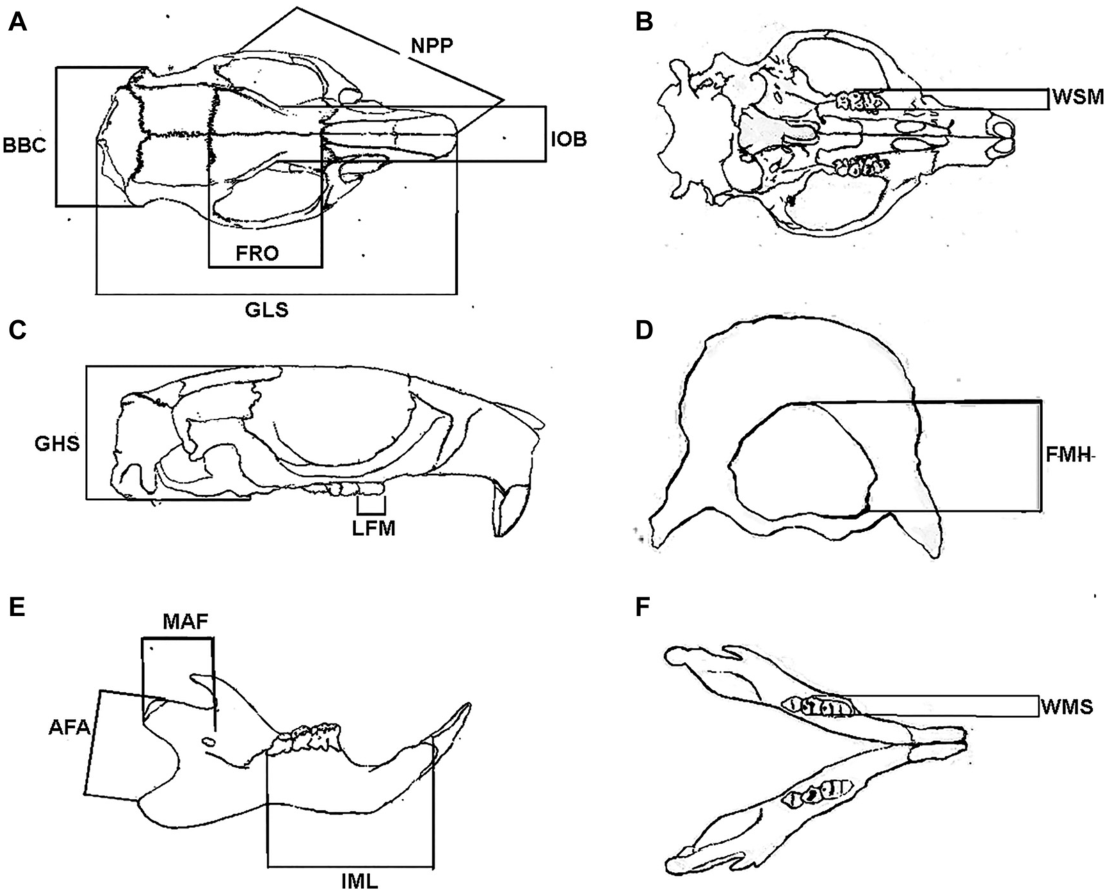


Figure 3: Dorsal (A), ventral (B), lateral (C), posterior (D) views of the cranium, and medial (E), and dorsal (F) views of the dentary showing abbreviations and reference points of cranio-dental measurements recorded from specimens of *R. norvegicus* and *R. rattus* from Alexandra and Tembisa Townships, and the University of Pretoria (UP) Experimental Farm, Gauteng Province, South Africa. 1) GLS: Greatest length of skull, taken from the anterior edge of the nasal bone to the posterior edge of the skull; 2) FRO: Greatest length of the frontals; 3) NPP: Distance measured from the anterior edge of the nasal bone to the anterior edge of the zygomatic arch; 4) BBC: Breath of brain-case; 5) IOB: Interorbital constriction, measured as the least distance between the orbits; 6) GHS: Greatest height of the skull, measured perpendicular to the horizontal plane; 7) FMH: Foramen magnum height; 8) LFM: Length of M^1; 9) WSM: Width of M^2; 10) AFA: Distance from the angular process to the mandibular condyle; 11) MAF: Distance from the mandibular foramen to the mandibular condyle; 12) IML: Distance from the posterior incisor to M^3; 13) WMS: Width of M^2.

for *R. norvegicus* from Tembisa Township; and range %SSQ = 1.52–25.05 for *R. rattus* from UP Experimental Farm (Table 2)].

The greatest length of the skull (GLS), the distance from nasal bone to the anterior edge of the zygomatic arch (NPP), and the distance from the angular process to the mandibular condyle (AFA) showed statistically significant age variation in all the three samples (Table 2). These cranio-dental measurements also contributed relatively more to the total variance due to age variation (Table 2). Only one cranio-dental measurement, the distance from the posterior incisor to M^3 (IML) in *R. norvegicus* from Alexandra Township, was statistically significant for both sexual dimorphism and age variation, and together with the width of M^2 (WSM) in *R. rattus* from UP Experimental Farm, were the only two cranio-dental measurements that showed a statistically significant interaction between sex and age (Table 2). The interaction between sex and age showed a negligible contribution in all three samples as indicated by the relatively small mean %SSQs (mean %SSQ = 7.88 for *R. norvegicus* from Alexandra Township); mean %SSQ = 2.84 for *R. norvegicus* from Tembisa Township; and mean %SSQ = 6.65 for *R. rattus* from UP Experimental Farm) (Table 2). In all the three samples, the largest %SSQs were due to *error* (i.e., *residual*) (mean % SSQ = 62.57 for *R. norvegicus* from Alexandra Township; mean %SSQ = 60.47 for *R. norvegicus* from Tembisa Township; and mean %SSQ = 49.90 for *R. rattus* from UP Experimental Farm (Table 2).

Tukey's *post hoc* test of *R. norvegicus* from Alexandra, the only locality with adequate age class samples for

Table 2: *F*-values and percent sum of squares (%SSQ) of each source of variation from a two-way analysis of variance (ANOVA) of five relative age classes (I–V) of male and female *R. norvegicus* from Alexandra and Tembisa Townships, and *R. rattus* from the University of Pretoria (UP) Experimental Farm, Gauteng Province, South Africa.

	***R. norvegicus* (Alexandra)**							***R. norvegicus* (Tembisa)**							***R. rattus* (UP experimental farm)**						
	***F*-values**			**%SSQ**				***F*-values**			**%SSQ**				***F*-values**			**%SSQ**			
Cranio-dental measurement	**Sex (S)**	**Age (A)**	**S × A**	**Sex (S)**	**Age (A)**	**S × A**	***Error***	**Sex (S)**	**Age (A)**	**S × A**	**Sex (S)**	**Age (A)**	**S × A**	***Error***	**Sex (S)**	**Age (A)**	**S × A**	**Sex (S)**	**Age (A)**	**S × A**	***Error***
GLS	0.59	18.08^{c}	2.13	0.78	56.55	6.00	36.67	1.36	7.97^{a}	0.56	6.15	50.88	2.07	40.90	0.43	11.36^{b}	2.96	5.55	57.02	9.25	28.17
FRO	0.35	0.90	0.97	1.54	4.99	6.49	86.98	0.36	8.85^{a}	0.04	1.70	51.66	0.16	46.49	0.26	7.73^{a}	1.64	4.33	51.09	6.86	37.73
NPP	2.25	15.26^{c}	3.13	0.22	50.04	9.65	40.09	2.28	19.56^{c}	0.27	5.52	64.96	0.71	28.81	1.80	9.15^{a}	0.02	10.78	44.89	0.10	44.23
BBC	2.28	5.13^{c}	2.49	0.01	23.44	12.31	64.25	0.24	1.93	0.67	2.32	24.88	4.15	68.64	0.74	5.02	0.09	6.64	35.55	0.54	57.27
IOB	0.31	2.71^{a}	1.37	0.61	17.61	7.78	74.00	0.28	0.68	1.77	0.78	12.87	11.97	74.39	1.03	0.68	0.74	15.90	4.84	6.04	73.22
GHS	2.28	12.90^{c}	3.17	0.30	44.85	10.75	44.10	2.33	12.37^{b}	0.00	7.63	56.91	0.00	35.46	0.70	2.84	4.09	12.83	24.38	19.64	43.16
FMH	0.12	0.10	0.15	2.13	0.76	1.08	96.03	0.87	0.77	0.15	6.17	6.79	1.18	85.86	0.34	0.19	0.04	4.86	5.47	0.41	89.26
LFM	1.11	1.08	1.10	0.38	8.42	7.13	84.06	0.01	1.08	0.07	0.18	8.33	0.59	90.89	0.33	6.40^{a}	0.02	1.52	54.68	0.08	43.71
WSM	0.29	0.07	0.53	1.05	1.28	3.81	93.86	1.93	1.32	0.46	15.93	3.08	3.26	77.74	2.55	0.04	5.16^{a}	4.54	9.44	31.36	54.66
AFA	0.60	11.72^{c}	1.33	3.11	49.50	4.39	43.00	0.27	4.98^{a}	1.44	2.17	42.71	6.38	48.73	0.81	17.00^{b}	0.86	4.89	66.63	2.50	25.98
MAF	0.00	8.61^{c}	1.66	6.39	34.68	6.67	52.25	0.19	4.95^{a}	0.02	1.02	35.76	0.14	63.08	1.51	12.51^{b}	0.48	9.26	54.83	1.80	34.11
IML	16.98^{c}	27.66^{c}	8.20^{c}	3.89	55.52	15.70	24.90	0.73	4.23	0.56	4.67	38.29	2.78	54.27	1.24	4.91	0.00	9.64	30.23	0.01	60.13
WMS	2.62	2.79^{a}	1.89	0.34	15.80	10.67	73.19	1.80	1.90	0.55	10.06	15.51	3.57	70.86	2.18	0.54	1.25	25.05	9.92	7.93	57.10
Mean				1.60	27.96	7.88	62.57				4.95	31.74	2.84	60.47				8.91	34.54	6.65	49.90

Cranio-dental measurements are defined and illustrated in Figure 3. Statistical significance: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

analysis showed that the following three groupings of age classes were not significantly different from each other (Table 3): 1) individuals of age class I being mainly in its own non-significant subset, with few individuals of age classes II–V; 2) individuals of age class II being in the same non-significant subset with individuals of age class III, with few individuals of age classes I, IV–V; and 3) individuals of age classes IV and V being mainly in the same non-significant subset of age classes, with a few individuals of age classes I–III. Although equivocal, the general pattern that emerged from the Tukey's *post hoc* tests of this sample suggested that age class I may represent juveniles, age classes II and III may be subadults, while age classes IV and V may represent adult specimens. The progression of cranial-dental dimensions from juveniles to adult individuals is best exemplified by the standard descriptive statistics of *R. norvegicus* from Alexandra (Table 4A) and Tembisa (Table 4B) Townships, and *R. rattus* from UP Experimental Farm (Table 4C) that showed a direct relationship between cranial-dental measurement magnitude and relative age.

3.2 Multivariate assessment

Because of some equivocal results of the Tukey's *post hoc* tests, multivariate PCA and UPGMA cluster analyses were also used to further evaluate sexual dimorphism and age variation in *R. norvegicus* and *R. rattus*. These analyses allowed the evaluation of the two species from the three sampling sites irrespective of within-cell sample size limitations of the sexes and age classes. Similar to the results of the univariate analyses, the results of the multivariate analyses were broadly similar.

The PCA of all three populations of *Rattus* generated 13 principal components (PC) axes, with most eigenvalues that explain the variables responsible for the variability, and the percent contribution to the total variance being attributed to the first two PC axes (*R. norvegicus* from Alexandra Township: eigenvalue for the first two PC axes = 7.87, total % variance contribution of the first two PC axes = 60.25%); *R. norvegicus* from Tembisa Township: 9.17, 70.48%; *R. rattus* from UP Experimental Farm: 9.14, 70.35%) (Table 5). The PCA scattergram of *R. norvegicus* from Alexandra Township, which was represented by all sexes and all five age classes, reflects age rather than sex as a major source of variation (Figure 4). The single individual of age class I tended to separate from the rest of the age classes. While there was no separation between individuals of age classes II and III, the individuals of these two age classes, with some overlaps, tended to separate from individuals of age classes IV and V. The PCA scattergram of *R. norvegicus* from Tembisa Township, which was represented by all sexes and age classes I (by a single individual), III, IV and V (by a single individual), also reflects age rather than sex as a major source of variation (Figure 4). The single individual of age class I tended to separate from the rest of the age classes. With a slight overlap (by two individuals), individuals of age classes III and IV were separate from each other. The single individual of age class V tended to separate from the rest of the age classes. The PCA scattergram of *R. rattus* from UP Experimental Farm (an analysis solely undertaken for comparison

Table 3: Tukey's *post hoc* tests of cranio-dental measurements (mm) of individuals of *R. norvegicus* of different age classes (I–V) that were sampled from Alexandra Township, Gauteng Province, South Africa.

Cranio-dental	Age class				
	I (*n* = 2)	II (*n* = 4)	III (*n* = 14)	IV (*n* = 36)	V (*n* = 7)
GLS	$33.98 \pm 3.1^{\mathrm{I}}$	$39.12 \pm 4.1^{\mathrm{II}}$	$41.69 \pm 2.8^{\mathrm{II,III}}$	$44.92 \pm 2.7\mathrm{I}^{\mathrm{V,V}}$	$47.31 \pm 1.2^{\mathrm{IV,V}}$
FRO	$11.64 \pm 0.2^{\mathrm{I,II}}$	$12.30 \pm 1.3^{\mathrm{I,II}}$	$20.26 \pm 0.9^{\mathrm{III}}$	$14.38 \pm 0.7^{\mathrm{IV,V}}$	$14.57 \pm 1.0\mathrm{I}^{\mathrm{V,V}}$
NPP	$24.21 \pm 2.4^{\mathrm{I}}$	$26.37 \pm 4.1^{\mathrm{I,II}}$	$29.17 \pm 2.5^{\mathrm{II,III}}$	$31.79 \pm 2.2^{\mathrm{III,IV,V}}$	$33.23 \pm 0.9^{\mathrm{IV,V}}$
BBC	$14.43 \pm 0.8^{\mathrm{I,II}}$	$15.27 \pm 1.7^{\mathrm{I,II}}$	$15.94 \pm 1.2^{\mathrm{III}}$	$16.56 \pm 0.7^{\mathrm{IV,V}}$	$16.45 \pm 1.0^{\mathrm{IV,V}}$
IOB	$6.71 \pm 0.9^{\mathrm{I,II}}$	$6.34 \pm 0.1^{\mathrm{I,II}}$	$6.45 \pm 0.7^{\mathrm{III}}$	$6.93 \pm 0.5^{\mathrm{IV,V}}$	$7.24 \pm 0.3^{\mathrm{IV,V}}$
GHS	$10.70 \pm 0.9^{\mathrm{I,II}}$	$12.11 \pm 1.1^{\mathrm{I,II}}$	$11.78 \pm 0.7^{\mathrm{I,II,III}}$	$12.88 \pm 0.6^{\mathrm{IV,V}}$	$13.14 \pm 0.6^{\mathrm{IV,V}}$
FMH	$5.02 \pm 0.3^{\mathrm{I,II}}$	$5.43 \pm 0.3^{\mathrm{I,II}}$	$5.26 \pm 0.8^{\mathrm{II,III}}$	$5.99 \pm 4.5^{\mathrm{IV}}$	$5.14 \pm 0.6^{\mathrm{I,II,III}}$
LFM	$3.24 \pm 0.3^{\mathrm{I,III}}$	$2.94 \pm 0.5^{\mathrm{II,III,IV}}$	$2.77 \pm 0.4^{\mathrm{II,III}}$	$3.03 \pm 0.4^{\mathrm{I,II,IV}}$	$2.73 \pm 0.3^{\mathrm{II,III,V}}$
WSM	$2.08 \pm 0.1^{\mathrm{I–V}}$	$2.06 \pm 0.1^{\mathrm{I–V}}$	$1.99 \pm 1.0^{\mathrm{I–V}}$	$2.01 \pm 0.2^{\mathrm{I–V}}$	$1.88 \pm 0.4^{\mathrm{I–V}}$
AFA	$6.29 \pm 0.4^{\mathrm{I}}$	$6.80 \pm 0.7^{\mathrm{II}}$	$7.29 \pm 0.9^{\mathrm{III}}$	$8.56 \pm 0.8^{\mathrm{IV,V}}$	$9.51 \pm 1.0^{\mathrm{III,IV,V}}$
MAF	$3.85 \pm 0.3^{\mathrm{I}}$	$4.40 \pm 0.8^{\mathrm{II}}$	$5.19 \pm 1.0^{\mathrm{III,IV,V}}$	$5.81 \pm 0.7^{\mathrm{III,IV,V}}$	$6.29 \pm 0.9^{\mathrm{III,IV,V}}$
IML	$8.40 \pm 5.7^{\mathrm{I}}$	$14.17 \pm 0.7^{\mathrm{II,III}}$	$14.89 \pm 1.7^{\mathrm{II,III}}$	16.02 ± 1.0	$17.00 \pm 1.0^{\mathrm{IV,V}}$
WMS	$1.54 \pm 0.6^{\mathrm{I–V}}$	$1.98 \pm 0.1^{\mathrm{II–V}}$	$1.81 \pm 0.3^{\mathrm{II–V}}$	$2.03 \pm 0.1^{\mathrm{II–V}}$	$1.85 \pm 0.4^{\mathrm{II–V}}$

Values are presented as mean ± 1 standard deviation (SD). Prescripts I–V represent non-significant subsets of relative age classes I–V ($P > 0.05$). *n* = sample size. Cranio-dental measurements are defined and illustrated in Figure 3.

Table 4: Standard descriptive statistics of cranio-dental measurements (mm) of males and females and age classes (I–V) of *R. norvegicus* from Alexandra (A) and Tembisa (B) Townships, and *R. rattus* from the University of Pretoria (UP) Experimental Farm (C), Gauteng Province, South Africa.

(A) Alexandra township

			Cranio-dental measurement												
Sex	**Age class**	**Statistics**	**GLS**	**FRO**	**NPP**	**BBC**	**IOB**	**GHS**	**FMH**	**LFM**	**WSM**	**AFA**	**MAF**	**IML**	**WMS**
Female	I (*n* = 1)	$\bar{X}$	31.75	11.45	22.49	13.83	6.05	10.06	4.78	3.46	2.22	5.99	3.59	12.50	1.10
		SD	–	–	–	–	–	–	–	–	–	–	–	–	–
		CV	–	–	–	–	–	–	–	–	–	–	–	–	–
	II (*n* = 2)	$\bar{X}$	36.86	11.40	23.29	14.31	6.20	11.20	5.40	3.33	2.01	6.47	3.88	13.59	2.05
		SD	3.09	0.35	0.16	2.30	0.06	0.18	0.49	0.18	0.01	0.33	0.54	0.40	0.07
		CV	8.38	3.10	0.70	16.06	1.03	1.58	9.04	5.32	0.35	5.03	14.05	2.97	3.45
	III (*n* = 8)	$\bar{X}$	42.72	13.65	29.90	16.38	6.70	12.01	5.36	2.84	2.23	7.41	5.62	15.69	1.87
		SD	3.15	1.04	2.60	1.03	0.79	0.79	0.81	0.47	1.37	1.14	0.90	1.48	0.38
		CV	7.37	7.61	8.69	6.28	11.85	6.55	15.17	16.63	61.75	15.42	15.95	9.44	20.11
	IV (*n* = 15)	$\bar{X}$	45.43	14.47	32.27	16.64	6.95	13.07	5.18	2.95	2.03	8.84	6.20	16.15	2.05
		SD	2.32	0.84	1.97	0.65	0.66	0.65	0.38	0.30	0.27	0.89	0.52	1.16	0.18
		CV	5.12	5.80	6.09	3.91	9.51	5.00	7.37	10.23	13.07	10.06	8.41	7.16	8.70
	V (*n* = 6)	$\bar{X}$	47.61	14.79	33.32	16.32	7.29	13.17	5.01	2.71	1.87	9.80	6.31	17.11	1.80
		SD	1.11	0.89	1.05	1.06	0.42	0.67	0.55	0.38	0.50	0.74	1.09	1.15	0.45
		CV	2.34	6.03	3.15	6.50	5.72	5.11	11.00	14.19	26.60	7.57	17.31	6.71	24.87
Male	I (*n* = 1)	$\bar{X}$	36.21	11.83	25.92	15.02	7.36	11.34	5.26	3.02	1.94	6.58	4.10	4.30	1.98
		SD	–	–	–	–	–	–	–	–	–	–	–	–	–
		CV	–	–	–	–	–	–	–	–	–	–	–	–	–
	II (*n* = 2)	$\bar{X}$	41.40	13.20	29.50	16.20	6.50	13.00	5.50	2.60	2.10	7.10	4.90	14.80	1.90
		SD	4.70	1.30	3.50	0.70	0.00	0.90	0.20	0.50	0.30	1.00	0.80	0.50	0.00
		CV	11.30	10.00	12.00	4.10	0.10	7.20	3.10	21.00	15.00	13.70	15.60	3.50	1.80
	III (*n* = 6)	$\bar{X}$	40.30	29.30	28.20	15.40	6.10	11.50	5.10	2.70	1.70	7.10	4.60	13.80	1.70
		SD	2.00	41.50	2.50	1.40	0.60	0.60	1.00	0.60	0.40	0.80	0.90	1.50	0.40
		CV	4.90	141.60	8.80	9.20	10.00	4.90	19.10	20.70	23.60	10.60	20.20	10.60	23.40
	IV (*n* = 20)	$\bar{X}$	45.00	14.4	31.70	16.60	7.00	12.80	6.70	3.10	2.00	8.40	5.60	16.10	2.00
		SD	2.50	0.50	2.10	0.70	0.60	0.60	6.10	0.60	0.30	0.80	0.70	0.80	0.20
		CV	5.50	3.70	6.60	4.50	8.10	5.00	91.10	18.40	13.40	9.00	12.50	4.70	9.60
	V (*n* = 1)	$\bar{X}$	45.50	13.20	32.70	17.30	7.00	13.00	5.90	2.80	1.90	7.80	6.20	16.40	2.20
		SD	–	–	–	–	–	–	–	–	–	–	–	–	–
		CV	–	–	–	–	–	–	–	–	–	–	–	–	–

(B) Tembisa township

Sex	Age class	Statistics	Cranio-dental measurement												
			GLS	FRO	NPP	BBC	IOB	GHS	FMH	LFM	WSM	AFA	MAF	IML	WMS
Female	III ($n = 3$)	$\overline{X}$	37.39	11.53	25.71	15.36	6.38	11.32	5.26	2.89	2.16	6.39	4.20	13.08	2.01
		SD	3.21	0.11	1.82	1.05	0.67	0.74	0.26	0.26	0.29	0.89	0.84	1.21	0.03
		CV	8.57	0.92	7.07	6.85	10.55	6.53	4.97	8.9	13.25	13.99	20.10	9.24	1.25
	IV ($n = 8$)	$\overline{X}$	43.90	13.88	29.21	16.48	6.85	12.84	5.48	3.06	2.11	8.29	5.20	14.71	2.16
		SD	3.02	1.08	1.58	0.88	0.27	0.44	0.29	0.34	0.13	0.92	0.88	0.93	0.14
		CV	6.88	7.78	5.41	5.35	3.91	3.46	5.33	11.08	6.30	11.07	16.87	6.35	6.31
Male	III ($n = 2$)	$\overline{X}$	36.63	11.23	23.9	15.53	6.78	10.65	5.49	2.85	2.09	6.77	3.92	13.02	2.16
		SD	3.92	2.80	0.33	0.33	0.06	1.76	0.04	0.49	0.04	1.12	0.43	0.83	0.04
		CV	10.72	24.93	1.39	2.09	0.83	16.54	0.77	17.37	2.03	16.62	11.02	6.36	1.64
	IV ($n = 4$)	$\overline{X}$	40.41	13.29	28.32	15.82	6.67	12.19	5.58	3.15	1.91	7.34	5.06	13.78	2.20
		SD	0.65	0.74	0.01	0.10	0.13	0.35	0.42	0.64	0.06	0.67	0.01	0.71	0.14
		CV	1.61	5.53	0.05	0.63	1.91	2.84	7.48	20.20	2.96	9.16	0.28	5.18	6.43

C) UP experimental farm

Sex	Age class	Statistics	Cranio-dental measurements												
			GLS	FRO	NPP	BBC	IOB	GHS	FMH	LFM	WSM	AFA	MAF	IML	
Female	III ($n = 1$)	$\overline{X}$	38.28	12.35	24.52	15.58	5.80	11.42	5.26	3.04	1.70	6.95	4.18	12.32	1.80
		SD	–	–	–	–	–	–	–	–	–	–	–	–	–
		CV	–	–	–	–	–	–	–	–	–	–	–	–	–
	IV ($n = 4$)	$\overline{X}$	40.48	12.83	27.77	16.62	5.79	11.32	5.12	2.58	1.81	8.01	5.14	13.34	1.98
		SD	2.39	0.36	1.61	1.07	0.21	0.63	0.51	0.21	0.06	0.67	0.62	0.43	0.23
		CV	5.91	2.77	5.79	6.46	3.68	5.58	10.04	8.34	3.35	8.34	12.14	3.23	11.83
Male	I ($n = 1$)	$\overline{X}$	30.35	11.13	18.24	12.05	5.08	9.30	5.36	3.02	1.73	5.52	3.48	10.25	1.74
		SD	–	–	–	–	–	–	–	–	–	–	–	–	–
		CV	–	–	–	–	–	–	–	–	–	–	–	–	–
	II ($n = 1$)	$\overline{X}$	40.86	12.98	28.79	16.87	6.01	11.16	5.77	3.07	1.86	7.91	5.2	13.53	1.90
		SD	–	–	–	–	–	–	–	–	–	–	–	–	–
		CV	–	–	–	–	–	–	–	–	–	–	–	–	–
	III ($n = 5$)	$\overline{X}$	36.86	12.11	25.87	15.88	5.84	11.08	5.34	3.18	1.87	6.94	4.36	12.83	1.77
		SD	2.12	0.37	2.01	0.66	0.60	0.33	0.12	0.23	0.07	0.43	0.53	0.90	0.06
		CV	5.74	3.04	7.79	4.17	10.28	2.99	2.22	7.24	3.71	6.19	12.16	6.99	3.32
	IV ($n = 3$)	$\overline{X}$	43.64	13.40	29.43	17.23	6.33	12.15	5.29	2.66	1.78	8.61	5.79	13.88	1.73
		SD	0.58	0.75	0.88	0.52	0.24	0.17	0.25	0.45	0.07	0.26	0.18	0.57	0.03
		CV	1.32	5.58	2.99	3.00	3.75	1.36	4.68	16.98	3.89	3.05	3.04	4.08	2.00

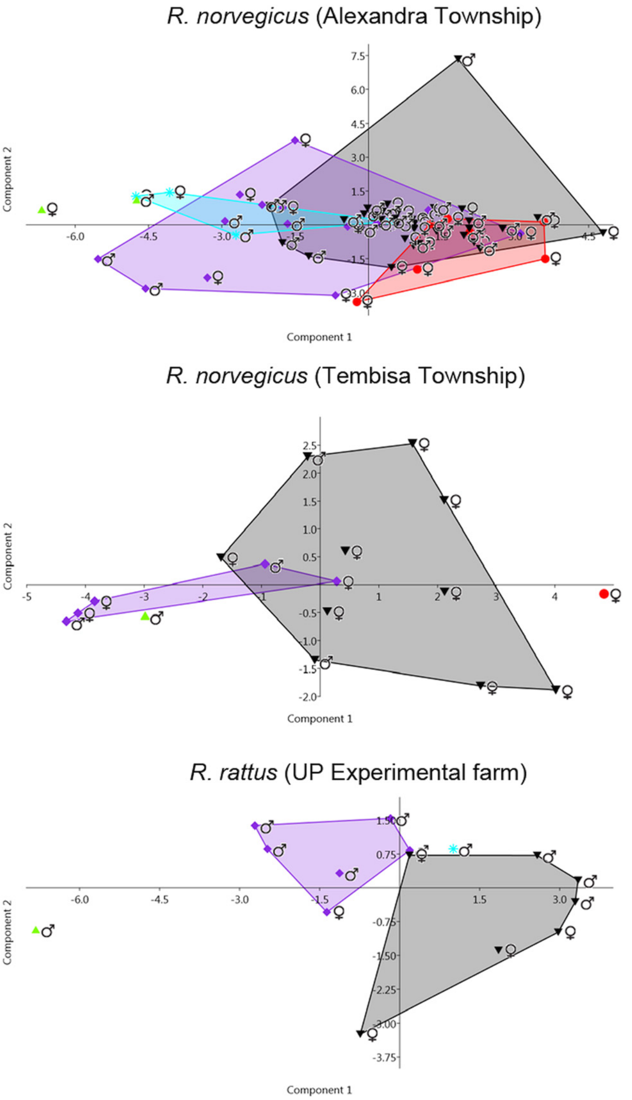
n = sample size; $\overline{X}$ = arithmetic mean; SD = ±1 standard deviation; CV = coefficient of variation. Cranio-dental measurements are defined and illustrated in Figure 3.

with the results of UPGMA cluster analysis because of the data's low KMO value), which was represented by all sexes and age classes I (by a single individual), II (by a single individual), III and IV, also reflects age rather than sex as a major source of variation (Figure 4). The single individual of age class I separated from the rest of the age classes. The single individual of age class II also separated from the rest of the age classes. Individuals of age classes III and IV were also separated from each other.

There were some cranio-dental measurements with relatively high loadings on the first principal components axis that were common in the PCAs of *R. norvegicus* from Alexandra (43.37% of the total variance) and Tembisa (58.59%) Townships, and *R. rattus* from UP Experimental Farm (58.10%) (Table 5), and these included: the greatest length of skull (GLS), the distance from nasal bone to the anterior edge of the zygomatic arch (NPP), the distance from the angular process to the mandibular condyle (AFA), the distance from the mandibular foramen to the mandibular condyle (MAF), and the distance from the posterior incisor to M^3 (IML). There were some cranio-dental measurements with relatively high loadings on the second principal components axis that were common in the PCAs of *R. norvegicus* from Alexandra (17.15% of the total variance) and Tembisa (11.89%) Townships, and *R. rattus* from UP Experimental Farm (12.25%), and these included: foramen magnum height (FMH) and width of M^2 (WSM). Collectively, these cranio-dental measurements also featured prominently either as statistically significantly different (except for the distance from the posterior incisor to M^3 (IML) in *R. norvegicus* from Tembisa and *R. rattus* from UP Experimental Farm) or contributed highly towards the total %SSQs in the univariate analyses (Table 2).

Table 5: The number of principal components (PC) axes, eigenvalues and percent (%) variances explained by each PC axis generated from each principal components analysis (PCA) of *R. norvegicus* from Alexandra and Tembisa Townships, and *R. rattus* from the University of Pretoria (UP) Experimental Farm, Gauteng Province, South Africa.

Cranial measurement	*R. norvegicus* (Alexandra township)		*R. norvegicus* (Tembisa township)		*R. rattus* (UP experimental farm)	
	Principal components		Principal components		Principal components	
	I	II	I	II	I	II
GLS	0.39	−0.16	0.32	−0.03	0.31	−0.04
FRO	0.04	0.00	0.32	0.05	0.31	−0.03
NPP	0.38	−0.13	0.32	−0.06	0.31	0.05
BBC	0.32	0.09	0.29	0.29	0.31	0.04
IOB	0.27	0.05	0.30	0.13	0.31	0.04
GHS	0.36	−0.02	0.29	−0.18	0.30	−0.06
FMH	0.04	0.46	0.06	0.70	0.03	0.62
LFM	0.04	0.59	0.32	−0.03	−0.13	0.62
WSM	0.07	0.45	0.16	−0.59	0.22	0.46
AFA	0.36	−0.09	0.32	0.07	0.31	−0.07
MAF	0.35	−0.08	0.32	0.09	0.31	−0.09
IML	0.30	−0.06	0.31	−0.13	0.31	0.04
WMS	0.19	0.40	−0.04	0.03	0.28	−0.02
%Variance	43.12	17.17	74.59	15.17	79.22	15.60


R. norvegicus (Alexandra Township)

R. norvegicus (Tembisa Township)

R. rattus (UP Experimental farm)

Figure 4: The first two axes from a principal components analysis (PCA) of cranio-dental data of *Rattus norvegicus* from Alexandra and Tembisa Townships, and *R. rattus* from the University of Pretoria Experimental Farm, Gauteng Province, South Africa. Tooth wear classes I: ▲; II: ✱; III: ◆; IV: ▼; V: ●. Convex polygons enclose individuals of the same tooth wear class.

Because of a low KMO value for the *R. rattus* population from UP Experimental Farm, the relatively low levels of variation explained by successive principal components to the total variance, and for comparative purposes, data for the three *Rattus* populations were further assessed by UPGMA cluster analysis. Similar to the PCA scattergram, the distance phenogram (Figure 5) of the UPGMA cluster analysis of *R. norvegicus* from Alexandra Township (cophenetic correlation coefficient (CCC) = 0.99), which was represented by all sexes and all five age classes, reflects age rather than sex as a major source of variation. It showed the following three main clusters: 1) Cluster I – comprising individuals of age class I (n = 2) and a mixture of two individuals of age class II, and three individual of age class III, suggesting that the young age class individuals are generally separated from the older individuals; 2) Cluster II – comprising mainly of individuals of age class III and a mixture of two individuals of age class II and two individuals of age class IV; and 3) Cluster III – except for one individual of age class II and three individuals of age class III, all individuals in this cluster comprise older individuals of age classes IV and V. The phenogram showed no discrete groupings of the sexes.

Similar to the PCA scattergram, the distance phenogram of the UPGMA cluster analysis of *R. norvegicus* from Tembisa Township (CCC = 0.73) (Figure 5), which was represented by all sexes and age classes I (by a single individual), III, IV and V (by a single individual), also reflects age rather than sex as a major source of variation. It showed three main clusters largely separating one individual of age class I, individuals of age class III (except for one individual of age classes IV, and individuals of age classes IV–V (except for one individual of age class III), with no discrete groupings of the sexes. Similarly, the distance phenogram of the UPGMA cluster analysis of *R. rattus* from UP Experimental Farm (CCC = 0.86) (Figure 5), which was represented by all sexes and age classes I (by a single individual), II (by a single individual), III and IV, also reflects age rather than sex as a major source of variation. It also showed three clusters showing the single individual of age class I, a cluster of individuals of age class II (except for two individuals of age class IV), and a cluster separating individuals of age class IV (except for one individual of age class III), with no discrete groupings of the sexes.

4 Discussion

The results of the univariate and multivariate analysis of *R. norvegicus* and *R. rattus* from urban and peri-urban areas of Gauteng Province, South Africa in this study showed no evidence of craniometric sexual dimorphism, however, both species showed significant ontogenetic variation between five age classes based on maxillary molar toothrow cusp eruption and wear. Several studies have indicated

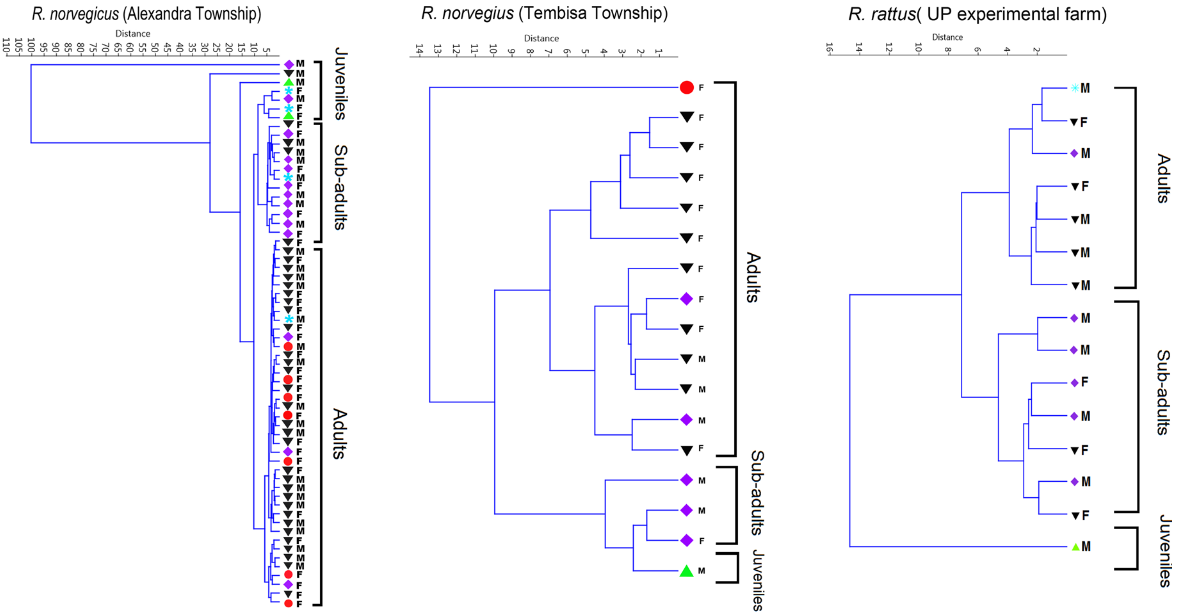


Figure 5: A distance phenogram from an unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of male (M) and female (F) *R. norvegicus* from Alexandra (Cophenetic correlation coefficient (CCC) = 0.99) and Tembisa (CCC = 0.73) Townships, and *R. rattus* from the University of Pretoria (UP) Experimental Farm (CCC = 0.86), Gauteng Province, South Africa. Tooth-wear classes I: ▲; II: ✱; III: ◆; IV: ▼; V: ●.

that the proximal variables that may exert an influence on the morphology of urban-dwelling rodents include ambient temperature (Porter et al. 2015; Rae et al. 2006), the lack of competition and predation (Angerbjörn 1986), and the quantity and quality of food available (Ventura and Lopez-Fuster 2000).

Wild populations of *R. norvegicus* and *R. rattus* are more likely to be exposed to a broader range of temperatures owing to the habitats in which they occur. Previous studies (e.g., Porter et al. 2015; Rae et al. 2006) have alluded to the effect of variation in ambient temperature and latitude on the cranio-dental morphology of *Rattus*. Laboratory strains of *R. norvegicus* that had been raised under colder ambient temperatures (5 °C) had subtle cranio-facial morphological differences on their facial shape and the volume of their maxillary sinuses when compared to a control group in which the ambient temperature had been kept at 22 °C (Rae et al. 2006).

Porter et al. (2015) compared *R. norvegicus* populations from temperate and tropical regions and demonstrated that latitude and seasonal variability could have an influence on the body size of male and female *R. norvegicus*. The sampled *Rattus* specimens in our study were sourced from Gauteng Province, which has a sub-tropical climate. Our craniometric analyses indicated that *R. norvegicus* and *R. rattus* lack sexually dimorphic cranio-dental morphological traits as similarly found by Ben-Faleh et al. (2012) in their study on the cranial morphology of *R. rattus*. The opportunistic manner in which our samples were acquired precluded further investigation into the effect that variables such as latitude, altitude, seasonal variability could have on the cranial morphological characteristics of male and female *Rattus*.

The results of the PCA (Figure 4; Table 5) generally reflected the pattern shown by the UPGMA cluster analysis (Figure 5). When univariate and multivariate results are collectively considered, the pooling of sexes and individuals into juveniles (i.e., tooth-wear class I), sub-adults (tooth-wear classes II and III), and adults (tooth-wear classes IV–V) for our trophic ecological study of *Rattus* from urban and peri-urban areas of Gauteng Province was justified. The broad pattern that emerged from the univariate and multivariate analyses is that if the tooth wear classes were to be visualised on a hypothetical growth curve, then individuals of age class I would be considered as juveniles, individuals of age classes II and III would be on the growth curve just before it stabilizes and therefore, representing sub-adults, while individuals of age classes IV and V would be considered as adults.

The results of the univariate Tukey's *post hoc* tests in our study (Table 3) were equivocal in supporting the case of which age classes to pool in our trophic ecological study of *Rattus* from urban and peri-urban areas of Gauteng Province. This constraint however, was resolved when the results of the univariate and multivariate analyses were interpreted collectively, showing that there is a progressive increase in cranio-dental dimensions from age classes I to V in *R. norvegicus* and *R. rattus* from urban and peri-urban South African populations (Table 4). The initial allocation of individuals into relative age classes indicated that individuals of *R. norvegicus* and *R. rattus* in this study can be assigned to five relative age classes, and if necessary, into much broader ageing categories to reflect juvenile, sub-adult, and adult individuals.

The assessment of ontogenetic variation in this study involved the assessment of maxillary molar eruption and wear to establish the relative age of individuals of *R. norvegicus* and *R. rattus* from Gauteng Province, South Africa. The erosion of the enamel overlaying the crown of molars can be correlated with relative age in laboratory strains of Norway rats (Sengupta 2011). The potential limitation in the use of molar eruption and wear to assess relative age in rats however, is that differences in their diet may lead to the over- or under-estimation of their relative age (Sengupta 2011). The results of the present study, however, suggest that the orderly tooth-wear structure observed may not have compromised the conclusions reached on relative ageing in *R. norvegicus* and *R. rattus* samples.

Only two cranio-dental measurements (the distance from the posterior incisor to M^3 (IML) in *R. norvegicus* from Alexandra Township and width of M^2 (WSM) in *R. rattus* from UP Experimental Farm) in the present study showed statistically significant interaction between sex and age (Table 2). Prado and Percequillo (2011) found that *Aegialomys xanthaeolus* (Family: Cricetidae) that they assigned to age class III exhibited inadequately defined differences in the molar tooth wear patterns and their broad range of cranio-dental measurements. Male *A. xanthaeolus* were found to grow larger than females resulting in the observed differences in their morphological features. Males and females from age classes IV to V, however, did not exhibit sexually dimorphic features (Prado and Percequillo 2011). The hypothesized mechanisms that resulted in the differences between the sexes and relative age included the misidentification and mis-assignment of individuals into correct age classes, as well as differences in their diet. Patton and Rogers (1983) concluded that the misidentification and mis-assignment of individuals may have resulted in the observed variation in individuals of age class III. Shine (1978) hypothesized that, at least in snakes, competition between males and females may result in morphological differences between them. Brandt and Pessôa (1994) found sexual dimorphism in relative age class III that was intermediate between juveniles and adults of the *Cerradomys langguthi* (Family: Cricetidae).

The largest %SSQ contribution to the total %SSQ in this study was due to *error* (i.e., *residual*) (Table 2), suggesting that apart from sex, age and their interaction, there may be other components of variation that may be responsible for the nature and extent of variation in *R. norvegicus and R. rattus* from Gauteng Province. Analyses based on the computationally simplistic %SSQs may be more appropriate for establishing the relative importance of cranio-dental variables when assessing sexual dimorphism and ontogenetic, and other sources of variation in rodents (Leamy 1983). Subsequent studies on partitioning sexual dimorphism and ontogenetic variation should focus on comparing individuals across populations while limiting or adjusting for additional sources of variation that may not be due to these two components of variation. For the analysis of sexual dimorphism and ontogenetic variation, cranio-dental variables with the smallest variances, and with large error components, may be the most appropriate for such analyses (Leamy 1983).

In conclusion, although *R. norvegicus* from Tembisa Township and *R. rattus* from UP Experimental Farm were represented by relatively small sample sizes compared to *R. norvegicus* from Alexandra Township which was represented by the largest sample size, all sexes and all age classes, craniometric analysis in this study (Table 1) showed the general lack of sexual dimorphism in the two species of *Rattus* from South Africa. These analyses however, showed evidence of statistically significant ontogenetic variation in *R. norvegicus* and *R. rattus*. Despite some of the relatively small sample sizes, the results in this study suggest that the sexual dimorphism and ontogenetic variation observed may not have compromised the conclusions reached on relative ageing in the two species of *Rattus*. Collectively, both univariate and multivariate results justified the pooling of the sexes as well as individuals into juveniles (i.e., individuals of tooth-wear class I), sub-adults (tooth-wear classes II–III), and adults (tooth-wear classes IV–V) for our trophic ecological study of *Rattus* in urban and peri-urban South African populations. Very few of the cranio-dental variables used in the present study showed statistically significant interaction between sex and age, and the interaction between these two components of variation did not account substantially to the total variance (Table 2). Previous studies have attributed the interaction between sexual dimorphism and age variation in rodents to differences in growth rates and competition for food resources among the sexes and age classes. The analysis based on SSQs revealed that the largest %SSQs to the total %SSQ was due to *error* (= *residual*) (Table 2), suggesting that apart from sex, age and the interaction between them, there may be other components of variation that may be responsible for the overall nature and extent of variation in *R. norvegicus* and *R. rattus* from urban and peri-urban areas of Gauteng Province, South Africa.

Research ethics: Samples were obtained under permit number CPF6 0032 from Gauteng Department of Nature Conservation, Johannesburg, South Africa. Permit number 13788 to conduct this study was granted under the terms of section 20 of the Animal Diseases Act (Act no. 35 of 1984) of the South African Department of Agriculture, Forestry and Fisheries. An animal ethics approval number EC025-10 to conduct this study was granted by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa.

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Author contributions: CC, CP and TZ conceptualized the study; GR collected and analysed the data, and all authors draughted and revised the manuscript.

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