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Article

Morphological divergence of Hermann's tortoise (*Testudo hermanni boettgeri*) in Albania

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Simple Summary: Morphology of chelonians is providing us basic information about development, evolution, biodiversity, biomechanics, behavior, ecology, and physiology. Furthermore, it has also played an important role in characterizing populations and analyzing the similarities between populations. This study investigates the morphological variation of Hermann's tortoise (*Testudo hermanni boettgeri*) between specimens from five different populations in Albania. Also, it is provided basic data on the morphological characteristics of the Hermann's tortoise. The results found that Hermann's tortoise in Albanian population were regionally diverged into 3 different populations that were situated in the northern (Shkodra), central (Tirana, Berati and Ballshi) and southern (Saranda) Albania. Moreover, the female individuals are larger and heavier than male individuals' overall population in Albania, and the females follow Bergmann´s rule. The morphological divergences may be due to molecular variations or environmental conditions of the regions.

Abstract: Testudines show phenotypic plasticity, and variation among specific populations within a species is widespread. Morphological differences between populations reflect ecological factors that drive adaptation to local conditions. In this context, gathered basic data on morphology of the Hermann's tortoise (*Testudo hermanni boettgeri*) to document their variation across different geographical regions. We surveyed Hermann's tortoises, in five different locales within Albania during April and May 2020 and measured 20 morphological characteristics, including carapace and plastron dimensions. We measured 188 tortoises (81 males, 107 females) in this study, and females were larger (P=.0001) and heavier (P=.0001) than males. Mean straight carapace length [SCL] and body mass were = 172.4 mm and 1128.8 g, respectively, for females and 151.3 mm and 735 g, respectively, for males. The overall Albanian *T. h. boettgeri* population were regionally diverged into 3 different populations that were situated in northern (Shkodra), central (Tirana, Berati and Ballshi) and southern (Saranda) Albania. The body size (curved carapace length; CCL) of females was positively correlated (r=0.216; P=0.025) with the latitude degree, in accordance with Bergmann's rule. However, there was no correlation between body size and degrees north latitude in males. These striking regional differences among Albanian *T. h. boettgeri* strongly suggest that further study of molecular variations in and reproductive output of Hermann's tortoises is warranted.

Keywords: Bergmann's rule, carapace morphometry, plastron scutes, Hermann's tortoises, *Testudo hermanni boettgeri*

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1. Introduction

Hermann's Tortoise (*Testudo hermanni*) is a terrestrial species that is widespread in the European Mediterranean region. It is one of the herpetofauna species of Albania which can be found in an area ranging from the shores of the sea to an altitude of approximately 1300 m in the mountains. However, most populations of Hermann's Tortoise are found below 500 m [1,2,3]. The most common habitats of the Hermann tortoise are agricultural lands, canals, hilly grasslands, areas of sparse vegetation, and land near forests. It is listed globally as ''near threatened'' according to the International Union for Conservation of Nature [4]. Due to the overuse of tortoises for the pet trade, serious declines in their populations have been observed [5]. In fact, the Hermann tortoise constitutes 13% of the world Testudo trade [6], and it is listed by the Bern Convention and European Habitat Directive as a species in need of strict protection. International trade of the species is regulated by Convention for International Trade of Wildlife Fauna and Flora [7]. The existence of *T. hermanni* is threatened by many additional factors such as rapid urbanization and concomitant habitat loss, climate change, increasing ambient temperatures, multiple summer fires, prolonged droughts or floods, and increased human activity [8].

Hermann's tortoises may be found only in Europe, the Balkans, and the Turkish Thrace. Based only on external morphology, there are two recognized subspecies: *Testudo hermanni hermanni* and *Testudo hermanni boettgeri* [9,10]. While *T. h. hermanni* inhabits the western part of the Po Valley river in Italy, *T. h. boettgeri* inhabits the Balkans and Turkey Thrace [7]. The tortoise found in Albania belongs to the subspecies Eastern Hermann's tortoise (*T. h. boettgeri*), which possesses external morphological features characteristic for the species such as: light yellow external coloration of the carapace; black pigmentation of the plastron unlike less defined than bands (i.e., discontinuous) and in some cases discolored; length of the inter pectoral suture is greater to or equal than the inter femoral suture; presence of inguinal scutes on either side of the shell to the extent of almost 100% of all individuals [11].

The carapace is high, almost oval form in females and sub-trapezoidal form in males. In the middle line of the carapace are situated a nuchal (cervical) scute, five vertebral scutes, four pleural scutes more laterally in either sides, and 12 marginal scutes surrounding the first. In the plastron, the scutes are nominated from the cranial part as gular, humeral, pectoral, abdominal, femoral, anal, and inguinal scutes [12-14].

The morphology of the chelonians shell can provide some detailed information about reproduction, locomotion, and protection against predators [15]. The overall shell shape in turtles and tortoises affects fecundity selection in females and sexual selection in males [16,17]. In fact, their shell morphology consists of a continuous set of sutured shell plates and free body parts covered with horny scales that can be measured individually and precisely; the role in mating and non-mating activities can be attributed to them. The adult body sizes in turtles offer a means to estimate the relationship between growth rate and body shape [18]. Furthermore, total body size is also an important morphological feature affected by ecological and sexual selection in chelonians [19,20] and can show geographic diversity [21,22,16].

Phenotypic plasticity and variation in specific populations is a widespread phenomenon in Testudo species [23,24]. For instance, differences in body size of *T. h. boettgeri* populations in Serbia and Montenegro have been reported [25]. Similarly, *T. h. boettgeri* populations in Trebinje (a municipality in Bosnia and Herzegovina) and the Pčinja River Valley (Serbia) in the Balkan Peninsula showed variation in carapace shape [26]. Hermann's tortoise populations between the Republic of Serbia and the Former Yugoslav Republic of Macedonia show morphological differences [15]. The size and shape of Italian Hermann's tortoises varies according to a North-South cline, following Bergmann's rule which proposes a positive relationship between mean body size and latitude [22]. Similarly, the carapace length of *Testudo graeca* was correlated with latitudes globally and locally [27], also following Bergmann's rule. Moreover, morphological divergence was

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also found in *T. graeca* populations from west-central Morocco [28]. Contrarily, the populations of *T. graeca* along the Mediterranean coast of Turkey are morphometrically homogenous [29]. Adult eastern populations of Hermann´s tortoises of the Balkans exhibit longer carapace length (>180 mm in females and >155 mm in males; [10]. Specimens with the longest carapace length were found in Bulgaria [30], whereas those with the shortest length were found in the south of the Peloponnese in Greece [31].

Morphological differences among populations give scientists clues about ecological and biological factors which drive adaptation. They can also help researchers gain insights into locomotion, fecundity and sexual selection, and health conditions of populations. Although studies on the morphological differences among Hermann's tortoise populations have been conducted in several countries in the Balkan peninsula, only one study of *T. hermanni* has been conducted for the Albanian population; it was conducted in southern Albania (Vlora) with a small sample [8]. Therefore, the aim of our investigation was to collect and analyze basic data on the morphological characteristics of the Hermann's tortoise in Albania and to assess any potential morphological variation between specimens from five different regions.

2. Materials and Methods

Study area

The study was conducted in the hills and mountains of five Albanian regions, distributed from north to south and from the sea side to the middle of the country during spring (April and May) of 2020. These regions were designated using the name of the majorcity located within/near the study area such as Shkodra, Tirana, Berati, Ballshi and Saranda (Figure 1).

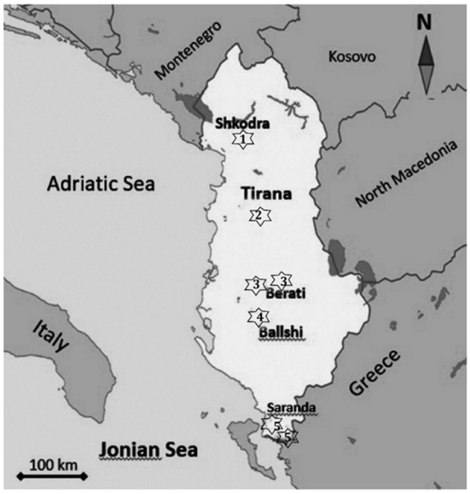

Figure 1. Location and distribution of the field survey regions of Hermann's tortoises in five regions of Albania.

The Shkodra region lies at 110 m above sea level and has a Mediterranean climate with hot summers with continental influences. The average yearly temperature varies from 14.5°C to 16.8°C. The average yearly precipitation is about 1,700 mm (from 34.1 mm in July to 229.8 mm in November; [32], which makes the area one of the wettest in Europe. The samples were collected from the rural areas of Maraç (41.9765831 N, 19.6661893 E).

The Tirana region has an average altitude of 521 m above sea level, and it has a humid subtropical climate. Temperatures vary throughout the year from an average of 6.7°C in January to 24°C in July. The average precipitation is about 1,266 mm per year, and the humidity ranges from 62 to 74% [32]. The samples were collected from the rural areas of Baldushk (41.1957547 N, 19.8395281 E).

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The Berati region lies an average of 53 m above sea level but contains hills from 130 – 200 m above sea level. Its climate is classified as warm and temperate. The average temperature is 15.7°C and ranges from 7.4°C (in January) to 24.4°C (in July and August). The average precipitation is 1032 mm annually [32]. The samples were collected from the rural areas of Ura Vajgurore (40.7652882 N, 19.8847051 E) and Lumas (40.812114 N, 20.0032559 E).

The Ballshi regions lies at 500 m above sea level. The average temperature for the last 30 years was 15.8°C and ranged from 5.8°C in January to 24.5°C in July. The average annual precipitation is 1041 mm [33]. The samples were collected from the rural areas of Aranitas (40.5977479 N, 19.8089151 E) in Ballshi region.

The Saranda region lies almost entirely at sea level but contains some land as high as 300 – 500m above sea level. Saranda has a typical Mediterranean climate with dry and hot summers and mild and wet winters. The average temperature is 15.6°C, which ranges from 1°C during winter to 28°C during summer. The average precipitation is 1370 mm annually [32]. The samples were collected from the rural areas of Çukë (39.8276987 N, 20.0610213 E) and Konispol (39.6592742 N, 20.1804537 E).

Data collection and measurements

Ethical approval for this study was obtained from the Ethics Commission of the Veterinary Faculty of Tirana, Albania (Nr. 179/20.05.2020). We randomly located and collected by free hands a total of 188 healthy and undamaged tortoises and evaluated them in their native wild habitat. All Hermann's tortoises were evaluated carefully for external morphologic characteristics to determine the correct gender and subspecies. Gender was determined by concavity of the plastron, tail length, and curve of supracaudal scutes which are more prominent in male tortoises than in female tortoises [13,14,25]. After measurements were recorded, tortoises were released at the same locations where they were captured. We used an electronic scale (1 gr error rate) to measure body mass, and a digital caliper (precision 0.01 mm) and tape measure (1 mm error) for external body measurements.

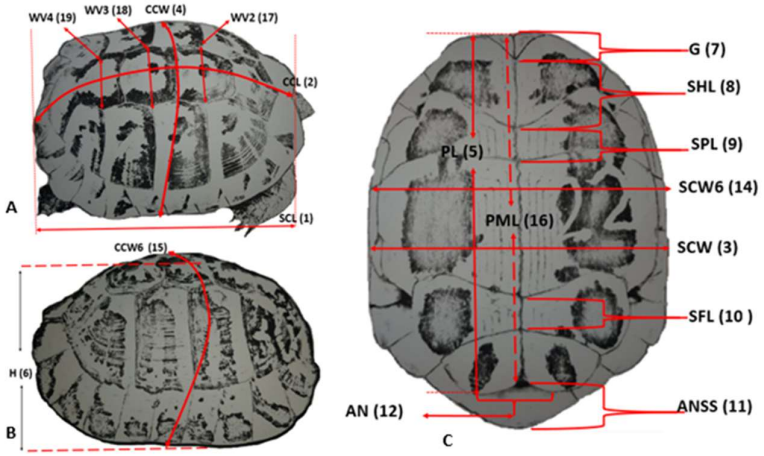

Figure 2. Measurement parameters of the carapace and plastron of Hermann's tortoise. Abbreviations are defined in the materials and methods section and are also listed in the footnote to Table 1. The numbers in parentheses indicate parameter number.

We evaluated 19 external measurements as shown in Figure 2 and as previously reported [28,6]. The morphometric dimensions recorded were 1: straight carapace length (SCL), 2: curved carapace length (CCL), 3: straight carapace width (SCW), 4: curved carapace width or maximal perimeter side to side (CCW), 5: plastron length (PL), 6: maximal high (H), 7: inter gular suture length (G), 8: inter humeral suture length (SHL), 9: inter pectoral suture length (SPL), 10: inter

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femoral suture length (SFL), 11: distance from anal notch to supracaudal scute tip (ANSS), 12: anal notch-between two anal scutes (AN), 13: tail length from cranial margin of cloaca to the tail´s tip (TL), 14: carapace width at the level of the 6th marginal scute (SCW6), 15: curved carapace width at the level of the 6th marginal scute (CCW6), 16: plastron midline length from gular notch to anal notch (PML), 17: maximal width of 2nd vertebra (WV2), 18: maximal width of 3rd vertebra (WV3), and 19: maximal width of 4th vertebra (WV4).

Body condition index (CI) in the tortoise was calculated using the ratio of observed body mass (M) to the predicted body mass (M') obtained from the relationship of body mass to SCL [34,35]. The best body condition index (CI) based on body mass is the log (M/M'), and this is equal to residuals from the regression of log M on log L [34]. This means that the distribution of log (M/M') did not deviate from normality, and it allowed the analysis of interaction effects in the analysis of variance.

Statistical analysis

The differences in body mass (M) and SCL between female and male tortoises were investigated by independent sample t-test. Differences in CI between regions were performed with a Univariate Test in a General Linear Model. Moreover, differences between male and female CI were analyzed with one-way ANOVA [35,7].

Multivariate comparisons of morphological dimensions between regions and sexes were performed with Multivariate Analysis of Covariance (MANCOVA, using SCL as a covariate). The equality of covariance matrices of dependent variables between groups was tested with Box's M test, and deviations from normality and homogeneity of covariance matrices were found ($P < 0.005$). Therefore, Pillai's trace test was used because it is most robust standard test in multivariate comparisons for deviations from normality and equality of covariance matrices [36,37]. The relationship between mean body size and latitude degree for north-south cline was tested with Spearman's rho (two tailed test) [47].

The degree of similarity among regions and sex were assessed by discriminant functional analysis (DFA) with stepwise selection (F for entry: 3.84; F for removal: 2.71); [28,6]. Population centroids with 95% confidence ellipses derived from the DFA were used to visualize relationships among the individuals of regions. Correct classification of regions into their groups were tested with the cross-validation test in DFA (regions were assigned to the samples using the canonical functions). This output shows the number of cases correctly and incorrectly assigned to each of region based on discriminant analysis. The percentage of correctly classified individuals gives a measure of the morphological distinctness of the samples. The number of misclassified individuals indicates the degree of intermingling between populations.

All statistical analyses were performed with IBM SPSS Statistics 20 software, and 95% confidence ellipses graphs were generated using XLSTAT (Addinsoft, version 2014.05.3). All means were presented with standard deviation (±) and minimum and maximum.

3. Results

A total of 188 adult Hermann's tortoises was collected from five regions in Albania. The sex ratio of the *T. hermanni boettgeri* sample we studied was female-biased with 1.3 females per male (107 females, 81 males). Distribution of tortoises by region was 28.2% in Berati, 26.1% in Saranda, 21.3% in Tirana, 13.3% in Ballshi, and 11.2% in Shkodra. Detailed descriptive statistics of morphological dimensions by sex and region are shown in Table 1.

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Table 1. Descriptive statistics (mean ◎ standard deviation) of morphological dimensions‡ of Albanian Hermann's tortoises stratified by region and sex

Dimension	Region	Male			Female			Combined Total		
		N	Mean ±Sd	Min-Max	N	Mean±Sd	Min-Max	N	Mean±Sd	Min-Max
Mass	**Berati**	13	688.8 ±276.9	300-1182	40	1090.6 ±351.4	300-1850	53	992±375.2	300-1850
	Shkodra	14	645.7 ±111.9	450-870	7	838.6 ±552.6	250-1550	21	710±329.3	250-1550
	Saranda	22	663.4 ±227.7	160-1150	27	1104.7 ±279.3	500-1620	49	906.6±337.8	160-1620
	Ballshi	18	813.9 ±227.1	350-1250	7	1071.4 ±494.9	310-1920	25	886±334.2	310-1920
	Tirana	14	878.6 ±274.0	242-1183	26	1306.2 ±264.7	539-1860	40	1156.6±335.6	242-1860
	Total	81	735.0 ±241.8	160-1250	107	1128.8 ±355.6	250-1920	188	959.2±367.3	160-1920
SCL	**Berati**	13	148.5 ±18.3	112-177	40	166.3 ±19.7	124-212	53	161.9±20.7	112-212
	Shkodra	14	145.4 ±13.8	130-170	7	155.7 ±42.4	100210	21	148.8±26.2	100-210
	Saranda	22	148.3 ±19.1	100-190	27	177.4 ±22.1	120-220	49	164.4±25.3	100-220
	Ballshi	18	159.0 ±16.3	121-185	7	169.1 ±32.1	112-216	25	161.8±21.6	112-216
	Tirana	14	154.8 ±20.8	101-174	26	182.0 ±13.1	139-207	40	172.5±20.7	101-207
	Total	81	151.3 ±18.2	100-190	107	172.4 ±22.9	100-220	188	163.3±23.4	100-220
CCL	**Berati**	13	192.8 ±23.9	149-238	40	209.1 ±24.6	155-253	53	205.1±25.2	149-253
	Shkodra	14	193.2 ±15.6	170-225	7	200.0 ±44.0	140-250	21	195.5±27.4	140-250
	Saranda	22	182.7 ±21.8	135-225	27	212.1 ±32.1	130-320	49	198.9±31.4	130-320
	Ballshi	18	210.1 ±21.2	160-245	7	211.1 ±36.0	142-260	25	210.4±25.3	142-260
	Tirana	14	203.8 ±27.3	131-231	26	227.8 ±15.2	177-255	40	219.4±23.1	131-255
	Total	81	195.9	131-	107	214.0 ±28.0	130-320	188	206.2±27.8	130-

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			±23.9	245						320
SCW	**Berati**	13	115.6 ±15.6	88-140	40	124.4 ±14.1	95-150	53	122.3±14.9	88-150
	Shkodra	14	120.0 ±9.0	100-130	7	124.3 ±25.1	90-150	21	121.4±15.7	90-150
	Saranda	22	167.9 ±31.6	110-206	27	167.5 ±41.0	120-250	49	167.7±36.7	110-250
	Ballshi	18	129.3 ±13.8	100-153	7	131.6 ±22.9	93-168	25	129.9±16.4	93-168
	Tirana	14	133.4 ±21.3	83-155	26	141.3 ±11.6	103-165	40	138.5±15.9	83-165
	Total	81	136.7 ±28.8	83-206	107	139.9 ±29.8	90-250	188	138.5±29.3	83-250
CCW	**Berati**	13	310.3 ±38.8	233-374	40	346.3 ±37.5	264-420	53	337.4±40.6	233-420
	Shkodra	14	183.9 ±15.3	155-215	7	200.0 ±53.2	130-260	21	189.3±32.6	130-260
	Saranda	22	279.7 ±64.2	170-353	27	266.3 ±76.2	160-410	49	272.3±70.7	160-410
	Ballshi	18	329.6 ±32.6	257-380	7	340.7 ±57.7	238-428	25	332.7±40.1	238-428
	Tirana	14	335.1 ±41.3	230-383	26	383.0 ±27.9	282-435	40	366.2±40.0	230-435
	Total	81	288.7 ±68.0	155-383	107	325.1 ±73.5	130-435	188	309.4±73.2	130-435
PL	**Berati**	13	119.8 ±14.9	94-144	40	147.4 ±23.2	106-245	53	140.6±24.5	94-245
	Shkodra	14	96.1 ±12.3	70-115	7	125.7 ±36.2	75-265	21	106.0±26.4	70-165
	Saranda	22	123.0 ±13.9	91-160	27	158.8 ±18.2	110-190	49	142.7±24.2	91-190
	Ballshi	18	134.4 ±12.0	111-155	7	157.1 ±24.1	113-193	25	140.8±18.9	111-193
	Tirana	14	138.9 ±21.2	90-180	26	162.2 ±12.0	119-181	40	154.1±19.2	90-181
	Total	81	123.1 ±20.3	70-180	107	153.1 ±22.6	75-245	188	140.2±26.2	70-245
SH	**Berati**	13	78.2 ±9.4	59-91	40	87.7 ±8.1	65-103	53	85.4±9.3	59-103
	Shkodra	14	79.6 ±6.0	70-90	7	87.1 ±22.3	60-115	21	82.1±13.7	60-115

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	Saranda	22	72.3 ±13.4	40-91	27	90.3 ±12.1	65-120	49	82.2±15.5	40-120
	Ballshi	18	83.3 ±8.3	64-95	7	86.3 ±14.4	57-102	25	84.1±10.2	57-102
	Tirana	14	77.8 ±10.0	54-91	26	92.4 ±7.0	69-107	40	87.3±10.7	54-107
	Total	81	77.9 ±10.6	40-95	107	89.4 ±10.8	57-120	188	84.4±12.1	40-120
GL	**Berati**	13	16.6 ±2.8	14-23	39	20.3 ±2.7	15-31	52	19.3±3.1	14.0-31
	Shkodra	14	20.9 ±3.9	10-25	7	20.0 ±7.1	15-35	21	20.6±5.0	10.0-35
	Saranda	22	14.7 ±3.3	10-20	27	18.6 ±3.9	10-26	49	16.8±4.1	10.0-26
	Ballshi	18	16.4 ±3.6	9-25	7	18.1 ±2.8	12-20	25	16.9±3.4	9.0-25
	Tirana	14	17.2 ±3.4	10-21	26	21.6 ±2.9	15-28	40	20.1±3.7	10.0-28
	Total	81	16.9 ±3.9	9-25	106	20.1 ±3.6	10-35	187	18.6±4.1	9.0-35
SHL	**Berati**	13	23.2 ±2.9	19-28	40	23.9 ±4.2	16-38	53	23.7±3.9	16-38
	Shkodra	14	19.6 ±3.7	10-25	7	21.4 ±8.5	10-30	21	20.2±5.6	10-30
	Saranda	22	21.7 ±4.0	15-30	27	26.0 ±4.2	12-32	49	24.1±4.6	12-32
	Ballshi	18	25.3 ±1.9	22-29	7	27.4 ±6.2	20-40	25	25.9±3.6	20-40
	Tirana	14	23.7 ±4.0	16-28	26	27.1 ±3.6	16-33	40	25.9±4.0	16-33
	Total	81	22.7 ±3.8	10-30	107	25.3 ±4.8	10-40	188	24.2±4.6	10-40
SPL	**Berati**	13	8.9 ±1.3	6-11	40	12.9 ±3.0	9-23	53	11.9±3.2	6-23
	Shkodra	14	10.6 ±2.5	5-15	7	14.3 ±8.4	5-30	21	11.8±5.3	5-30
	Saranda	22	10.9 ±3.4	6-19	27	14.5 ±3.6	10-20	49	12.9±3.9	6-20
	Ballshi	18	9.4 ±1.6	6-13	7	14.0 ±1.5	11-15	25	10.7±2.6	6-15
	Tirana	14	9.0 ±2.1	5-12	26	12.3 ±2.8	8-20	40	11.2±3	5-20
	Total	81	9.9 ±2.5	5-19	107	13.3 ±3.6	5-30	188	11.8±3.6	5-30
SFL	**Berati**	13	9.9 ±3.6	6-19	40	10.7 ±3.0	6-23	53	10.5±3.2	6-23

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	Shkodra	14	14.9 ±3.1	8-20	7	19.3 ±7.3	10-30	21	16.4±5.2	8-30
	Saranda	22	9.0 ±1.9	5-13	27	10.0 ±2.4	5-15	49	9.6±2.2	5-15
	Ballshi	18	10.2 ±2.3	7-14	7	11.4 ±3.8	6-17	25	10.5±2.8	6-17
	Tirana	14	10.9 ±2.1	6-14	26	11.4 ±1.9	8-16	40	11.2±2	6-16
	Total	81	10.8 ±3.2	5-20	107	11.3 ±3.8	5-30	188	11.1±3.5	5-30
ANSW	**Berati**	13	30.2 ±6.7	16-40	40	30.3 ±5.6	22-48	53	29.9±6.9	2-48
	Shkodra	14	30.0 ±5.5	20-40	7	29.3 ±10.6	15-40	21	29.8±7.3	15-40
	Saranda	22	30.0 ±7.8	13-45	27	34.4 ±6.6	22-45	49	32.4±7.4	13-45
	Ballshi	18	30.1 ±7.3	15-48	7	27.9 ±11.2	13-43	25	41.4±61.7	13-335
	Tirana	14	28.4 ±6.3	14-35	25	30.4 ±4.3	15-35	39	29.6±5.1	14-35
	Total	81	29.7 ±6.7	13-48	106	31.1 ±6.6	13-48	187	32.0±23.4	2-335
ANW	**Berati**	13	44.3 ±9.8	27-58	40	35.3 ±5.1	22-47	53	37.5±7.6	22-58
	Shkodra	14	44.6 ±10.3	20-60	7	36.4 ±16.5	20-60	21	41.9±12.9	20-60
	Saranda	22	44.0 ±6.8	27-55	27	38.1 ±4.4	30-47	49	40.7±6.3	27-55
	Ballshi	18	49.7 ±6.2	35-61	7	34.4 ±7.9	22-48	25	45.4±9.6	22-61
	Tirana	14	51.3 ±10.7	28-72	26	39.6 ±5.1	29-51	40	43.7±9.3	28-72
	Total	81	46.7 ±8.9	20-72	107	37.0 ±6.6	20-60	188	41.2±9.0	20-72
TL	**Berati**	8	39.6 ±7.8	30-50	32	23.3 ±4.6	17-34	40	26.6±8.5	17-50
	Shkodra	14	31.8 ±7.5	15-50	7	24.0 ±12.5	15-50	21	29.2±9.9	15-50
	Saranda	22	35.7 ±10.3	10-55	27	29.6 ±8.6	15-60	49	32.3±9.8	10-60
	Ballshi	18	30.6 ±7.9	18-50	7	15.7 ±3.4	10-21	25	26.4±9.7	10-50
	Tirana	14	42.4 ±10.9	20-55	26	23.0 ±3.3	15-29	40	29.8±11.6	15-55

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	Total	76	35.4 ±10.0	10-55	99	24.4 ±7.3	10-60	175	29.2±10.1	10-60
SCW6	**Berati**	8	114.3 ±12.5	99-133	32	116.6 ±32.1	13-179	40	116.1±29.1	13-179
	Shkodra	14	121.8 ±12.5	100-150	7	118.6 ±26.1	80-150	21	120.7±17.6	80-150
	Saranda	22	105.1 ±34.9	50-150	27	98.1 ±38.6	60-165	49	101.3±36.7	50-165
	Ballshi	18	119.2 ±12.7	95-140	7	125.6 ±22.4	88-162	25	121.0±15.8	88-162
	Tirana	14	120.2 ±17.0	83-140	26	137.5 ±10.5	100-157	40	131.4±15.4	83-157
	Total	76	115.3 ±22.7	50-150	99	117.8 ±32.2	13-179	175	116.7±28.4	13-179
CCW6	**Berati**	8	201.3 ±32.7	171-271	32	217.3 ±37.7	160-379	40	214.1±36.9	160-379
	Shkodra	14	178.6 ±12.3	160-200	7	188.6 ±41.8	130-240	21	181.9±25.4	130-240
	Saranda	22	168.5 ±22.7	100-195	27	205.5 ±40.6	90-340	49	188.9±38.3	90-340
	Ballshi	18	194.1 ±18.5	151-230	7	208.3 ±37.2	140-263	25	198.1±25.1	140-263
	Tirana	14	195.2 ±24.8	134-220	26	226.6 ±17.1	165-260	40	215.6±24.9	134-260
	Total	76	184.8 ±24.7	100-271	99	213.9 ±35.6	90-379	175	201.2±34.4	90-379
MPL	**Berati**	8	114.1 ±12.2	96-130	32	139.0 ±13.7	110-173	40	134.1±16.7	96-173
	Shkodra	14	115.4 ±8.9	100-130	7	137.1 ±33.9	95-180	21	122.6±22.5	95-180
	Saranda	22	109.3 ±13.1	80-150	27	144.6 ±21.1	79-170	49	128.8±25.1	79-170
	Ballshi	18	121.3 ±10.6	102-142	7	148.4 ±23.8	103-182	25	128.9±19.4	102-182
	Tirana	14	118.0 ±12.9	84-134	26	151.2 ±12.4	112-172	40	139.6±20.3	84-172
	Total	76	115.4 ±12.3	80-150	99	144.3 ±18.7	79-182	175	131.7±21.6	79-182
WV2	**Berati**	8	32.5 ±2.2	30-36	32	36.8 ±4.8	29-51	40	36.0±4.7	29-51
	Shkodra	14	25.2 ±3.7	20-30	7	29.3 ±9.3	20-40	21	26.6±6.2	20-40

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	Saranda	21	31.4 ±6.4	20-42	27	35.8 ±5.0	21-42	48	33.9±6.0	20-42
	Ballshi	17	30.6 ±3.0	26-36	7	36.6 ±5.3	27-42	24	32.4±4.6	26-42
	Tirana	14	30.7 ±3.1	23-35	26	36.4 ±3.0	26-42	40	34.4±4.0	23-42
	Total	74	30.0 ±4.9	20-42	99	35.9 ±5.2	20-51	173	33.4±5.8	20-51
WV3	**Berati**	8	37.3 ±5.3	33-49	32	43.0 ±9.7	32-90	40	41.9±9.2	32-90
	Shkodra	14	30.4 ±4.6	25-40	7	33.6 ±10.3	20-45	21	31.4±6.9	20-45
	Saranda	20	36.5 ±5.1	26-48	27	41.4 ±5.2	26-50	47	39.3±5.7	26-50
	Ballshi	17	35.1 ±3.5	30-41	7	40.4 ±7.3	28-50	24	36.6±5.4	28-50
	Tirana	14	35.3 ±4.1	24-40	26	42.2 ±3.7	30-50	40	39.8±5.1	24-50
	Total	73	34.8 ±5.0	24-49	99	41.5 ±7.4	20-90	172	38.7±7.3	20-90
WV4	**Berati**	8	35.3 ±5.1	29-42	32	36.6 ±4.4	27-49	40	36.4±4.5	27-49
	Shkodra	14	28.6 ±4.3	20-36	7	28.6 ±7.5	20-40	21	28.6±5.3	20-40
	Saranda	20	32.7 ±6.5	19-46	27	36.4 ±5.5	22-46	47	34.8±6.2	19-46
	Ballshi	17	34.6 ±4.6	26-42	7	35.3 ±7.3	23-46	24	34.8±5.4	23-46
	Tirana	14	36.1 ±5.4	20-41	25	39.2 ±4.1	30-48	39	38.1±4.8	20-48
	Total	73	33.29 ±5.7	19-46	98	36.6 ±5.6	20-49	171	35.2±5.9	19-49

‡ SCL denotes straight carapace length; CCL denotes curved carapace length; SCW denotes straight carapace width, CCW denotes curved carapace width or maximal perimeter side to side; PL denotes plastron length; H denotes maximal height; G denotes inter gular suture length; SHL denotes inter humeral suture length; SPL denotes inter pectoral suture length; SFL denotes inter femoral suture length; ANSS denotes distance from the anal notch to the supracaudal scute tip; AN denotes the anal notch between the two anal scutes; TL denotes tail length from the cranial margin of the cloaca to the tail´s tip; SCW6 denotes the carapace width at the level of the 6th marginal scute; CCW6 denotes the curved carapace width at the level of the 6th marginal scute; PML denotes the plastron midline length from the gular notch to the anal notch; WV2 denotes the maximal width of 2nd vertebra; WV3 denotes the maximal width of the 3rd vertebra; WV4 denotes the maximal width of 4th vertebra

The mean body mass was 735 ±241.8g (range: 160-1250g) for males and 1128.8 ±355.6 g (range: 250-1920g) for females, with the peak frequency distributions ranging between 800 and 1000g (28.4%) for males, and 1000 and 1200g (32.7%) for females. The mean SCL was 151.3 ±18.2mm (range: 100-190mm) for males and 172.4 ±22.9mm (range: 100-220mm) for females, with the peak frequency distributions ranging between 139 and 152mm (30.9%) for males, and 178 mm and 191

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mm (27.1%) for females. Females were heavier (t = -8.57, df=186, p=0.0001) and larger (t = -6.81, df=186 p=0.0001) than males.

When differences among regions were tested using SCL as a covariate in MANCOVA, differences were observed (Pillai's Trace=2.302, F=10.274, p=0.001). In addition, differences were also found for males (Pillai's Trace=2.982, F=8.013, p=0.001) and females (Pillai's Trace=2.242, F=5.035, p=0.001) among the regions.

There was no correlation (P>0.05) between mean body size (SCL and CCL) and latitude degree for north-south cline in males, rejecting Bergmann's rule. On the other hand, the CCL of females was positively correlated with degrees north latitude (n=107, r=0.216, p=0.025), following Bergmann's rule.

The body condition index (CI) of Hermann's tortoises ranged from -0.24 to 0.62 with a mean CI of 0.00 in all regions. The mean CI of 0 indicates that the observed body mass is equal to the predicted body mass. Descriptive statistics of CI by region are shown in Table 2. The mean CI differed (F=2.612, df=4, p=0.037) among regions. The observed mass ratio to the predicted mass was positive in the Tirana and Berati regions, whereas it was negative in the other regions. The mean CI was influenced (F=10.492, df=1, p=0.001) by sex and ranged from -0.23 to 0.36 (mean of -0.02 ±0.08) in males and from -0.22 to 0.61 (mean of 0.01 ±0.10) in females. The frequency distribution of CI by sex is illustrated in Figure 3.

Table 2. Descriptive statistics of the body condition index (CI) of Hermann's tortoises in five regions of Albania.

Regions	N	Mean ±StdDev	Min - Max
Shkodra	21	-0.01 ±0.13	-0.19 - 0.43
Tirana	40	0.03 ±0.06	-0.06 - 0.36
Berati	53	0.00 ±0.08	-0.23 - 0.19
Ballshi	25	-0.02 ±0.04	-0.10 - 0.08
Saranda	49	-0.02 ±0.12	-0.22 - 0.61
Total	188	0.00 ±0.09	-0.23 - 0.61

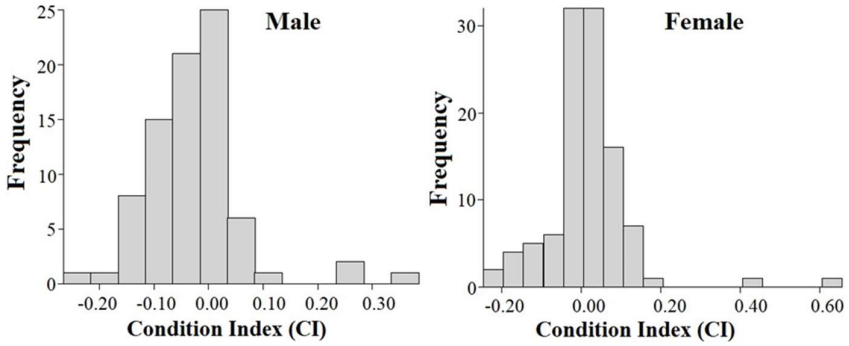

Figure 3. Frequency distributions of body condition index (CI) in male (n= 81) and female (n=107) Hermann's tortoises in Albania

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In discriminant function analysis, the first canonical function accounted for the largest amount of between group variability (84.9 %) while the second, third and fourth accounted for 13.2%, 1.4% and 0.5% respectively. Plotting DF1 and DF2 explained 98.2% of the between-group variation and revealed clear between population differences. The Saranda and Shkodra populations were the most isolated from each other and from all other samples (Figure 4), suggesting that there is limited intermingling between these Saranda and Shkodra populations. With respect to correct initial classification of individuals into their original populations, 83,5% of original classifications were correct (Table 3). Not unexpectedly, the proportion of Saranda and Shkodra samples correctly classified into their original group was the highest (100%).

Table 3. Percentage‡ of initial classifications that were correct, shown by region.

Regions	Berati	Shkodra	Saranda	Ballshi	Tirana	Total
Berati	**69.8**	0.0	0.0	5.7	24.5	100.0
Shkodra	0.0	**100.0**	0.0	0.0	0.0	100.0
Saranda	0.0	0.0	**100.0**	0.0	0.0	100.0
Ballshi	4.0	0.0	0.0	**68.0**	28.0	100.0
Tirana	7.5	0.0	0.0	10.0	**82.5**	100.0

‡ Percentages within rows sum to 100%

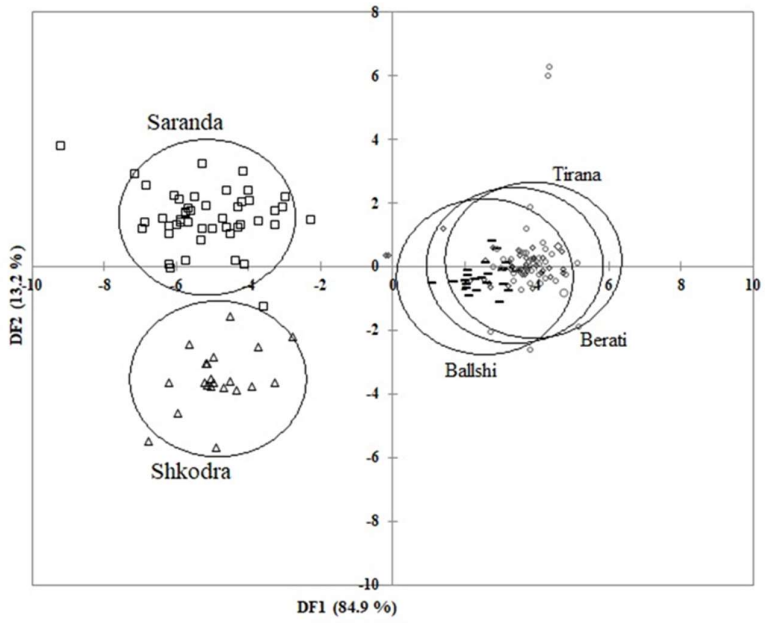

Figure 4. Discriminant function analysis plot with 95% confidence ellipses based on morphological analysis among the five regions in Albania

In order to discern which morphometric characters differentiate populations, pooled within-group correlations between discriminating variables and standardized canonical discriminant functions were produced with DFA. This analysis revealed that the observed differences were

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mainly from carapace dimensions such as SCW and CCW, followed by (in order of decreasing influence) body mass, PL, G, SFL, AN, TL, SCW6 and WV2.

Discriminant function analysis by sex among the regions was also performed. Plotting DF1 (56.9%) and DF2 (40.5%) explained 97.4% of the between-group variation and revealed clear population differences by region in males. The Saranda and Shkodra populations were the most isolated from each other and from all other samples (Figure 5). The initial classification of individuals into their proper population was correct in 90.1% of cases. The proportion of samples initially classified into their correct group was the highest (100%) in Saranda, Shkodra and Ballshi; Tirana was 78.6%, and Berati was 61.5% correctly classified initially. The observed differences were mainly from carapace dimensions such as CCL, SCW and CCW, followed PL, AN, TL, SCW6.

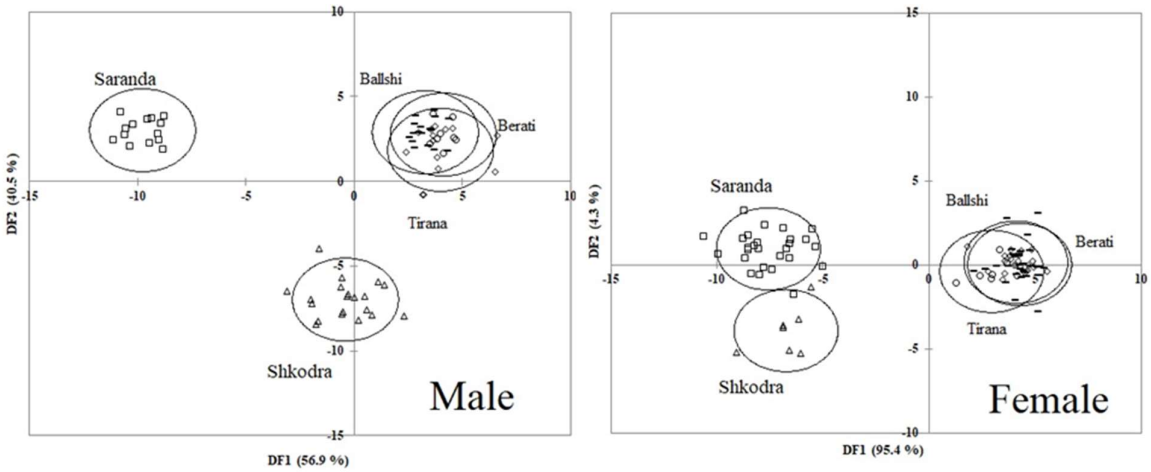

Figure 5. Discriminant function analysis plot with 95% confidence ellipses based on the morphological analysis by the male and female among the five regions in Albania

Plotting DF1 (95.4%) and DF2 (4.3%) explained 99.7% of the between-group variation and revealed clear differences between populations by region in females. The Saranda and Shkodra populations were the most isolated from each other and from all other samples (Figure 5). Seventy-eight-point five percent (78.5%) of samples were initially classified correctly into the proper group. The proportion of samples initially correctly classified was highest (100%) for Saranda and Shkodra samples, followed by Tirana (92.3% initial correct classification), Ballshi (71.4%), and Berati (52.5%). The observed differences were mainly from carapace dimensions such as SCW and CCW, followed by SFL and SCW6.

4. Discussion

This was the first study of Hermann's tortoises with samples obtained from nearly all regions of Albania, and it was the largest-ever study of Hermann's tortoises in Albania (188 specimens studied). The overall population studied was female-biased similar to the only other study conducted in Albania [8]. A female-biased population was also reported in Serbia and the Former Yugoslav Republic of Macedonia [15], as well as in the central Balkans [25]. In contrast, a male-biased population was observed in Greece [38] and in the former Yugoslavia-Montegenero [39]. These differences in sex bias among the various studies may be due to differences in maturity timing between the sexes [40], differences mortality rates of the sexes [41], or some unquantified environmental influence such as high ambient temperature or aberrant rainfall pattern.

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The mean body mass of Albanian Hermann's tortoises was 735 ±241.8g (range: 160-1250g) for males and 1128.8 ±355.6g (range: 250-1920g) for females, and the mean SCL was 151.3 ±18.2mm (range; 100-190mm) for males and 172.4 ±22.9mm (range: 100-220mm) for females. Our result showed that females were significantly larger and heavier than males, and this is consistent with previous studies [42,25,15,7]. In the Vlora region of Albania Hermann's tortoises were130-180 mm for males and 150-200 mm for females [8]. Those measurements are similar to our results for overall Albania, but they are smaller than tortoises found in the study of eastern populations of adult Hermann's tortoises of the eastern Balkans that had an average carapace length of more than 180mm in females and more than 155mm in males [10]. The Hermann's tortoises of Limnaji (Montenegro) were similar in size (151mm for males and 165mm for females) to the Albanian tortoises, but the Starcevo island (Montenegro) population (132mm for males and 145mm for females) were smaller [25]. The Albanian population from the present study was of similar body size to the Turkish Thrace population (153 mm for males and 175mm for female) [7]. Hermann's tortoises of Serbia (populations of the eastern, central and southern), however, were larger in body size than the Albanian population [17,25]. The largest Hermann's tortoises (346mm) were found in Bulgaria [5], whereas smallest (153mm in females) were found in the south of the Peloponnese in Greece [42].

These similarities or differences in body size of Hermann's tortoises may be associated with the latitude at which each population lives because previous studies showed that Hermann's tortoises at higher (more northern) latitudes have larger body size. The body size of Italian Hermann's tortoise showed variation in the North-South cline following Bergmann's rule [22], and body size of Hermann's tortoises in Greece was strongly correlated with latitude [42]. Similar results were reported for *Testudo graeca* [27]. Also, some chelonians support Bergmann's rule, whereas some reject it [43].

In our study, the CCL of females was positively correlated with latitude, but the same was not true for males. This CCL correlation may have an effect on the reproductive output of the female tortoises. The northern latitudes have a shorter reproductive season as the climate will be colder and will have fewer hours of daylight per day in the colder months, thus limiting the number of successive clutches [44]. Moreover, larger females in northern latitudes lay larger clutches, but produce smaller eggs [27]. In a review of clutch size of reptiles [45], some species of tortoises had larger clutches in the north. Clutch size is also associated with female body size in Hermann's tortoises [46]. Moreover, clutch size and egg size is negatively correlated in all tortoises [47]. The tortoises are an ectotherm, it can be disadvantageous have a larger body size because of the time required for warming up a larger mass of tissues [48]. In females of increasing body size, laying more eggs with a decreased egg size may be an effort to increase reproductive success within a shorter breeding season due to colder climate. However, in this study we did not investigate reproductive output of Albanian Hermann's tortoises. Unfortunately, there are also no studies on reproductive output of Hermann's tortoise in Albania. The effect of body size in north-south cline on the reproductive output of Albanian tortoises should be studied in the future.

The body condition index (CI) of Hermann's tortoises for overall Albania is zero, which means the observed body mass is equal to the predicted body mass and relative mass. However, the CI showed a mean negative value in males and a mean positive value in females and was significantly different between males and females. Similar differences were also reported from France [49] and the Turkish Thrace [7]. The CI can be affected by habitat conditions, food and water availability, and tortoise activities [35,49]; it increases in spring and decreases in summer [49]. Our study was conducted during the mating season, and males tend to feed less frequently during the mating season. Also, the CI showed geographic variations where the central part of Albania (Tirana and Berati) had a positive value, whereas the other regions had a negative value. This difference may be related to the stability of the environmental factors of each region, such as habitat conditions and/or

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food and water availability. However, the geographical location within Greece did not affect the mass-to-length relationship and CI of Hermann's tortoises.

Hermann's tortoises showed regional morphological differences in Albania. As a result of these differences, three groups (northern, central and southern) evolved from each other. These three groups were: Shkodra in the north, Tirana, Ballshi and Berati in the center, and Saranda at the south. The important characteristics that played a role in the morphological separation of regions were generally carapace measurements (SCW, CCW, SCW6 and SCL). Similar regional differences were reported between tortoise populations in Serbia and Montenegro, and the Montenegrin population was characterized by small body size and mass [29]. The Hermann's tortoise populations of the Trebinje (Bosnia and Herzegovina) and Pcinja River Valley (Serbia) were distinguished from each other based on carapace shape analyses with the geometric morphometry [26].

Regional differences in morphology may be due to the survival rate, growth rate, mortality rate, genetics of the populations, or north-south cline (i.e., Bergmann's rule). Variation in adult survival rates would be sufficient to explain differences in adult size among populations in Greece [50]. This means larger the size might have higher survival rate, and it might explain why some populations show variations in size. Although adult survival rate is negatively correlated with growth rate, variation in growth rate in the population limits variation in adult size. Differences in adult mortality rate among the populations may also explain variation in adult size. This situation may be due to the high environment temperature caused by frequent fires [42] or due to predation [51]. The low or high mortality rate affects the number of adult individuals leaving the population, and this affects the mean body size of the population. The effect of mortality rate on mean size of the adult population was reported for other chelonian species like sea turtles [52].

Another reason for regional differences may be due to molecular differences, although we did not examine this factor in the present study. Two populations of Hermann's tortoises were distinguished genetically between Trebinje (Bosnia and Herzegovina) and the Pcinja River Valley of Serbia [26]. Molecular variations may have been influenced by the biogeographical history of southern Europe, and geological barriers such as Alps and the Dinaric mountain systems may have divided the populations. In Albania, although there are some geographical barriers between the regions of this study (such as the large rivers Mati, Shkumbini, Semani and Vjosa), we do not think that these barriers completely separate these regions. We hypothesize that the morphological changes of tortoises may have been caused by environmental conditions of the regions, the availability of food and water, or other factors. Morphological variations may be the result of phenotypic flexibility, which is related to environmental conditions and plays a major role in the external morphology of tortoises.

5. Conclusions

Female Albanian Hermann's tortoises are larger and heavier than males which is compatible with previous studies. Also, the existence of a female-biased population was determined, and the Albanian population was morphologically divided into three groups (northern, central, and southern). Among the reasons for this divergence, besides the phenotypic flexibility caused by environmental conditions, may have been the survival and mortality rates of the Hermann’s tortoise population in each region of Albania. This study has paved the way for future investigations concerning the population status of the species, such as recruitments to the population with the reproductive output, clutch size, and its relationship with latitude. In particular, our findings strongly suggest that the molecular variations in and reproductive output of the Albanian Hermann's tortoise should be investigated in the near future.

Author Contributions:

Conceptualization: S.D., B.S.; Methodology: S.D., B.S., O.G.; Software: B.S., O.G., T. S.; Investigation: S.D., T.J.; Writing—original draft preparation: S.D., B.S., O. G., Writing—review and editing: T.S., T.J. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest.

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