Herpetology Notes, volume 12: 229-233 (2019) (published online on 26 January 2019)

# Sexual dimorphism of *Testudo* tortoises from an unstudied population in northeast Greece

Konstantina N. Makridou[1,*], Charalambos T. Thoma[1], Dimitrios E. Bakaloudis[1], and Christos G. Vlachos[1]

**Abstract.** Sexual dimorphism in tortoise species is prominent, but may vary geographically. Here, we investigated sexual dimorphism of *Testudo hermanni* and *T. graeca* from previously unstudied populations in northeast Greece. Females of each species illustrated higher mean values in almost all traits and exhibited better body condition than males. The longer tails and smaller plastron found in males of *T. hermanni*, and the wider anal notch found in males of *T. graeca* are believed to be linked with mating success and courtship behavior. When compared to other regions, both our species were found to be larger and heavier in most cases. Our results may be used in future comparative studies, providing additional insights for both species.

**Keywords.** *Testudo hermanni*, *Testudo graeca*, morphometry, Scale mass index, Greece

## Introduction

Sexual dimorphism in body size and shell shape is prominent in tortoise species and has been shown to be driven by natural selection (e.g. to increase female fecundity), sexual selection (e.g. to increase male reproductive success), or a combination of the two (Bonnet et al., 2001; Djordjevíc et al., 2011; Willemsen and Hailey, 2003). In addition, some tortoise species exhibit considerable geographic variation in the degree of sexual dimorphism in body size and shell shape. Kaddour et al. (2008) and Djordjević et al. (2011,2013) found that sexual size dimorphism in body size is more prominent than that of shell shape across populations and species. Geographic variation in tortoise species body size appears to be driven by latitude (Ljubisavljević et al., 2012; Werner et al., 2016; Willemsen and Hailey, 1999) and altitude (Willemsen and Hailey, 1999) or can change to a North-South cline (Sacchi et al., 2007).

In this study, we use morphometry as a tool to investigate sexual dimorphism of two tortoise species; *T. hermanni boettgeri* (Mojsisovics, 1889) and *T. graeca ibera* (Pallas, 1814), found in northeast Greece. Although, morphometry and sexual dimorphism of tortoises has been previously studied in several regions of Greece (Willemsen and Hailey, 1999, 2003), populations from Evros district remain unstudied. Our results aim to offer additional insights from a region where tortoises have never been studied before and provide new data that may be used in future comparative studies.

[1] School of Forestry and Natural Environment, Aristotle University of Thessaloniki, P.O. Box 241, 541 24 Thessaloniki, Greece.
[*] Corresponding author. E-mail: makridkn@for.auth.gr

## Materials and Methods

The study was conducted in Evros district, at the northeastern part of Greece (41° 79′-40° 72′ N, 26° 63′-25° 61′ E). The area is characterized by a sub-Mediterranean climate. However, considerable spatial variation in environmental conditions and land cover types exist despite its relatively small size (ca. 4065 km$^2$). Mean monthly temperatures range from 4$^0$C in January to 25$^0$C in July, mean annual precipitation is 664 mm (Bakaloudis et al., 2009), and altitudes range from sea level to 984 m. Agricultural areas dominate the northern and eastern part of the area, whereas forests and shrubs mainly cover the western and central portions.

Fieldwork was conducted from 2015 to 2016 throughout Evros district, covering the spectrum of available habitats proportional to the area of each habitat type (De Frutos et al., 2007). Observers recorded the exact location (using a handheld GPS device), species and sex of each individual tortoise encountered. Morphometric traits were measured immediately after an individual tortoise was captured (Stubbs et al., 1984). Sex was determined based on tortoise external characteristics (e.g. concavity of plastron and longer tails in males). Juveniles (< 10 cm straight carapace length) for which gender could not be determined were excluded from the analysis (Willemsen and Hailey, 2003). Before release, each individual was marked (using a non-toxic marker) with a unique code to avoid double-counting.

For each individual, a total of 13 traits were measured: Straight carapace length (SCL), curved carapace length (CCL), midline plastron length (MPL), mid body carapace width (CW1), posterior carapace width (CW2), carapace height (CH), pectoral scute length (PSL) and width (PSW), femoral scute length (FSL) and width (FSW), anal notch width (ANW), tail length (TL), and body mass (Mass) (for full description of traits see Table 1). Traits SCL and CH were measured to the nearest mm using a specially constructed caliper marked with a millimeter scale, having a fixed perpendicular block at the zero mark and a second sliding perpendicular block referred to as ''tortometer'', CCL was measured with a flexible measuring tape, and all other straight-line traits were measured using digital calipers (0.1 mm precision). In addition, body mass of each individual was measured using a digital balance (1 g precision). All traits were measured by a single observer to avoid measurement error due to observer variability (Thoma et al., 2018).

Differences in mean trait sizes between sexes of each species were investigated using independent samples t-tests (parametric or non-parametric where appropriate). Scaling patterns of tortoise traits (log transformed) on body size (log SCL) were examined via standard major axis (SMA) regression, as this approach is more suitable in estimating scaling effects between sexes of a given species (Warton et al., 2006). Moreover, 95% confidence intervals of slope estimates were calculated to test for deviations from isometry. Finally, body condition of each individual was calculated using the scaled mass index (Peig and Green, 2009), which uses the equation: $SMI = Mi\ [L_0/Li]\ ^\wedge\ b_{sma}$, where Mi and Li are the body mass and the straight carapace length of individual i, $b_{sma}$ is the scaling exponent estimated by the SMA regression of body mass on straight carapace length and $L_0$ is the arithmetic mean value for the study population. Differences in body condition between sexes of a species where then compared using independent samples t-tests (parametric or non-parametric where appropriate). All analyses were run in R (R Core Development Team, 2016). SMA regression analysis was carried out using the package *smatr* (Warton et al., 2012).

## Results

A total of 113 individuals (77 females, 36 males) of *T. hermanni* and 118 individuals (76 females, 42 males) of *T. graeca* were analyzed. *T. hermanni* and *T. graeca* females had higher mean values in all but two traits in comparison to their male counterparts (Table 1). Males of both species exhibited higher mean values for traits ANW (45.03 ± 7.32 and 46.14 ± 4.99, respectively) and TL (58.28 ± 7.77 and 44.74 ± 5.4, respectively) when compared to females (ANW: 30.8 ± 3.64 and 35.37 ± 6.06, respectively and TL: 37.97 ± 5.2 and 33.88 ± 4.64, respectively).

A positive allometrical relationship ($P < 0.05$) was found between SCL and all other traits for male and female individuals of *T. hermanni*. However, females did not show a statistically significant relationship for traits ANW ($P = 0.638$) and PSL ($P = 0.076$). Differences in the slope between sexes were detected in MPL (Likelihood ratio = 5.698, df = 1, $P = 0.017$), TL (Likelihood ratio = 4.945, df = 1, $P = 0.026$) and PSL (Likelihood ratio = 4.007, df = 1, $P = 0.045$) (Table1). Similarly, male and female individuals of *T. graeca* illustrated a positive allometrical relationship ($P < 0.05$) between SCL and all other traits, with the exception of CH ($P = 0.36$) and ANW ($P = 0.07$) in males. Differences in the slope between sexes were found only for trait ANW (Likelihood ratio = 4.135, df = 1, $P = 0.042$) (Table 1).

Scale mass index for male and female individuals of *T. hermanni* ($b_{sma} = 2.704$, $L_0 = 162.5$ and $b_{sma} = 2.242$, $L_0 = 191.77$, respectively) (Fig. 1A) and *T. graeca* ($b_{sma} = 2.686$, $L_0 = 192.2$ and $b_{sma} = 2.704$, $L_0 = 213.67$, respectively) (Fig. 1B) were used to estimate body condition, which was found to differ significantly ($P < 0.001$) between sexes within species. Specifically, *T. hermanni* females (1434.66 ± 158.41) had a higher mean body condition value than males (947.03 ± 129.49) (W = 2717, $P < 0.001$). The same pattern was also observed between female (2062.04 ± 255.19) and male (1541.47 ± 190.74), individuals of *T. graeca* ($t = 12.54$, $P < 0.001$).

## Discussion

Research on tortoise species' sexual dimorphism from previously unstudied regions may enhance our knowledge of the species biology, also allowing geographical patterns to be investigated among different populations. Our results showed that female individuals of both *T. hermanni* and *T. graeca* were larger, heavier and had better body conditions than males.

**Table 1**. Mean trait values (± standard deviation) for *T. hermanni* and *T. graeca*. Whenever statistically significant ($P < 0.05$), the sex with the higher mean value is shown in bold. Slopes (upper and lower 95% confidence intervals) and *P* values; resulting from the standard major axis regression analysis of each trait, using straight carapace length as a covariate.

| | | | ***T. hermanni* (77 females, 36 males)** | | | ***T. graeca* (76 females, 42 males)** | | |
|---|---|---|---|---|---|---|---|---|
| **Trait** | **Description** | | **Mean (± SD)** | **Slope (95%CI)** | ***P*** | **Mean (± SD)** | **Slope (95%CI)** | ***P*** |
| SCL | Length from the outermost projection of the cervical scute to the tip of the supracaudal | ♀ | **191.77** (15.27) | – | – | **213.64** (21.38) | – | – |
| | | ♂ | 162.5 (17.63) | – | – | 192.2 (16.66) | – | – |
| Mass | Weight measured in g | ♀ | **1439.74** (259.98) | 2.242 (1.966-2.557) | 0.051 | **2091.05** (532.98) | 2.704 (2.394-3.054) | 0.942 |
| | | ♂ | 967.78 (300.98) | 2.704 (2.359-3.1) | | 1558.1 (377.77) | 2.686 (2.326-3.102) | |
| CCL | Domed length from the tip of the cervical scute to the tip of the supracaudal scute | ♀ | **235.97** (18.45) | 0.979 (0.872-1.09) | 0.906 | **267.12** (27.51) | 1.083 (0.987-1.187) | 0.316 |
| | | ♂ | 215.22 (23.97) | 0.989 (0.879-1.112) | | 249.48 (21.23) | 0.997 (0.869-1.143) | |
| CH | Vertical high from the most dorsal point of carapace and the most ventral point of plastron | ♀ | **86.28** (5.8) | 0.856 (0.696-1.053) | 0.819 | **99.63** (8.6) | 0.879 (0.746-1.035) | 0.321 |
| | | ♂ | 77.45 (6.99) | 0.829 (0.694-0.991) | | 91.83 (8.05) | 1.022 (0.792-1.32) | |
| MPL | Length from the notch between the gular scutes to the notch formed by anal scutes | ♀ | **155.17** (9.96) | 0.758 (0.646-0.888) | **0.017** | **185.33** (19.33) | 1.074 (0.969-1.191) | 0.334 |
| | | ♂ | 122.43 (13.34) | 0.996 (0.849-1.17) | | 156.3 (13.33) | 0.976 (0.823-1.157) | |
| CW1 | Width at the middle of the abdominal scute, at the front edge of the marginal scute | ♀ | **144.77** (9.08) | 0.85 (0.75-0.964) | 0.324 | **165.84** (16.62) | 1.023 (0.919-1.138) | 0.136 |
| | | ♂ | 129.89 (13.56) | 0.942 (0.8-1.109) | | 150.04 (10.92) | 0.86 (0.701-1.055) | |
| CW2 | Width at the junction of the femoral and anal scutes in the midline | ♀ | **150.45** (9.65) | 0.84 (0.733-0.963 | 0.281 | **165.64** (16.01) | 0.937 (0.84-1.046) | 0.768 |
| | | ♂ | 140.83 (14.51) | 0.937 (0.807- 1.088) | | 155.98 (12.78) | 0.969 (0.797-1.178) | |
| ANW | Width between the tips of the two anal scutes | ♀ | 30.8 (3.64) | 1.53 (1.245-1.881) | 0.685 | 35.37 (6.06) | 1.694 (1.414-2.029) | **0.042** |
| | | ♂ | **45.03** (7.32) | 1.44 (1.161-1.786) | | **46.14** (4.99) | 1.292 (1.056-1.58) | |
| TL | Length from the anterior edge of cloaca to the tip of the tail | ♀ | 37.97 (5.2) | 1.674 (1.408-1.99) | **0.026** | 33.88 (4.64) | 1.349 (1.073-1.697) | 0.942 |
| | | ♂ | **58.28** (7.77) | 1.234 (1.003-1.518) | | **44.74** (5.4) | 1.333 (1.051-1.692) | |
| PSL | Length of the pectoral scute at mid-seam | ♀ | **22.87** (3.54) | 1.752 (1.349-2.277) | **0.045** | **22.61** (4.11) | 1.816 (1.395-2.362) | 0.736 |
| | | ♂ | 18.91 (2.55) | 1.194 (0.903-1.578) | | 20.85 (3.87) | 1.945 (1.437-2.632) | |
| PSW | Width of the pectoral scute at mid-seam | ♀ | **55.95** (4.18) | 1.041 (0.846-1.282) | 0,499 | **64.62** (6.69) | 1.032 (0.915-1.163) | |
| | | ♂ | 47.3 (5.14) | 0,959 (0.841-1.093) | | 58.64 (5.15) | 0.973 (0.8-1.183) | |
| FSL | Length of the femoral scute at mid-seam | ♀ | **35.86** (4.34) | 1.478 (1.251-1.745) | 0.562 | **44.02** (7.87) | 1.675 (1.428-1.963) | 0.928 |
| | | ♂ | 28.61 (4.3) | 1.378 (1.156-1.641) | | 36.7 (5.62) | 1.653 (1.307-2.091 | |
| FSW | Width of the femoral scute at mid-seam | ♀ | **41.29** (3.67) | 1.082 (0.907-1.291) | 0.811 | **45.27** (5.37) | 1.156 (1.012-1.321) | 0.258 |
| | | ♂ | 36.8 (4.73) | 1.115 (0.937-1.326) | | 41.77 (4.32) | 0.985 (0.77-1.26) | |

Sexual dimorphism was evident in both tortoise species, with female individuals exhibiting greater mean values in most measured traits. However, when straight carapace length was used as a covariate in the SMA analysis, fewer traits were found to differ significantly between species' sexes. *T. hermanni* males had smaller midline plastron length, longer tails and longer pectoral scutes than females, whereas *T. graeca* males had wider anal notches compared to females. For both species, differences in the above mentioned traits may benefit male individuals during mating or courtship (Hailey and Willemsen, 2003; Kaddour et al., 2008). More specifically, the smaller plastron found in males of *T. hermanni* may be linked to increased mobility, allowing them to chase female individuals during the mating season and facilitate mounting (Hailey and Loumbourdis, 1990). Likewise, the larger plastron in female individuals may be explained by fecundity selection, under which the larger plastron provides increased ventral protection as well as increased space for body reserves and clutches (Bonnet et al., 2001). Additionally, longer tails found in males of *T. hermanni* serve, in combination with the 'claw' at the tip of their tail, in stimulating females during courtship (Bonnet et al., 2001; Keswick and Hofmeyr, 2015). Conversely, *T. graeca* males use a different courtship behavior (Hailey and Willemsen, 2000), due to the lack of tail 'claw'. In their case, the wider anal notch in males allows them to move their tail more freely, which is believed to promote mating success (Kaddour et al., 2008). Although

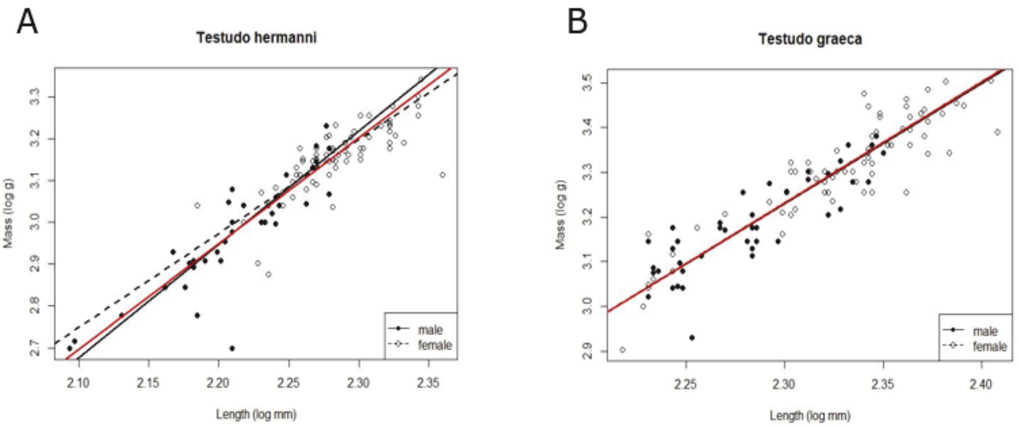


**Figure 1**. Allometric scaling relationship of body mass and straight carapace length for; (A) *T. hermanni* and (B) *T. graeca.* Lines represent the standard major axis regression slopes for sexes (straight line = males, dash line = females, red line = common slope).

pectoral scutes were found to be significantly longer in males of *T. hermanni*, this particular trait has no known functional importance, but can be used along with the length of the femoral scute to differentiate between the subspecies *T. hermanni hermanni* and *T. hermanni boettgeri* (Willemsen and Hailey, 2003). Finally, given the larger size and weight of female individuals in both species, their body condition was found to be better than that of males. This finding was expected as the value of this index is positively associated with greater values of mean size and weight. However, seasonal variation between sexes and body condition may occur (Willemsen and Hailey, 2002).

Mean straight carapace length values of *T. hermanni* (191.77 mm females and 162.5 mm males) and *T. graeca* (213.64 mm females and 192.2 mm males) found in our study area were higher than those previously reported in other parts of Greece (Willemsen and Hailey, 1999, 2003), with the exception of *T. hermanni* populations from Deskati (214 mm females and 195 mm males) and Kastoria (197 mm females and 177 mm males) (Willemsen and Hailey, 1999). This may be because the former two regions are situated at higher altitudes (650-800 m), and tortoise body size is expected to increase with increased elevation (Willemsen and Hailey, 1999). Moreover, when comparing our results to those reported in other countries at higher latitudes, we found cases where *T. hermanni* illustrated lower (Ljubisavljević et al., 2012), as well as higher (Djordjevíc et al., 2011, 2013) mean straight carapace length values. In the former case, lower values may be attributed to the relatively small sample size used in that particular study or perhaps the lower altitudinal range characterizing the study area. Conversely, *T. graeca* individuals found in our study area were consistently larger and heavier (2091.05 g females and 1558.1 g males) than those reported in other parts of the world (Türkozan and Olgun, 2005; Cogălniceanu et al., 2010). Latitudinal and altitudinal differences characterizing each region may perhaps explain these differences, with larger tortoises expected at higher latitudes (Sacchi et al., 2007; Werner et al., 2016) and higher altitudes (Willemsen and Hailey, 1999).

In conclusion, our results suggest that *T. hermanni* follows the general pattern of geographic variation, according to which increasing body size is positively associated with increasing latitude and/or altitude. On the other hand, no such clear pattern was evident for *T. graeca.* This may perhaps be explained by the limited number of studies regarding the species. Thus, further research is required so as to better understand and indentify the drivers of geographic variation among *T. graeca* populations.

**Acknowledgements.** We would like to thank the Soufli Forestry Service for kindly providing KNM and CTT free accommodations during the sampling period. We also thank the reviewers for their constructive comments, which helped improve the manuscript. This study is part of a Ph.D. thesis (KNM), funded by the A.G. Leventis Foundation Scholarship program.

## References

Bakaloudis, D.E., Vlachos, C.G., Chatzinikos, E., Bontzorlos, V., Papakosta, M. (2009): Breeding habitat preferences of the turtledove (*Streptopelia turtur*) in the Dadia-Soufli National Park and its implications for management. European Journal of Wildlife Research **55:** 597–602.

Bonnet, X., Lagarde, F., Henen, B.T., Corbin, J., Nagy, K.A., Naulleau, et al. (2001): Sexual dimorphism in steppe tortoises (*Testudo horsfieldi)*: influence of the environment and sexual selection on body shape and mobility. Biological Journal of the Linnean Society **72:** 357–372.

Cogălniceanu, D., Samoilă, C., Tudor, M., Tallowin, O. (2010): An extremely large spur-thighed tortoise male (*Testudo graeca*) from Măcin Mountains National Park, Romania. Herpetology notes **3:** 045–048.

De Frutos, A., Olea, P.P., Vera, R. (2007): Analyzing and modelling spatial distribution of summering lesser kestrel: The role of spatial autocorrelation. Ecological Modelling **200:** 33–44.

Djordjevíc, S., Djurakíc, M., Golubovíc, A., Ajtíc, R., Tomovíc, L., Bonnet, X. (2011): Sexual body size and body shape dimorphism of *Testudo hermanni* in central and eastern Serbia. Amphibia-Reptilia **32:** 445–458.

Djordjević, S., Tomović, L., Golubović, A., Simović, A., Sterijovski, B., Djurakic, M., Bonnet, X. (2013): Geographic (in-)variability of gender-specific traits in Hermann's tortoise. Herpetological Journal **23:** 67–73.

Hailey, A., Loumbourdis, N.S. (1990): Population ecology and conservation of tortoises: demographic aspects of reproduction in *Testudo hermanni*. Herpetological Journal **1:** 425–434.

Hailey, A., Willemsen, R.E. (2000): Population density and adult sex ratio of the tortoise *Testudo hermanni* in Greece: evidence for instrict population regulation. Journal of Zoology, London **251:** 325–338.

Hailey, A., Willemsen, R.E. (2003): Changes in the status of tortoise populations in Greece 1984–2001. Biodiversity and Conservation **12:** 991–1011.

Kaddour, K.B., Mouden, E.H., Slimani, T., Bonnet, X., Lagarde, F. (2008): Sexual dimorphism in the Greek Tortoise: A test of the body shape hypothesis. Chelonian Conservation and Biology **7:** 21–27.

Keswick, T., Hofmeyr, M.D. (2015): Sexual dimorphism and geographic variation in the morphology of a small southern African tortoise *Psammobates oculifer*. Amphibia-Reptilia **36:** 55–64.

Ljubisavljević, K., Džukić, G., Vukov, T.D., Kalezić, M.L. (2012): Morphological variability of the Hermann's tortoise (*Testudo hermanni*) in the Central Balkans. Acta Herpetologica **7:** 253–262.

Peig, J., Green, A.J. (2009): New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos **118:** 1883–1891.

R Core Development Team (2016): R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sacchi, R., Pupin, F., Rosa, D.P., Fasola, M. (2007): Bergmann's rule and the Italian Hermann's tortoises (*Testudo hermanni*): latitudinal variations of size and shape. Amphibia-Reptilia **28:** 43–50.

Stubbs, D., Hailey, A., Pulford, E., Tyler, W. (1984): Population ecology of European tortoises: review of field techniques. Amphibia-Reptilia **5:** 57–68.

Türkozan, O., Olgun, K. (2005): A Preliminary Survey of *Testudo graeca* Linnaeus 1758 Specimens from Central Anatolia, Turkey. Turkish Journal of Zoology **29:** 255–262.

Thoma, C.T., Makridou, K.N., Bakaloudis, D.E., Vlachos, C.G. (2018): Evaluating the potential of tree-dimensional laser surface scanning as an alternative method of obtaining morphometric data. Annales Zoologici Fennici **55:** 55–66.

Warton, D.I., Duursma, R.A., Falster, D.S., Taskinen, S. (2012): smatr 3 - an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution **3:** 257–259.

Warton, D.I., Wright, I.J., Falster, D.S., Westoby, M. (2006): Bivariate line-fitting methods for allometry. Biological Reviews of the Cambridge Philosophical Society **81:** 259–291.

Werner, Y.L., Korolker, N., Sion, G., Göçmen, B. (2016): Bergmann's and Rensch's rules and the spur-thighed tortoise (*Testudo graeca*). Biological Journal of the Linnean Society **117:** 796–811.

Willemsen, R.E., Hailey, A. (1999): Variation of adult body size of the tortoise *Testudo hermanni* in Greece: proximate and ultimate causes. Journal of Zoology, London **248:** 379–396.

Willemsen, R.E. , Hailey, A. (2002): Body mass condition in Greek tortoises: regional and interspecific variation. Herpetological Journal **12:** 105–114.

Willemsen, R.E. , Hailey, A. (2003): Sexual dimorphism of body size and shell shape in European tortoises. Journal of Zoology, London **260:** 353–365.

*Accepted by Eric Munscher*