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Testudo graeca tripolitania, a new taxon of spur-thighed tortoise found in Libya at risk of exploitation for the international pet trade

Stephen F. Poterala[1,*], Paul Rattay[1], Aaron S. Johnson[1], Murad S.A. Buijlayyil[2], Askin Kiraz[2], Ahmad M.S. Ajaj[3]

[1] Turtle and Tortoise Preservation Group. 1042 N Higley Rd. Suite. 105, Mesa, AZ, USA.
[2] Near East University. Near East Boulevard, Nicosia 99138, Cyprus.
[3] Albarari Organization for Conservation of Nature. Gharyan, Libya.

*Correspondence: spoterala@gmail.com

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The spur-thighed tortoise, *Testudo graeca,* is a widely distributed and deeply diversified species inhabiting areas of Africa, Asia, and Europe. For decades, populations of *T. graeca* from North Africa have been exploited for the international pet market. In particular, *T. graeca* of Libyan origin have been commercially imported into the United States several times since 2021. Using mitochondrial DNA (mtDNA) sequencing, we show that these imported tortoises include *T. g. cyrenaica* and a novel lineage originally reported in 2017 from a displaced specimen from a market in Libya. That novel lineage inhabits northwestern Libya, and we show that wild populations near Gharyan share the same mtDNA haplotype as those now sold as pets in the USA. Populations of *T. graeca* in northwestern Libya, previously identified as *T. g. nabeulensis,* are reassigned to a **new subspecies *Testudo graeca tripolitania*** described herein. Additional field work is needed to determine the mtDNA haplotypes of populations in central and southern Tunisia and to locate the subspecies boundary between *T. g. nabeulensis* and *T. g. tripolitania*.

Key words: Africa; holotype; mtDNA; phylogeny; Testudines.

The spur-thighed tortoise, *Testudo graeca,* Linneaus, 1758, is a diverse species with a large native range, extending approximately 6700 kilometers from Morocco to Iran, and inhabits a wide range of climatic conditions (Türkozan *et al.*, 2023). The divergence of *T. graeca* is generally acknowledged to have occurred on multiple temporal scales as the species' range expanded over time (Fritz *et al.*, 2009, Graciá *et al.*, 2017a). The differentiation of six primary clades (five in Asia, and the other one in North Africa and Europe) occurred first during the Pliocene (7.95-3.48 million years ago). The Asian clades are currently recognized as *T. g. armeniaca, T. g. buxtoni, T. g. ibera, T. g. terrestris,* and *T. g. zarudnyi.* Differentiation within the North African clade occurred later, within the early- to mid-Pleistocene (3.47-1.44 million years ago), to yield five North African subspecies: *T. g. graeca, T. g. marrokensis, T. g. whitei, T. g. nabeulensis,* and *T. g. cyrenaica.* The nomenclature of *T. graeca* was disrupted in 2020, with the discovery that the type location of *T. graeca* Linneaus

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1758 was actually in Agadir, Morocco, and had been recorded incorrectly (as being in Algeria) in historic publications (SCHWEIGER & GEMEL, 2020). To correct this mistake, *T. g. soussensis* was re-designated as *T. g. graeca*, and the subspecies in Algeria and northeastern Morocco (previously recognized as *T. g. graeca*) was designated by the Turtle Taxonomy Working Group as *T. g. whitei* (RHODIN *et al.*, 2021). The arrival of *T. g. whitei* and *T. g. nabeulensis* into Europe is comparatively recent, having occurred in the late Pleistocene (FRITZ *et al.*, 2009; GRACIÁ *et al.*, 2013) and in prehistoric times (VAMBERGER *et al.*, 2011; GRACIÁ *et al.*, 2017a,b).

The range of *T. graeca* in Asia is largely contiguous, with a presumption of some gene flow at subspecies boundaries. The range of *T. graeca* in North Africa shows evidence of both parapatric and allopatric distribution, with biogeographic barriers at some subspecies boundaries but also significant differences in habitat preference between subspecies (ANADÓN *et al.*, 2015; GRACIÁ *et al.*, 2017a). Differentiation of the North African subspecies may have been driven by oscillation between wet and arid climates during the mid-Pleistocene, with the species' range being repeatedly fragmented during arid periods (LAMBERT, 1983; FRITZ *et al.*, 2009).

Prior to DNA studies, the identity of various *T. graeca* populations was widely debated in literature, with many new subspecies, species, and genera being defined on the basis of morphological analysis between 1986 and 2004 (CHKHIKVADZE & TUNIEV, 1986; HIGHFIELD, 1990; CHKHIKVADZE & BAKRADSE, 1991, 2002; PERÄLÄ, 1996, 2001, 2002; PIEH, 2000; WEISSINGER, 2000; PIEH & PERÄLÄ 2004; BONIN *et al.* 2006, CHKHIKVADZE *et al.*, 2011). Currently ten extant subspecies are recognized, and the monophyletic status of *T. graeca* has been firmly established based on mitochondrial DNA (mtDNA) phylogeny using the *cyt-b* gene (FRITZ *et al.*, 2007, 2009). Additional diversity has been proposed, including up to four distinct sub-clades of *T. g. buxtoni* (RANJBAR *et al.*, 2022).

The present study focuses on a new North African lineage first reported by GRACIÁ *et al.* (2017a). Our initial objective was to use mtDNA sequencing to identify a cohort of 28 Libyan *Testudo graeca* specimens imported into the United States via the pet trade. This was based on observation that the imported tortoises varied greatly in appearance and size and the finding that they could not be accurately identified morphologically. After finding that some tortoises represented this undescribed lineage, a further study was conducted to geographically locate the lineage, provide a detailed physical description, and assign taxonomic nomenclature. Our primary goal in this endeavor is to facilitate conservation efforts for *Testudo graeca* in Libya, particularly given the pressing concern surrounding the sudden appearance of this lineage in the USA pet trade. We highlight the vulnerability of these tortoises to exploitation, alongside other North African lineages, and emphasize that there is a critical need for enhanced protection of these tortoises within their native range.

MATERIALS AND METHODS

The captive Libyan spur-thighed tortoises in this work were studied with the permission of private keepers. All animals were originally part of commercial importations into the United States from Egypt, which were accompanied by a US Fish & Wildlife clearance (USFWS Form 3-177), indicating that CITES import and export permits were reviewed. DNA samples were collected from a total of 28 tortoises housed within four private facilities in the United States. Both choanal swabs and shed epidermis were submitted to a commercial lab (Gendika B.V., Van Beresteijnstraat 22B, 9641 AB Veendam, Netherlands) for determination of the *cyt-b* mtDNA haplotypes using sequencing methods reported in Fritz *et al.* (2009). Mitochondrial DNA containing the complete *cyt-b* gene and ~20 base pairs (b.p.) of the adjacent tRNA-THR gene was amplified via PCR. The primers used were CytB 5′-AAC CAT CGT TGT WAT CAA CTA C-3′ (Spinks *et al.*, 2004) and Mt-E-Rev2 5′-GCR AAT ARR AAG TAT CAT TCT GG-3′ (Praschag *et al.*, 2007). Samples were sequenced using an ABI 3130 (Applied Biosystems, Foster City, California). Chromatograms were analyzed in UGENE, checked manually, and were aligned to published sequences from GenBank (Clark *et al.*, 2016). Both forward and reverse sequences were analyzed for most tortoises. We have followed the convention of Fritz *et al.* (2009) in the labeling of *T. graeca cyt-b* haplotypes from North Africa. Additionally, a total of 44 morphological measurements on the captive tortoises were collected following the methods of Pieh & Perälä (2004) to facilitate future morphological studies. These parameters are defined in Table 1. Despite veterinary care, some captive tortoises died during this study. Three tortoises were preserved

Table 1: Description of morphological measurements collected for *Testudo graeca* in this study.

Parameter	Description	Parameter	Description
CL	Max. carapace length	V5-w	5th vertebral width
CU	Carapace length along curvature	SUP-l	Midline supracaudal length
HE	Max. height	SUP-d	Max. supracaudal width (dorsal)
PL	Max. plastron length	SUP-v	Max. supracaudal width (ventral)
PL-m	Midline plastron length	GU-l	Max. gular length
MI	Median width (marginals 5-6)	GU-m	Midline gular length
MA	Max. width (marginals 7-9)	GU-w	Max. combined gular width
NU-l	Nuchal length	GU-h	Gular height
NU-w	Nuchal width	HUM-w	Max. combined humeral width
C1	1st costal length	PEC-w	Max. combined pectoral width
C2	2nd costal length	ABD-w	Max. combined abdominal width
C3	3rd costal length	FEM-w	Max. combined femoral width
C4	4th costal length	AN-w	Max. combined anal width
V1-l	1st vertebral length	HUM-m	Midline humeral seam length (left)
V2-l	2nd vertebral length	PEC-m	Midline pectoral seam length (left)
V3-l	3rd vertebral length	ABD-m	Midline abdominal seam length (left)
V4-l	4th vertebral length	FEM-m	Midline femoral seam length (left)

as wet specimens by fixation in 95% ethanol and storage in 75% ethanol, this method being chosen to best preserve DNA for future studies. These specimens are housed in the collection of the Field Museum Chicago under accession numbers FMNH 289175, 289176, and 289177.

Subsequent to mtDNA analysis of the captive tortoises, a field study in Libya was arranged to locate wild *T. graeca* matching the novel lineage from GRACIÁ *et al.* (2017a). Published range maps indicate two different ranges for *T. graeca* in Libya: in the northwest (Jabal Nafusa mountains and the adjacent coast of Tripolitania) and in Cyrenaica (FRITZ *et al.*, 2007, 2009; Es-

Table 2: Summary of the *Testudo graeca* belonging to the E lineage included in this study. For parameter abbreviation explanation, see Table 1.

Specimen ID	GenBank Accession Number	Specimen Accession Number	Sex	CL (mm)	HE (mm)	MA (mm)	WT (g)	Location / Origin
US-I1-001*	PP942654	FMNH 289175	F	145.7	86.2	111.3	649.9	Origin unknown (Imported to the USA in 2021)
US-I1-002	PP942655		F	141.3	78.2	105.1	574.7	
US-I1-003	PP942656		F	136.0	75.1	100.9	538.1	
US-I1-004	PP942657		F	140.1	84.1	104.7	658.1	
US-I1-005	PP942658		F	122.4	75.8	91.5	408.8	
US-I1-006*	PP942659		F	122.0	68.9	96.2	367.9	
US-I1-007	PP942660		M	116.9	67.3	87.9	303.4	
US-I1-008*	PP942661	FMNH 289176	M	110.7	60.9	85.4	321.1	
US-I1-009*	PP942662	FMNH 289177	M	123.0	62.0	92.2	353.9	
US-I1-010	PP942663		F	152.3	87.6	115.1	666.4	
US-I1-011*	PP942664		F	145.7	80.0	111.3	476.4	
US-I1-012*	PP942665		F	142.1	77.1	108.1	497.8	
US-I2-001	PP942666		M	142.6	74.2	110.3	595.3	Origin unknown (Imported to the USA in 2022)
US-I2-002*	PP942667		M	112.6	65.5	84.1	250.0	
US-I2-003	PP942668		M	119.5	73.6	93.3	367.4	
US-I2-004	PP942669		M	140.9	75.0	98.3	480.4	
LY-GRN-001	PP942670		M	120	66	91	386	Near Gharyan, Libya (GPS coordinates withheld)
LY-GRN-002	PP942671		F	137	76	100	576	
LY-GRN-003	PP942672		M	111	62	80	286	
LY-GRN-004	PP942673		M	109	62	80	274	
LY-GRN-005	PP942674		F	135	78	106	605	
LY-GRN-006	PP942675		F	152	79	109	614	
LY-GRN-007	PP942676		F	133	76	101	487	

*Deceased

coriza *et al.*, 2022). The authors made efforts to inquire within wildlife conservation groups in Libya as to whether *T. graeca* might exist between these two regions (e.g. along the Gulf of Sirte). Field work was ultimately conducted in northwestern Libya, since tortoises in Cyrenaica have been relatively well sampled and documented to be *T. g. cyrenaica*. Over a period of one week, a group of six people (operating as two three-person teams) was dispatched to search five sites in November of 2022. Four sites were within the Jabal Nafusa Mountains, all within an 8 km radius of Gharyan, Libya. The fifth site was on an alluvial plain approximately 12 km northwest of Gharyan. These initial searches did not follow a rigorous methodology, as citizen scientists were recruited for the effort, and the primary goal was simply to locate tortoises in their natural habitat and obtain DNA samples. Due to time constraints, only basic morphological data (CL, HE, MA, and WT, see Table 1 for details), photographs, choanal swabs, and small samples of loose epidermis were collected on wild tortoises before they were released. Mitochondrial DNA analysis of these tortoises was performed as described above.

Figure 1: Wild *Testudo graeca* and associated habitat near Tarhunah, Jabal Nafusa mountains, Libya (Willi Schneider, unpublished data).

Results

The 2021-2022 USA imports of Libyan spur-thighed tortoises were found to include distinct small and large morphotypes. The larger tortoises were generally consistent with *T. g. cyrenaica* as described by Pieh & Perälä (2002), both in size (CL of ~15-17 cm) and visual appearance (in particular, mottled carapace patterning). Some of the imported tortoises were smaller (~11-14 cm) and exhibited more uniform carapace patterning than the rest, typically without mottling. After submitting samples from both morphotypes to a commercial lab, we were able to generate partial (600-800 b.p.) *cyt-b* sequences for each tortoise. The relatively short sequence lengths reflect poor amplification, which we primarily attribute to suboptimal sample quality (and probably, swab technique). Despite multiple attempts at rerunning samples and analyzing both forward and reverse sequences, we were unable to obtain significantly improved results and made the decision to proceed with the obtained data. All of the partial sequences obtained matched exactly either the C1, C2

(*T. g. cyrenaica*), or E1 (undescribed lineage) haplotypes. In particular, out of the 28 tortoises tested among the USA imports, we identified 16 animals belonging to the E lineage (Table 2).

Regarding the search for wild tortoises within Libya, feedback obtained from wildlife conservation groups was consistent with the existence of a gap in the range of *T. graeca* at the Gulf of Sirte along the central coast of Libya, as we could find no reliable records of the species in this region. As a result, field work was conducted in northwestern Libya, in regions where SCHNEIDER & SCHNEIDER (2008) had previously reported *T. graeca* to occur, and where no DNA sampling had been reported in the past. Tortoises found by SCHNEIDER & SCHNEIDER (2008) and the associated habitat are shown in Fig. 1 (Willi Schneider, unpublished data). Using these records and habitat images to inform the search effort, five sites were searched in the vicinity of Gharyan, Libya. A total of seven *T. graeca* were found at two sites, both within the Jabal Nafusa mountains at ~700 m elevation. At both sites, tortoise habitat consisted of flat terrain or gentle hills with partial vegetation cover. In general, only grasses and herbaceous plants were present at these sites, including several species of Asteraceae. Interestingly, the sites where tortoises were found were the closest in proximity to human development and residential areas surrounding Gharyan. Of the remaining sites where *T. graeca* was not found, two were also within the Jabal Nafusa Mountains, but at lower elevations in areas with steeper and more rocky hillsides. The last site was located on an alluvial fan northwest of the Jabal Nafusa Mountains. This site was topographically flat but was heavily disturbed by agricultural activity. Data for the wild tortoises found are provided in Table 2. *Testudo graeca* individuals found near Gharyan fall within the range of sizes observed for captive E1 lineage tortoises from the 2021-2022 USA imports. As for the captive specimens, we were able to recover partial mtDNA *cyt-b* sequences (~600-800 b.p.) for the wild tortoises. For six of the seven wild tortoises, partial *cyt-b* sequences exactly matched the E1 haplotype. The other tortoise (LY-GRN-003) differed from the E1 haplotype by a single mutation at position 693 of the *cyt-b* gene, indicating the presence of a second haplotype within clade E.

DISCUSSION

We agree with GRACIÁ *et al.* (2017a) that clade E represents a unique undescribed lineage, and the present study helps establish greater confidence in this finding. In general, mtDNA phylogeny has given clear, consistent, and defensible relationships between *Testudo* species and clades/subspecies. In further support to this approach, MIKULICEK (2013) found nuclear amplified fragment length polymorphism clusters to be largely congruent with mtDNA clades, and GRACIÁ *et al.* (2017b) showed similar consistency between mtDNA results and nuclear (microsatellite) data for *T. g. graeca* and *T. g. marrokensis*. Phylogenetically, mtDNA differentiation of the E1 haplotype from other North African *T. graeca* subspecies was shown to be similar to differentiation between other described subspecies, supporting a subspecies-level description

(Graciá *et al.*, 2017a).

We have concluded that a detailed morphological study of this lineage will require additional field work, as many prior studies have failed to capture the extent of morphological variability within each subspecies. For example, Escoriza *et al.* (2022) reported that *T. g. nabeulensis* exhibits extreme variation in size, with populations of larger specimens (CL up to 24.8 cm) not being noted in many prior studies. The average CL of tortoises in this study (males 12.1 cm, females 13.9 cm) was comparable to that of *T. g. nabeulensis* from northern Tunisia (males 12.1 cm, females 13.0 cm) studied by Pieh & Perälä (2002). Populations near Sfax, Tunisia (Escoriza *et al.*, 2022) were even smaller (males 11.1 cm, females 12.1 cm), but without mtDNA testing we cannot be certain that these tortoises are *T. g. nabeulensis*. The E lineage tortoises in this study were very high-domed in profile, with an average (± standard deviation) normalized shell height (HE/CL) of 0.56 ± 0.028.

We hypothesize that E lineage *T. graeca* are geographically isolated from both *T. g. cyrenaica* and *T. g. nabeulensis.* The mixing with *T. g. cyrenaica* in import groups almost certainly occurred in captivity, as the small range of *T. g. cyrenaica* has been well established and thoroughly sampled without the discovery of clade E haplotypes in wild tortoises. Likewise, no *T. g. nabeulensis* (clade A) haplotypes were found in either the wild tortoises from the Jabal Nafusa mountains or the 2021-2022 USA imports. Based on these factors, we infer that a significant wild population of E lineage spur-thighed tortoises exists and represents a new North African subspecies of *T. graeca*. We found no evidence of a prior description of the lineage. The name *Testudo flavominimaralis* (Highfield & Martin, 1989), not recognized as valid, was assigned to a displaced tortoise of North African origin. The identity of this specimen is uncertain, as it lacks a type locality and has a considerably lower shell profile (HE/CL = 0.487) than the clade E tortoises in this work. Clade E is described herein:

Testudo graeca tripolitania n. subsp.

Tripolitanian Tortoise

Holotype – An adult male (US-I1-008) with the E1 *cyt-b* haplotype, imported into the United States in 2021, and believed to originate from northwestern Libya. The holotype specimen, shown in Fig. 2, is typical in size, appearance, and morphology for the subspecies, except for having scute anomalies. Specifically, the nuchal is miss-

Figure 2: Holotype of *Testudo graeca tripolitania* n. subsp.

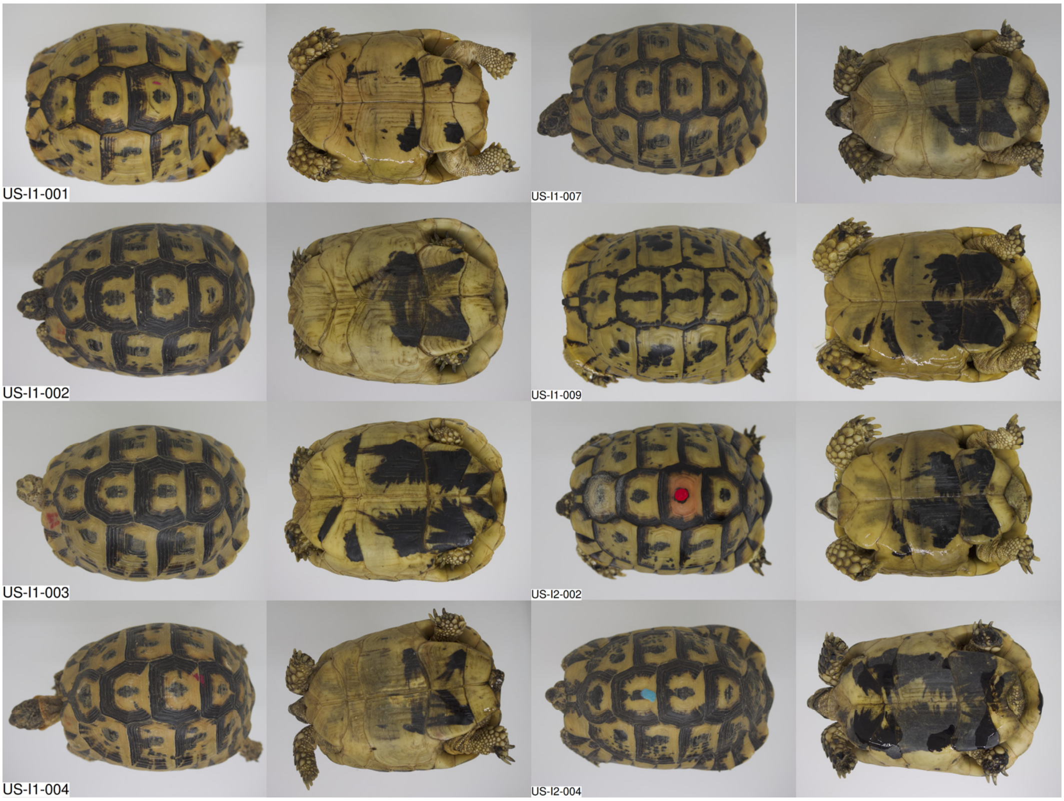

Figure 3: *Testudo graeca tripolitania* n. subsp. Females (left pictures) and males (right pictures) imported into the USA in 2021-2022.

ing (typically present but varies in size) and the supracaudal scute is divided (more commonly undivided). Scute anomalies are common in *T. graeca* and should not be regarded as diagnostic (MIRA-JOVER *et al.*, 2024). The holotype died of unknown causes in 2022 and was fixed in 95% ethanol and preserved in 75% ethanol. The holotype is stored at the Field Museum of Natural History as accession number FMNH 289176.

Paratypes – A total of 15 additional tortoises (seven males and eight females) were sampled from USA commercial importations in 2021 and 2022 and were verified to have the same *cyt-b* haplotype (E1) as the holotype specimen. Male US-I1-009 and female US-I1-001 died during the course of this study, and are preserved as wet specimens at the Field Museum (FMNH 289177 and 289175, respectively). Four additional tortoises (females US-I1-006, US-I1-011, US-I1-012, and male US-I2-002) died in captivity, and the remaining tortoises are still living in private facilities in the United States. A subset of these tortoises are shown in Fig. 3 to exemplify typical variability in coloration and pattern. For comparison, wild tortoises (three males and four females) found near Ghar-

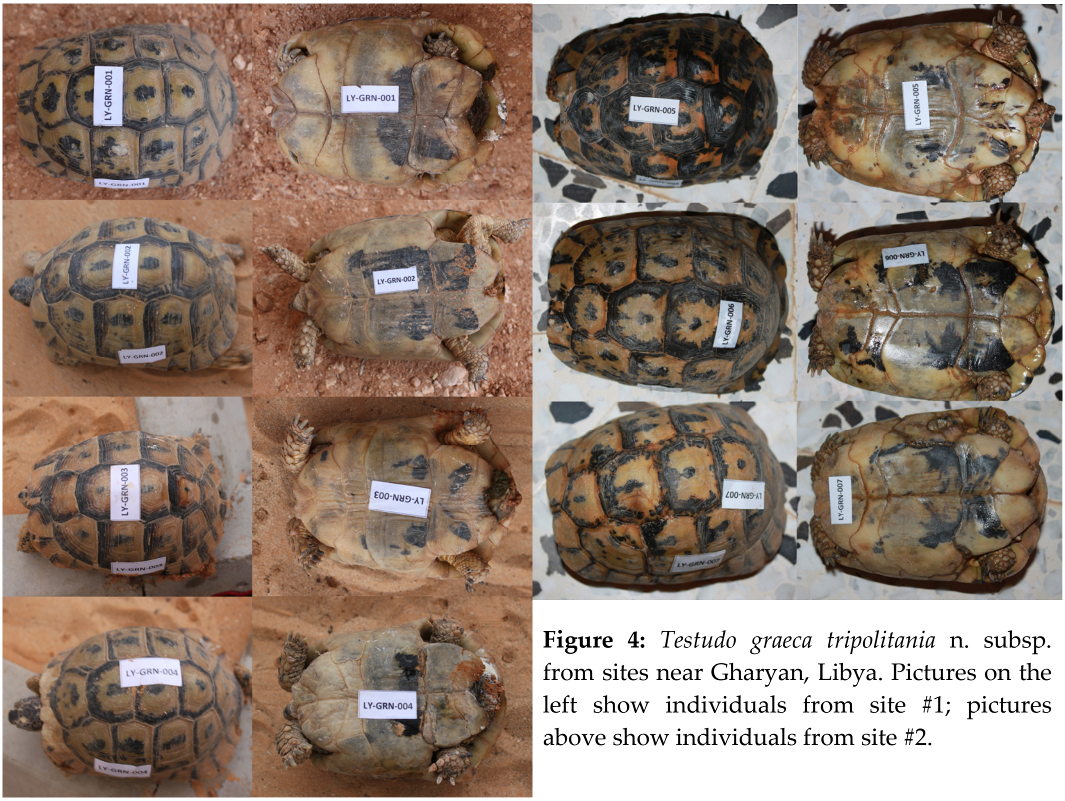

Figure 4: *Testudo graeca tripolitania* n. subsp. from sites near Gharyan, Libya. Pictures on the left show individuals from site #1; pictures above show individuals from site #2.

yan, Libya, are shown in Fig. 4. These tortoises are also morphologically consistent with the holotype, not considering scute anomalies. Six of these wild tortoises share the E1 *cyt-b* haplotype, and the seventh is identified as belonging to clade E but having a previously unreported haplotype.

Description and Comparison – The carapace is rounded in appearance and very high-domed in profile, with a yellow-tan or yellow-orange base color and bold black patterning. The typical pattern consists of a central black spot on each vertebral or costal scute, combined with a black rim along the anterior and lateral edges of each vertebral scute and along the anterior (and sometimes ventral) edges of each costal scute. Typically, black pigment also fills a triangular region between the areola and the anterior edge of most marginal scutes. The plastron is yellow-tan with sharply contrasting and irregular black patterning. The head and limbs are predominantly yellow with intermixed black pigment, particularly on the top of the head and forelimb scales. Complete morphological parameters are given in Table S1. All tortoises appear mature with no evidence of recent growth. We also examined the relationship between CL and HE/CL for North African *T. graeca* (Fig. 5). Comparative data for other North African subspecies were derived from HIGHFIELD (1990), PIEH (2000), PIEH & PERÄLÄ (2002, 2004) and TIAR-SAADI *et al.* (2022) and are presented as average values for males and females in

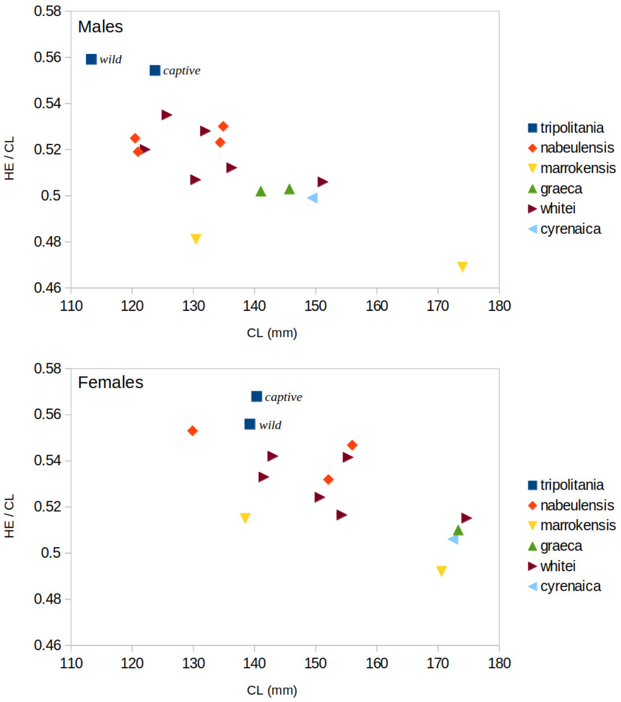

Figure 5: Plot of normalized shell height (HE/CL) *versus* straight carapace length (CL) for North African *Testudo graeca* subspecies. Each symbol represents the average of a study population (captive and wild groups of *T. g. tripolitania* n. subsp. are indicated)

each studied population. Averages are used because most authors have elected to withhold morphology data on individual tortoises. For *T. g. tripolitania* n. subsp., we show separate averages for the 16 captive tortoises and for the seven wild tortoises from Gharyan, Libya.

Genetic differentiation between *T. g. tripolitania* n. subsp. and other recognized *T. graeca* subspecies was previously discussed by GRACIÁ *et al.* (2017a). Support for differentiation of *T. g. tripolitania* n. subsp. was found to be similar to that for other North African subspecies, and this lineage was found to have diverged from *T. g. graeca* during the Pleistocene. The dominance of a single *cyt-b* haplotype within the population may indicate low genetic diversity relative to other *T. graeca* subspecies, though we do find evidence that at least one additional haplotype exists, and more diversity may yet be discovered by further range surveys.

Remarks – Morphological diagnosis of subspecies in *T. graeca* is fraught with difficulty, as evidenced by an extensive list of now-invalidated genera, species, and subspecies. Despite this, there are distinct morphological characteristics of *T. g. tripolitania* n. subsp., which are useful for diagnosis. These tortoises are very small in size, similar to the smallest documented *T. g. nabeulensis* and *T. g. whitei*. In addition, the subspecies exhibits a very high shell profile and has minimal flaring of the marginal scutes. The carapace is rounded in profile and is highest at the 3rd vertebral scute, with a dome-shaped appearance. The shell is wider toward the posterior side, typically being widest at the 9th marginal scute. The carapace has a yellow, yellow-tan, or yellow-orange base color with highly contrasting black patterning (Figs. 3, 4). Most individuals show a well-defined pattern like the holotype, but in some cases the black pigmentation may be irregular or show mottling as in *T. g. cyrenaica*. The plastron is typically yellow with irregular and asymmetric black pigmentation, but may be predominantly black in some individuals. The head may range from nearly entirely yellow to mostly black, while the forelimbs are predominantly yellow with occasional black leg scales. While some *T. g. cyrenaica* exhibit similar coloration, adults can typically be distinguished by differences in size and HE/CL ratios. These tortoises are difficult to distinguish from *T. g. nabeulensis* and *T.g. white*i, as tortoises from Algeria or the northern Tunisian coast are very similar in both size and appearance (Highfield, 1990; Rhodin *et al*., 2021; Tiar-Saadi *et al*., 2022). *Testudo g. terrestris* also exhibits well-defined yellow and black carapace patterning and a similar overall appearance, but typically lacks the characteristic bold pigmentation on the head and forelimbs (Rhodin *et al*., 2021).

Etymology – The subspecific name *tripolitania* references the historical region of Tripolitania in northwestern Libya, where the tortoises are native. Tripolitania is derived from Greek, meaning "three cities".

Type Locality – Northwestern Libya. In the absence of exact locality data for the holotype, we refer to ICZN Art 76.1.1, "If capture or collection occurred after transport by artificial means, the type locality is the place from which the name-

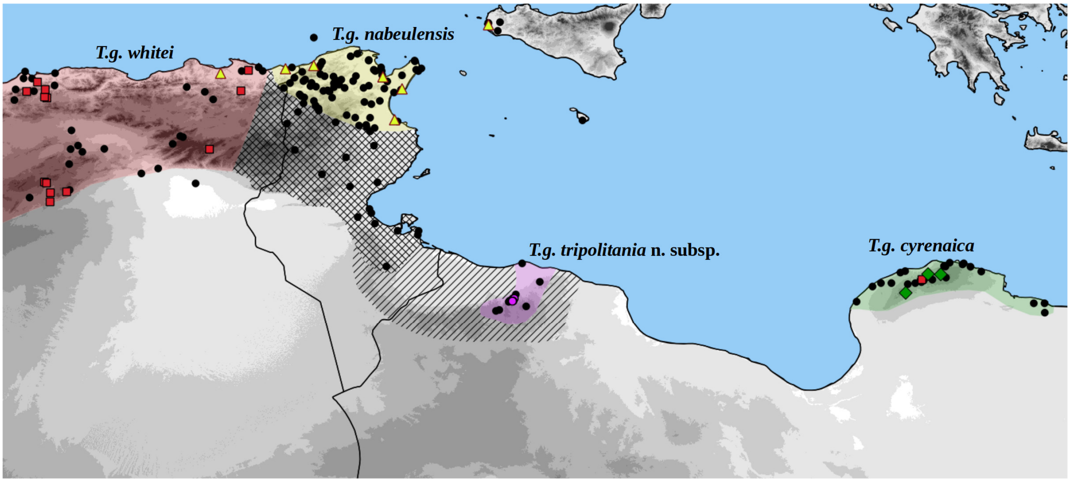

Figure 6: Range for *Testudo graeca* in eastern Algeria, Tunisia, and Libya. Filled symbols indicate localities for *T. g. whitei, nabeulensis, cyrenaica,* and *tripolitania* n. subsp. with mtDNA confirmation, including data from Fritz *et al*. (2009), and Anadón *et al*. (2015). Black dots indicate records without mtDNA confirmation from Anadón *et al*. (2015), Schneider & Schneider (2008), and Rhodin *et al*. (2021). The cross-hatched region indicates areas where *T. graeca* occurs but the subspecies identity is uncertain. The single-hatched region indicates areas where the presence of *T. graeca* is uncertain.

bearing type, or its wild progenitor, began its unnatural journey." We have collected evidence to show that populations in northwestern Libya are the wild progenitor of the type specimen, with populations near Gharyan being consistent genetically and morphologically with the type. The presence of spur-thighed tortoises in northwestern Libya was reported by HIGHFIELD (1990) and was further documented by SCHNEIDER & SCHNEIDER (2008). These tortoises were historically assigned to *T. g. nabeulensis* based on appearance, and this interpretation was followed by other authors investigating mtDNA phylogeny of the species, including FRITZ *et al.* (2007, 2009), despite being approximately 400 kilometers from the nearest mtDNA verified records in northern Tunisia.

Range and Ecology – Additional field work is necessary to confidently establish the range of *T. g. tripolitania* n. subsp. Because previous range maps suggest a contiguous range for *T. graeca* from Tunisia to Libya (FRITZ *et al.*, 2009; RHODIN *et al.*, 2021), the location of a subspecies boundary is unclear. An updated map for *T. graeca* in Libya, Tunisia, and western Algeria is proposed in Fig. 6. We restrict identification of subspecies to regions where it is well supported by mtDNA sampling. Consequently, there is a large region where *T. graeca* is present but not adequately identified: from Sfax, Tunisia, south to Tataouine, and west to approximately 100 kilometers beyond the Algerian border. We also identify regions where *T. graeca* has not been recorded but may plausibly occur, particularly between Tataouine, Tunisia and the Jabal Nafusa mountains in Libya. If allopatric speciation is considered, there are two plausible locations for a biogeographic barrier between *T. g. nabeulensis* and *T. g. tripolitania* n. subsp. The first is near the salt lake Chatt el Djerid in central Tunisia, where low elevation and lack of vegetation limit tortoise habitat to a narrow strip along the Gulf of Gabes. The second is near the Tunisia-Libya border, another area of lower elevation where *T. graeca* has so far not been reported. We also consider the strong evidence for niche partitioning in North African *T. graeca* reported by ANADÓN *et al.* (2015). The known habitat of *T. g. tripolitania* n. subsp. in the Jabal Nafusa range is relatively warm and dry, with low vegetation cover and annual precipitation of 150-250 mm. This habitat contrasts with that of *T. g. nabeulensis* in northern Tunisia, where annual precipitation is 300-1200 mm and vegetation cover is higher. Potential *T. graeca* habitat in central and southern Tunisia (from the Gulf of Gabes south to the Libyan border) is more similar to that of Gharyan, Libya, than to northern Tunisia, and we find plausible that the range of *T. g. tripolitania* n. subsp. could extend into Tunisia.

Conservation Remarks – Our foremost concern is the ongoing importation of a new lineage of *T. graeca* in the USA pet trade, along with *T. g. cyrenaica*, and the dubious nature of these imports. These *T. graeca* shipments have been consistently approved for legal commercial import into the USA despite serious reason for concern, namely, their export from a country where they are not native (Egypt) but which is geographically adjacent to their native range in Libya. Illegal transport of *T. graeca* from Libya to Egypt is frequent, as personally recounted by authors of this work who have assisted Libyan authorities in caring for and releasing confiscated tortoises. We urge CITES to ensure that international trade of North African *T. graeca* is legal and biologically sustainable, as populations of these tortoises are at risk of depletion without oversight and enforcement of existing laws. Moreover, we request that USFWS considers the concerns raised herein when reviewing any future importation of *T. graeca*.

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