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An evaluation of the taxonomic validity of *Testudo werneri*

Omar Attum[1,*], Sherif Baha El Din[2], Salvador Carranza[3], Ryan Earley[4], E. Nicholas Arnold[5], Bruce Kingsbury[1]

Abstract. The Egyptian tortoise *Testudo kleinmanni* was recently split into two species on the basis of apparent differences in shell morphology and markings. *Testudo kleinmanni* was restricted to areas west of the Nile river and a new form, *T. werneri*, was described which occurred east of the Nile river (Perälä, 2001). However, when the morphometric analysis on which this decision was based (Perälä, 2001) was adjusted to allow for experiment-wise Type I error, by using *P*-value corrections, the proportion of the 46 characters that differed significantly between the two populations fell from 36.9% to only 13% in males and from 39.1% to just 8.7% in females. We then conducted a new morphometric analysis using our own data set that showed minor significant variation in morphometric and plastron markings between populations. An analysis of mitochondrial DNA based on 393 base pairs of the 12S rRNA gene, also showed near uniformity of western and eastern populations. Genetic divergence was only 0.2%, with the only consistent difference being a single G – A substitution at position 205. Based on the revised interpretation of Perälä (2001) results, our morphometric analysis on our own data set, and the molecular evidence, the variation observed between populations is normal within a species and therefore *T. werneri* is not a distinct independent evolutionary lineage and should not be considered a separate species from *T. kleinmanni*.

Keywords: *Testudo kleinmanni*, *Testudo werneri*, Egypt, morphology, mitochondrial DNA, 12S rRNA.

Introduction

The genus *Testudo* occurs throughout much of the Mediterranean basin and is represented in the southeastern section by the smallest species of the genus, the Egyptian tortoise, *Testudo kleinmanni* Lortet, 1883. The Egyptian tortoise's range extends from the western Negev in Israel, through northern Egypt to Cyreniaca and possibly Tripolitania in Libya (Baha El Din et al., 2002). Recently, Perälä (2001) using solely shell morphology, argued that *T. kleinmanni* was in fact two species, separated by the Nile river. *Testudo kleinmanni* occurred west of the Nile and *T. werneri* east of the Nile.

On the basis of reservations about the statistical procedures used by Perälä (2001) to test differences between populations, and our extensive studies of animals from both populations, we believe the splitting of *T. kleinmanni* into two species is inappropriate. This is especially so as the use of shell morphometrics and coloration is known to sometimes be unreliable in Chelonian taxonomy, being highly plastic and variable within species, and affected greatly by environmental factors (Leuck and Carpenter, 1981; Lovich and Earnst, 1989; Germano, 1993; Rowe, 1997; Willemsen and Hailey, 1999; Austin et al., 2003; Palkovacs et al., 2003; Spinks et al., 2004; Carretero et al., 2005). Morphometric variation of shell characteristics can also be of low taxonomic value because of significant correlations with carapace length indicating that these features vary with growth and therefore age (Mosiman, 1956; Tinkle, 1962; Lovich and Earnst, 1989; Leuck and Carpenter, 1981). A combination of these factors may explain why some species described on shell morphometric basis do not always agree

1 - Center for Reptile and Amphibian Conservation and Management, Indiana-Purdue University Fort Wayne, 2101 E. Coliseum Blvd., Fort Wayne, IN 46805, USA
2 - Nature Conservation Sector, Egyptian Environmental Affairs Agency, 3 Abdalla El Katib St., Dokki, Cairo, Egypt
3 - Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain
4 - Department of Biology, California State University, Fresno, 2555 East San Ramon Avenue M/S SB73, Fresno, CA 93740, USA
5 - Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD, U.K.
*Corresponding autor: omarattum@yahoo.com

with genetic data (Germano, 1993; Austin et al., 2003; Palkovacs et al., 2003; Spinks et al., 2004; Carretero et al., 2005; Fritz et al., 2005).

Some of the statistical methods used to distinguish *T. werneri* as a separate species are inappropriate (Holm, 1979; Parckard and Boardman, 1999; Garcia, 2003). For example, 46 separate univariate analyses of variance were used to test differences between populations for morphometric shell characteristics without taking into account Type I errors, in which differences are falsely found to be statistically significant. While, partitioning the data set might increase ease of interpretation, it is necessary in this situation to avoid spurious results by making adjustments to the *P* value level of significance to control for Type I error. By failing to do this, Perälä (2001) analysis is vulnerable to experiment-wise Type I errors because the likelihood of accepting false significant differences increases with the number of non-mutually exclusive hypotheses tested (Sokal and Rohlf, 1981).

The other major statistical flaw was the use of ratios to control for body size by dividing measurements of shell characteristics by carapace length. The use of such simple ratios seldom eliminates the effect of body size, introduces major problems in statistical analysis, and can lead to spurious data interpretation (Mosimann, 1958; Iverson, 1982; Atchley et al., 1976; Packard and Boardman, 1988; Albrecht et al., 1993; Allison et al., 1995; Packard and Boardman, 1999). This is problematic because spurious correlations among the ratios and other variables can result in between group statistical comparisons that are considered unreliable (Atchley et al., 1976; Albrecht et al., 1993; Allison et al., 1995; Raubenheimer, 1995; Garcia-Berthou, 2001). The more superior and widely accepted statistical procedure for adjusting for body size is analysis of covariance, ANCOVA (Atchley et al., 1976; Atchley, 1978; Atchley and Anderson, 1978; Phillips, 1983; Reist, 1985; Packard and Boardman, 1988; Jackson and Somers, 1991; Garcia-Berthou, 2001).

We therefore decided to re-examine Perälä's hypothesis that populations west and east of the Nile valley are taxonomically different. We first reinterpreted Perälä's (2001) morphometric data using P value corrections, then conducted a new independent morphometric analysis using our own data set, and finally used molecular techniques to estimate genetic differences between the two populations in question.

Materials and methods

Material examined

We examined a total of 136 specimens; these were grouped according to geographic locality, east and west of the Nile. The analysis included 34 eastern animals (12 males and 22 females) and 104 western animals (49 males and 55 females). Following are the details of examined material:

Materials from east of the Nile were (FMNH 58720); 31 live animals measured in the field from Zaranik Protected Area, North Sinai (31°05′N, 33°25′E); and two live individuals whose measurements were taken in the Negev by Jarmo Perälä and deposited in the British Museum of Natural History (JP 97.3.36; JP 97.3.37). For west of the Nile we used (FMNH 79184, 79185, 79186, 79187, 79188, 79190, 79191, 79192, 79193, 82667); two live animals measured in the field from Omayed Protected Area (30°45′N, 29°10′E); and 102 live animals of Libyan origin that were confiscated from an animal dealer upon their arrival in Egypt.

Morphological data

We used two statistical methods to correct Perälä's *P* values; the sequential Dunn-Sidak method and the more conservative Bonferroni corrections, which minimize Type I errors by adjusting the *P* values for multiple tests (Day and Quinn, 1989; Quinn and Keough, 2002). For our own morphometric measurements, we considered twenty-two of the twenty-four variables Perälä (2001) found to be significant. We did not measure the minimum distance between the right eye and tympanum and gular scute height (the minimum distance between gular scute/humeral midline crossing and dorso-median gular lip surface), because it was difficult to take those measurements from live animals. The twenty morphometric variables as defined by Perälä (2001) are maximum (not midline) carapace length (CL), curved carapace length over the dome, from the anterior tip of nuchal scute to the posterior tip of supracaudal (CU), maximum mid-body width within central bridge area not affected by a potential flaring of anterior or posterior marginals (MI), maximum width of shell at posterior marginals (MA), maximum shell height (HE), maximum inner width of anterior shell opening (ASO-w), maximum (combined) humeral scute width (HUM-w), maximum (combined) femoral scute

width (FEM-w), anal midline (interanal) seam length (AN-m), maximum (combined) anal scute width (AN-w), maximum (combined) pectoral scute width (PEC-w), maximum (combined) abdominal scute width (ABD-w), maximum width of first vertebral scute (V1-w), maximum width of second vertebral scute (V2-w), maximum width of third vertebral scute (V3-w), maximum width of fourth vertebral scute (V4-w), maximum width of fifth vertebral scute (V5-w), maximum median length of first vertebral scute (V1-l), maximum median length of second vertebral scute (V2-l), maximum median length of third vertebral scute (V3-l), first costal length as the minimum straight-line distance between the anteriormost and posteriormost contact points with adjacent (normally first and fifth) marginal scutes (C1), maximum dorsal width of supracaudal scute (SUP-d), and, maximum median length of supracaudal scute (SUP-l). We also examined animals for the presence of pectoral and abdominal chevron like markings or blotches while making morphological measurements.

Statistical analysis

We analyzed our data by using separate ANCOVAs, with maximum carapace length as the covariate, to compare the shell characteristics of tortoises between east and west of the Nile. We corrected P values for the multiple tests by using the sequential Dunn-Sidak method (Day and Quinn, 1989; Quinn and Keough, 2002). We analyzed males and females separately, with carapace length as the covariate, distribution (west or east of the Nile) as a fixed factor, and the remaining morphometric characteristics as the dependent variables. The ANCOVA assumption of homogeneity of slopes was tested using a protocol described in Zar (1996; p. 355). Regression slopes between the covariate (CL) and other variables were statistically similar between populations in both sexes (all slope comparisons; females: $t < 2.289$, $P > 0.025$, $df = 67$; males: $t < 2.268$, $P > 0.027$, $df = 55$); the sequential Dunn-Sidak adjusted p-value was $P = 0.0023$.

We also used a Principal Component Analysis (PCA) to examine multivariate clustering of shell characteristics between populations and to compare our results with those of Perälä's (2001). We compared the PCA scores between populations through the use of MANOVA. If the MANOVA was significant, we then performed follow-up ANOVAs to determine which components were significantly different between populations. All morphometric data were log transformed prior to analysis to normalize the data and only animals in which all characters were available (to measure) were included in the analysis. Males and females were analyzed separately.

We compared the presence of pectoral and abdominal markings on animals from west and east of the Nile by using Fisher's exact tests. Tortoises with any morphological malformations, injuries, or abnormal growth/missing features were not used in the morphometric analysis but were included in the categorical analysis of examining the presence/absence of abdominal and pectoral markings.

Molecular data

In order to investigate the genetic differences of populations east and west of the Nile, a segment of the mitochondrial 12S rRNA gene was sequenced for 19 specimens from west of the Nile and 2 from east of the Nile. Two extra sequences of *T. kleinnmani* were downloaded from GenBank: DQ080048 – Parham et al. (2006) and AF175332 – van der Kuyl et al. (2002). Unfortunately the sequence by Parham et al. (2006) did not have locality data and therefore it is not possible to know if the animal originated from west or east of the Nile. The sequence by van der Kuyl et al. (2002) was obtained from two specimens from Libya and belongs to *T. kleinmanni*. Locality data and GenBank accession numbers for the specimens included in the molecular analysis are shown in table 1.

Genomic DNA was extracted from tissue samples using the DNeasy Tissue Kit (Quiagen®). The primers used in both amplification and sequencing were 12Sa (5′-AAA CTG GGA TTA GAT ACC CCA CTA T-3′) and 12Sb (5′-GAG GGT GAC GGG CGG TGT GT-3′) (Kocher et al., 1989). The gene fragment was amplified by the polymerase chain reaction (PCR) using the same standard protocols and conditions described in Carranza et al. (1999), which consisted of an initial 90 s at 94° followed by 35 cycles of 94° for 30 s, 45° for 45 s, and 72° for 1 min, and then by a single cycle at 72° for 10 m. Successful PCR bands were cut out and purified using the QIAquick PCR purification kit from Quiagen®. The clean PCR products were sequenced using an automated sequencer following the manufacturer's protocols.

Results

The re-evaluation of Perälä's (2001) results using the sequential Dunn-Sidak method and the Bonferroni P value corrections revealed a smaller number of significant morphological differences between the two populations. When the P values for the 46 separate tests of the morphometric characters are adjusted using the conservative Bonferroni corrections, only 10 characters for both sexes remained significantly different for the two populations (table 2). When using sequential Dunn-Sidak corrections only 6 male and 4 female characters remained significantly different between eastern and western populations; a further 3 male and 2 female characters were ambiguous as significance could not be assigned because the number of decimal places needed for the test was not provided in Perälä (2001). By adjusting for experimentwise Type I error, the percent of characters that

Table 1. Details of genetic material and sequences used in the present study.

Taxa	Locality	Accession Numbers Cyt *b*	Codes
Testudo kleinmanni	Egypt, west of the Nile river	DQ991957	E10023-16
Testudo kleinmanni	Egypt, west of the Nile river	DQ991956	E10023-17
Testudo kleinmanni	Egypt, west of the Nile river	DQ991955	E10023-18
Testudo kleinmanni	Egypt, west of the Nile river	DQ991954	E10023-19
Testudo kleinmanni	Libya, west of the Nile river	DQ991953	E10023-20
Testudo kleinmanni	Libya, west of the Nile river	DQ991952	E10023-21
Testudo kleinmanni	Libya, west of the Nile river	DQ991948	E10023-22
Testudo kleinmanni	Libya, west of the Nile river	DQ991958	E10023-23
Testudo kleinmanni	Libya, west of the Nile river	DQ991947	E10023-24
Testudo kleinmanni	Libya, west of the Nile river	DQ991951	E10023-25
Testudo kleinmanni	Libya, west of the Nile river	DQ991946	E10023-26
Testudo kleinmanni	Libya, west of the Nile river	DQ991945	E10023-27
Testudo kleinmanni	Libya, west of the Nile river	DQ991950	E10023-29
Testudo kleinmanni	Libya, west of the Nile river	DQ991944	E10023-30
Testudo kleinmanni	Libya, west of the Nile river	DQ991949	E10023-31
Testudo kleinmanni	Libya, west of the Nile river	DQ991943	E10023-32
Testudo kleinmanni	Egypt, west of the Nile river	DQ991942	E19066-1
Testudo kleinmanni	Egypt, west of the Nile river	DQ991940	E19066-2
Testudo kleinmanni	Egypt, west of the Nile river	DQ991941	E19066-3
Testudo kleinmanni	Libya, west of the Nile river	AF175332	van der Kuyl et al. 2002
Testudo werneri	Egypt, east of the Nile river (Zaranik)	DQ991959	E10023-36
Testudo werneri	Egypt, east of the Nile river (Zaranik)	DQ991960	E10023-39

were reported to differ significantly by Perälä between the two populations was reduced from 36.9% to 13% for males and from 39.1% to 8.7% for females.

The ANCOVAs showed that overall there was no significant difference in the shell characteristics between the different populations, when the effects of carapace length were taken into consideration. Although a few of the morphometric characteristics had initial P values of less than 0.05, these were not significant after they had been adjusted appropriately for multiple tests (tables 3 and 4).

The PCA indicated that two components accounted for 82% and 83% of the morphometric variation in males and females respectively (tables 3, 4). The MANOVA showed that at least one of the PCA components exhibited a significant difference between the populations of males ($F_{2,56} = 4.86$, $P = 0.011$) and females ($F_{2,68} = 18.21$, $P < 0.0001$). Component 1, which accounted for 75% of the variation in males and 77% in females, indicated no significant difference between populations, respectively ($F_{1,57} = 2.28$, $P = 0.14$; $F_{1,69} = 0.58$, $P = 0.45$). Component 2, which explained 7% of interpopulation variation in males and 6% in females, showed that males and females from the eastern population had significantly lower scores than the western population, respectively ($F_{1,57} = 7.02$, $P < \alpha_{adj}0.025$; $F_{1,69} = 35.61$, $P < \alpha_{adj}0.025$). Component 2 appears to be heavily influenced by differences in the vertebral scutes and by the costal length for males and females (tables 3 and 4).

The occurrence of abdominal markings was not significantly different according to Fisher's exact tests, in males ($P = 0.58$) from east (100%, $n = 11$) and west (89.4%, $n = 47$) of the Nile or females ($P = 1.00$) from east (100%, $n = 21$) or west (96%, $n = 53$) of the Nile. Also, Fisher's exact tests showed no significant difference ($P = 0.19$) in the occurrence of pectoral markings in males from east (72.7%, $n = 11$) and west (48.9%, $n = 47$) of the Nile. However, eastern females were significantly ($P < \alpha_{adj}0.025$) more likely to

Table 2. A re-analysis of the data on differences in morphometric characteristics (ratio with carapace length) between eastern and western *Testudo* populations presented in Perälä (2001) Table 4. Perälä's (2001) univariate results (*P*-value) for each character in males and females are presented. B refers to Bonferroni corrections and the number of characters adjusted for is depicted in parentheses. SDS refers to the sequential Dunn-Sidak adjustment. * = remained significant after adjustment, NS = non-significant after adjustment, M = marginal, significance could not be assigned because the number of decimal places needed for the test was not provided in Perälä (2001).

Male Character	Perälä's P-value	B (46)	SDS (46)
CU	<0.0001	*	*
MI	0.035	NS	NS
MA	<0.0001	*	*
GU-h	0.001	NS	M
HE	0.001	NS	M
PEC-w	0.02	NS	NS
ABD-w	0.025	NS	NS
FEM-w	0.009	NS	NS
AN-1	0.008	NS	NS
V1-w	<0.0001	*	*
V2-w	0.002	NS	NS
V3-w	<0.0001	*	*
V4-w	0.002	NS	NS
V1-l	<0.0001	*	*
V2-l	0.001	NS	M
V3-1	<0.0001	*	*
C1-l	0.018	NS	NS
Female Character	**Perälä's P-value**	**B (46)**	**SDS (46)**
CL	0.032	NS	NS
CU	<0.0001	*	*
MI	0.002	NS	NS
MA	<0.0001	*	*
Gu-h	0.031	NS	NS
HE	0.001	NS	M
ASO-w	0.007	NS	NS
HUM-w	0.024	NS	NS
PEC-w	<0.0001	*	*
ABD-w	0.002	NS	NS
AN-w	0.049	NS	NS
V3-w	0.033	NS	NS
V4-w	0.001	NS	M
V5-w	0.004	NS	NS
V3-1	0.003	NS	NS
SUP-d	0.049	NS	NS
SUP-l	<0.0001	*	*
EYE-TYMP	0.005	NS	NS

have pectoral markings (81%, $n = 21$) than western females (49%, $n = 53$).

The results of the DNA analysis suggest that the eastern and western populations of *Testudo* are very similar, differing consistently in just one base pair out of a total of 393 (G – A substitution at position 205 of the 12S rRNA fragment sequenced for this study). Moreover, of the 20 *T. kleinmanni* analyzed for this study, there is one specimen from Libya (GenBank accession numbers DQ991958) that also presents one mutation (an A – G substitution) at position 263 of the 12S rRNA gene fragment sequenced. This indicates that although the two specimens of *T. werneri* analyzed for this study are differentiated by a single point mutation from *T. kleinmanni*, this small difference can be considered as part of the genetic variability of *T. kleinmanni*.

Discussion

Reevalutation of Perälä's (2001) analysis and the results of our own study suggest that there are minor morphometric differences between the eastern and western populations of the Egyptian tortoise. The degree of variation encountered is to be expected in a species that has large geographic distribution and is much lower than found in some other chelonians (Germano, 1993; Carretero et al., 2005; Fritz et al., 2005). These minor differences are corroborated by our re-evaluation of Perälä's (2001) results. Although, slightly more morphometric differences were apparent in Perälä's (2001) analysis than what we found using our own data, this could be due to differences in statistical methods, sampling bias, sample size and origin of animals measured. For example, Perälä (2001) used a very small sample for the shell character CU, with just three eastern females and three males. Our sample size of animals from east of the Nile was also quite small, but larger than that used by Perälä (2001). Furthermore, Perälä (2001) included individuals without known geographic data in his analysis and classified them as belonging to western or eastern populations based on his "preliminary comparative examination of the material". This circular procedure, animals being assigned to groups by some of the supposed distinguishing characters that are to be

Table 3. Mean (mm) shell characteristics and statistical results for male tortoises found east and west of the Nile. Sample size, $n = 11$ (East) and $n = 48$ (West). $df = 1, 59$ for all ANCOVA tests. ^{NS} = Not significant because the P value is less than the sequential Dunn-Sidak adjustment value, P_{adj}. PCA 1 and PCA 2 refer to the two components extracted from the PCA analysis and the loadings of each variable. PC1-S and PC2-s refer to the mean scores of the components.

	East		West		ANCOVA	PCA	
	Mean	SD	Mean	SD		PCA1	PCA2
CL	90.8	6.6	97.9	10.1		0.954	−0.157
CU	109.2	7.3	124.9	13.9	$F = 0.62$ $P = 0.44$	0.906	−0.131
MI	62.3	4.7	65.7	6.1	$F = 0.22$ $P = 0.64$	0.917	−0.246
MA	65.1	5.0	68.3	6.3	$F = 0.18$ $P = 0.68$	0.931	−0.233
HE	45.3	2.1	49.0	3.8	$F = 1.43$ $P = 0.24$	0.879	0.031
PEC-w	56.6	3.6	58.2	5.6	$F = 1.49$ $P = 0.28$	0.902	−0.247
ABD-w	57.9	3.6	60.9	5.8	$F = 0.97$ $P = 0.33$	0.924	−0.249
ASO-w	41.0	2.5	43.4	4.2	$F = 5.14$ $P > \alpha_{adj}0.0023$	0.941	−0.076
HUM-w	39.0	2.2	41.4	3.5	$F = 1.46$ $P = 0.23$	0.935	−0.063
FEM-w	39.8	2.4	43.3	4.2	$F = 0.38$ $P = 0.54$	0.895	−0.258
AN-w	32.6	3.3	34.6	3.4	$F = 2.52$ $P = 0.12$	0.846	−0.404
AN-m	19.6	2.7	22.2	2.4	$F = 3.28$ $P = 0.075$	0.826	−0.257
V1-w	18.4	2.1	21.0	2.4	$F = 0.79$ $P = 0.38$	0.820	0.340
V2-w	21.0	1.3	22.6	2.1	$F = 4.42$ $P > \alpha_{adj}0.0024$	0.833	0.409
V3-w	23.4	1.5	25.3	2.7	$F = 3.14$ $P = 0.82$	0.843	0.427
V4-w	20.5	1.8	23.1	3.2	$F = 1.76$ $P = 0.19$	0.842	0.349
V5-w	23.9	2.3	26.3	3.8	$F = 1.97$ $P = 0.17$	0.836	0.031
V1-l	15.4	1.5	17.9	1.9	$F = 0.12$ $P = 0.73$	0.829	0.155
V2-l	17.8	1.8	19.7	2.2	$F = 0.002$ $P = 0.97$	0.865	0.289
V3-l	16.8	1.6	18.9	2.4	$F = 0.39$ $P = 0.54$	0.805	0.428
C1-l	26.9	2.3	30.0	2.9	$F = 0.18$ $P = 0.89$	0.922	0.219
SUP-d	22.9	3.9	26.1	3.0	$F = 1.25$ $P = 0.27$	0.698	0.040
SUP-l	13.6	2.0	16.9	2.6	$F = 0.69$ $P = 0.41$	0.719	−0.278
PC1-S	−0.41	0.93	0.09	1.00			
PC2-S	−0.69	0.59	0.16	1.01			
					Total eigenvalues	17.26	1.59
					Total % of variance	75.0%	6.9%

assessed in the subsequent analysis, introduces bias and could lead to spurious conclusions.

Our results also suggest that abdominal and pectoral plastron markings cannot be used to consistently distinguish between the two populations. We found no significant differences in male pectoral and abdominal markings between the two populations. Although western females were significantly less likely to have pectoral markings than eastern females, 49% of the former still had pectoral markings. The discrepancy between our results and Perälä's (2001) is, among others, probably due to his inclusion of juveniles or animals of unknown locality in his analysis. Moreover, shell color has low taxonomic value as it has been shown to be influenced by physical wear, exposure to the sun, carapace length, and age (Leuck and Carpenter, 1981; Zug, 1991). Certainly, the faintness of plastron markings in many adult *T. kleinmanni* from west of the Nile appears to be due to shell wear, probably produced by the more abrasive substrates in this area which often consist of rock and coarser sand than in the east (S. Baha el Din and O. Attum, unpubl. data).

The minor morphometric differences between western and eastern populations are in agreement with our molecular analysis, which showed that there was a single point mutation between the two eastern *T. werneri* and the twenty western *T. kleinmanni* specimens analyzed for this study. This very low level of genetic variability between eastern and western populations is much lower than the mini-

Table 4. Mean (mm) shell characteristics and statistical results for female tortoises found east and west of the Nile. Sample size, $n = 20$ (east) and $n = 51$ (west). $df = 1, 71$ for all ANCOVA tests. ^{NS} = Not significant because the P value is less than the sequential Dunn-Sidak adjustment value, P_{adj}. PCA 1 and PCA 2 refer to the two components extracted from the PCA analysis and the loadings of each variable. PC1-S and PC2-s refer to the mean scores of the components.

	East		West		ANCOVA	PCA	
	Mean	SD	Mean	SD		PCA1	PCA2
CL	109.4	11.9	117.8	8.9		0.950	−0.212
CU	131.8	14.6	154.5	14.2	$F = 1.47$ $P = 0.22$	0.934	0.074
MI	78.3	7.3	81.8	6.2	$F = 4.10$ $P > \alpha_{adj} 0.0027$	0.921	−0.255
MA	78.9	7.3	83.1	6.3	$F = 3.36$ $P = 0.071$	0.937	−0.187
HE	55.9	5.5	62.3	4.8	$F = 0.25$ $P = 0.62$	0.907	0.031
PEC-w	68.9	6.8	72.1	5.9	$F = 2.51$ $P = 0.12$	0.902	−0.314
ABD-w	71.8	7.5	75.9	6.2	$F = 1.74$ $P = 0.19$	0.927	−0.249
ASO-w	49.2	4.4	53.0	4.1	$F = 3.45$ $P = 0.68$	0.948	−0.119
HUM-w	47.1	4.4	49.3	4.2	$F = 1.32$ $P = 0.26$	0.890	−0.247
FEM-w	50.8	6.6	54.6	5.7	$F = 1.77$ $P = 0.19$	0.905	−0.041
AN-w	38.0	4.6	39.5	3.3	$F = 0.93$ $P = 0.34$	0.884	−0.290
AN-m	25.5	3.4	27.9	2.9	$F = 0.19$ $P = 0.89$	0.869	−0.147
V1-w	21.5	1.7	24.8	2.3	$F = 5.24$ $P > \alpha_{adj} 0.0023$	0.738	0.548
V2-w	26.3	2.2	29.0	2.7	$F = 4.17$ $P > \alpha_{adj} 0.0026$	0.854	0.418
V3-w	30.8	3.2	33.5	3.4	$F = 2.53$ $P = 0.12$	0.883	0.247
V4-w	26.5	3.0	29.1	2.9	$F = 0.12$ $P = 0.91$	0.870	0.148
V5-w	27.8	3.4	31.5	3.2	$F = 2.43$ $P = 0.12$	0.756	−0.113
V1-l	19.0	1.5	22.0	2.2	$F = 4.73$ $P > \alpha_{adj} 0.0024$	0.853	0.257
V2-l	22.8	2.5	25.4	2.6	$F = 0.10$ $P = 0.92$	0.832	0.346
V3-l	21.8	2.1	24.8	2.5	$F = 1.47$ $P = 0.23$	0.917	0.172
C1-l	33.7	3.0	36.8	3.2	$F = 2.85$ $P = 0.96$	0.907	0.179
SUP-d	26.3	3.6	29.5	3.0	$F = 0.22$ $P = 0.64$	0.813	−0.079
SUP-l	14.6	2.6	19.2	2.1	$F = 0.96$ $P = 0.76$	0.763	−0.005
PC1-S	−0.15	1.32	0.6	0.85			
PC2-S	−0.93	0.61	0.36	0.89			
					Total eigenvalues	17.75	1.32
					Total % of variance	77.2%	5.7%

mum level of genetic variability found for exactly the same mitochondrial region among five species of *Testudo* (*T. hermanni*, *T. horsfieldii*, *T. graeca*, *T. kleinmanni* and *T. marginata* – between 8 and 24 differences with an average of 18.3 changes; van der Kuyl et al., 2002); three species of *Chelonoides* (*Ch. carbonaria*, *Ch. denticulata* and *Ch. nigra* – between 21 and 30 differences with an average of 25.6 changes; Le et al., 2006); and three species of *Geochelone* (*G. sulcata*, *G. elegan*s and *G. platynota* – between 5 and 17 differences with an average of 12.6 changes; Le et al., 2006). In fact, the level of genetic variability between *T. werneri* and *T. kleinmanni* is even lower than the within-species level of genetic variability in *Testudo* (*T. h. hermanni* and *T. h. boettgeri* – 3 changes; *T. g. ibera* and *T. g. graeca* – 4 changes; van der Kuyl et al., 2002; Harris et al., 2003). The taxonomic status of other subspecies of *T. graeca* like for instance *T. g. soussensis* and *T. g. whitei* is very doubtful and therefore have not been included in our comparisons (see Harris et al., 2003). These results, together with the morphological evidence, strongly indicates that eastern and western populations of the Egyptian tortoise should be considered as a single species. The name *T. kleinmanni*, should be used in its earlier sense with *T. werneri* regarded as a junior synonym of it.

One of the main reasons for the lack of variation between western and eastern populations is that the Nile has not acted as a continuous biogeographical barrier and isolated related popu-

lations. The river flowed only intermittently, sometimes drying up completely, over a period of a million years in the early Pleistocene (Said, 1993). It began to flow more vigorously some 800,000 years ago, after making its first connection with the Ethiopian Highlands. But even during the late Pleistocene and Holocene the river's flow fluctuated considerably (Said, 1993). The tenuous nature of the river has allowed ample opportunities for terrestrial fauna to move from one side to the other over an extended period of time. The great similarity of the herpetofauna on both sides of the Nile corroborates this (Baha El Din, 2006).

In addition, we have not observed any major "discrepancies in ecological adaptations between the Israeli and mainland African populations" (Perälä, 2001). For example, although *T. kleinmanni* in Libya are often found in rockier deserts, west of the Nile in Egypt, they often occur in habitats similar to those found east of the Nile in Sinai and Israel. In addition, the distribution of *T. kleinmanni* in Sinai and Israel is not restricted solely to sand dunes. Widely ranging species often exhibit differences in habitat use between separated populations, which may lead to intraspecific morphometric variation (Reinert, 1993). Carapace size and shape plasticity may represent local adaptations to different habitats, productivity, environmental conditions, and clutch frequency (Arnold, 1979; Frits, 1983; Rowe, 1997; Willemsen and Hailey, 1999; Fritz et al., 2005). Thus, we would expect *T. kleinmanni* that occur in rocky deserts to have slightly different morphology than those occurring in sand dunes, as is found in *T. graeca* species in North Africa (Carretero et al., 2005) or that *T. kleinmanni* occurring in more productive environments would have a slightly different morphology than *T. kleinmanni* occurring in less productive environments as found in *T. marginata* (Fritz et al., 2005).

Based on the above evidence we conclude that *Testudo werneri* should not be treated as a full species.

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