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Research paper

Re-examination and illustration of shells of interspecific hybrid tortoises of *Testudo horsfieldii* (Gray, 1844) and *Testudo h. hermanni* (Gmelin, 1789) (Testudines: Testudinidae) from the collection of Walter Kirsche in the Dahme-Heideseen nature park, Prieros, Germany

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Abstract

In this paper, we have re-examined and assessed the only existing shells of two interspecific hybrid tortoises of *Testudo horsfieldii* and *Testudo h. hermanni* from the Walter Kirsche collections. It is the first detailed examination and measurements of important preserve shells of hybrid tortoises of *Testudo* taxa. An overview of the systematic relationship and the literature debate is given.

Keywords: *Tortoise Hybrid;Testudo horsfieldii;Testudo h. hermanni; Morphology; Re-Examination.*

1. Introduction

Interspecific crosses among various species and subspecies of genus *Testudo* have been documented and literature citations of these interspecific *Testudo* hybridizations compiled by different authors (Mayer 1992a & 1992b, Bruekers 1995, Veidt& Fritz 2001, Soler et al. 2011 & 2012). The German anatomist Walter Kirsche (Born June 21, 1920; Died June 30, 2008) was a specialized neuro-anatomist and was looking for an animal with very slow ontogenetic development for his embryological studies. So he used tortoises, which he bred on his extensive private property for his studies. For many years he kept European land tortoises (*Testudo h. hermanni*) and Russian tortoises (*Testudo horsfieldii*) and at last he got hybrid specimens of these two *Testudo* species there. In the case of personal observation by one of us (HVK) has visited examined and photographed the specimens of hybrid tortoises in the collection Centre in the nature park of the estate in Prieros on 14th July, 2021.

The Russian tortoise (*Testudo horsfieldii* / *Agrionemys horsfieldii*), also commonly known as the Afghan tortoise, the Central Asian tortoise, Horsfield's tortoise, four-clawed tortoise, and the Russian steppe tortoise is a threatened species of tortoise in the family Testudinidae. The species is endemic to Central Asia. This species is traditionally placed in *Testudo*. Due to distinctly different morphological characteristics, the monotypic genus *Agrionemys* was proposed for it in 1966. Today, *Agrionemys horsfieldii* is currently being accepted. *A. h. horsfieldii* (Gray, 1844) is indigenous land tortoise to Afghanistan, Pakistan and southern Central Asia among three described sub-species.

In September 1968 two Russian tortoises flew to the Moon, circled it, and returned safely to Earth on the Russian Zond 5 mission. Accompanied by mealworms, plants, and other life forms, they were the first Earth creatures to travel to the Moon. The species can spend as much as 9 months of the year in dormancy.Russian tortoises can live up to 50 years (Siddiqi (2018).

Hermann's tortoise (*Testudo hermanni*) is a species in the genus Testudo. Two sub-species are known: the western Hermann's tortoise (*T. h. hermanni*) and the eastern Hermann's tortoise (*T. h. boettgeri*). *Testudo hermanni* can be found throughout southern Europe.

The western population (*T. h. hermanni*) is found in eastern Spain, southern France, the Balearic islands, Corsica, Sardinia, Sicily, south and central Italy (Fritz &Havas (2007).

2. Materials and methods

Repository: The specimen #17 is present in the estate of Walter Kirsche in the Dahme-Heideseen Nature Park, Section N5 of the State Office for the Environment, PO Box 60 10 61, 14410 Potsdam, Arnold-Breithor-Str. 8, 15754 Heidesee, OT Prieros, Germany.

Coding of the measuring distances: CL med - median carapace length; CL - carapace length; CW - plastron width; CH - carapace height; PLT - total plastron length; PLW - median plastron length; AnN - anal notch; PBax - axillary plastron width; PBing - inguinal plastron width; BrLsin - left bridge length; BrLdex - right bridge length; BI - bridge index; L-B-I - carapax length-width index; L-H-I - carapax length-height index; CerL - cervical length; CerW - cervical width; C1L - first central length; C1B - first central width; C2L - second central length; C2B - second central width; C3L - third central length; C3B - third central width; C4L - fourth central length; C4B - fourth central width; C5L - fifth central length; C5B - fifth central width; CauL - caudal length; CauW - caudal width; GuL - gular length; GuW - gularwigth; PeL - pectoral length; PeW - pectoral width; HuL - humeral length; HuW - humeral width; AbdL - abdominal length; AbdW - abdominal width; FeL - femoral length; FeW - femoral width; AnL - anal length; AnW - anal width; L – lateral scutes; M – marginal scutes (Figure 1).

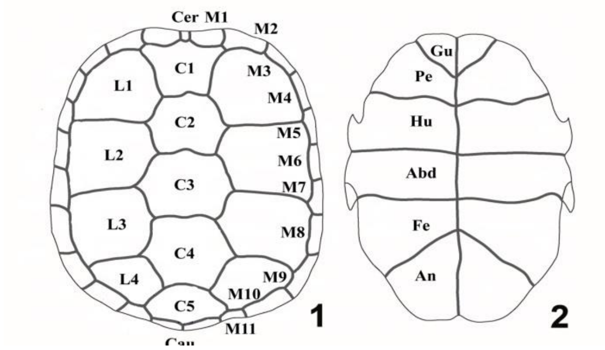

Fig. 1: Schematic Turtle Shell; 1 – Carapax2 – Plastron.

The cluster analysis is carried out with the software package PHYLIP, a free program package for parsimony algorithms. For the determination of discrete features, the cladograms are generated with TreeView © 2000. For comparisons to version from 3.6, see the website created by Joseph Felsenstein (University of Washington): https://evolution.genetics.washington.edu/phylip.html [19.01.2021], and see the Treeview program: http://priede.bf.lu.lv/ftp/pub/GIS/datu_analiize/TreeView/treeview_manual.html [19.01.2021].

Character coding: 1 - more round, flat shell shape, 2- undivided anal shield, 3 - light skin color, 4 - four claws of all extremities, 5 - front pigmented areas of the horny scutes, 6 - longitudinally oval shell shape, 7 - end nail on the tail.

Data matrix: *Testudo horsfieldii* 1111000, hybrid11111100, hybrid20111111, *Testudo hermanni* 0000111 (Kirsche, 1984).

Simplified 3D representation: In the course of the documentation of the turtle shell, a 3D model was also created using photogramming. For this purpose, several detailed shots of the tank with an overlap of at least 60% were made with a digital SLR camera from Nikon d5100 and a very light-sensitive lens by Sigma with a focal length of 40 to 60 mm, in the case of the shell there are 260 images. These were then aligned using Structure-for-Motion with the software Metashape from Agisoft (https://www.agisoft.com) and converted into a 3D model. Finally, the model from Metashape can be exported in any 3D format and viewed or further processed, e.g. in 3D viewer: https://www.microsoft.com/en-us/p/windows-view-3d/9nblggh42ths?activetab=pivot:overviewtab.
#3D-link here (As supplement material)

3. Description of the hybrid specimen

3.1. Proportions and coloring

With a length-width index of 1.21 and a length-height index of 1.85, the shell is short oval and moderately curved. The shell characteristics of the hybrids described and figured by Kirsche (1984) at the age of 7 months have changed their shell patterns considerably since hatching. The dark pigmentation of the carapace horny scutes has increased considerably in all animals, but this increase is not as pronounced in the *Testudo graeca* young animals. In his figure 2 it becomes clear that the phenotype of the carapace drawing of the both hybrids corresponds to the phenotype of *Testudo h. hermanni*. The hybrid 2 died at the age of 7 months without any externally recognizable symptoms; an indication that hybrids are often less vigorous. Hybrid 1 (present here) had also died in the meantime, at the age of one year and a few months immediately after the first wintering outdoors. At the age of 10 months, the hybrid 1 still showed the same shell patterns. The posterior areas of the carapace horny scutes are still like that of *Testudo h. hermanni* without pigment. In contrast, in *Testudo horsfieldii* the dark pigmentation of the horny scutes is found in the middle or rear area. The intermediate character is evident in the characters distribution between the parents and the two hybrids. They show the characteristics of *Testudo horsfieldii*, such as the more round, flat shell shape (hybrid 1), the undivided anal shield, the light skin color and four claws of all extremities as well as those of *Testudo h. hermanni*, like the front pigmented areas of the horny scutes, the longitudinally oval shell shape (hybrid 2), the front pigmented areas of the horny scutes and an end nail on the tail (Kirsche 1984).
The basic color of the horn scutes is gray-yellow. From the black centers of the areoles, clear rays run anteriorly and laterally, sometimes with a reddish-brown tinge (Plate 1).

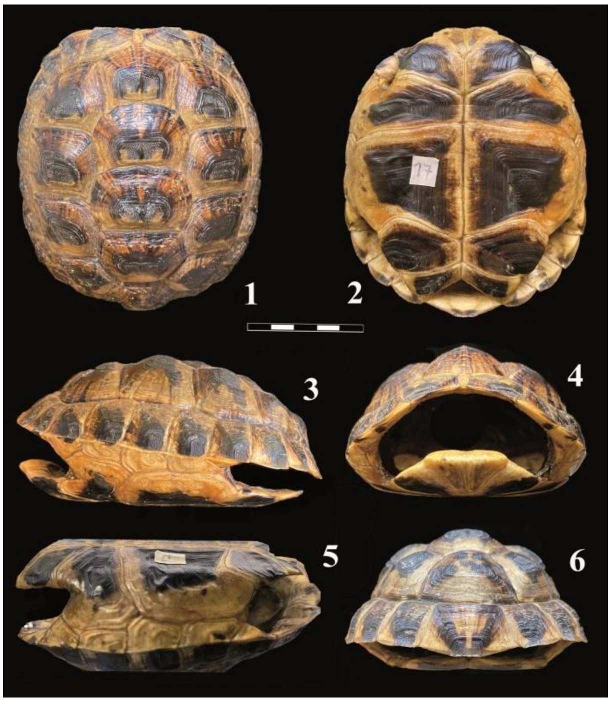

Plate 1: Shell of Hybrid of *Testudo horsfieldii* and *Testudo h. hermanni*; 1 - Dorsal View, 2 - Ventral View, 3 - Lateral View from Left, 4 - Frontal View, 5 - Right Bridge and 6 - Caudal View. (Scale 5 Cm: Photo by Dr. Karl).

3.2. Cluster analysis

One most parsimonious tree found ((*Testudo hermanni*: 3.00, hybrid 2:0.00):3.00,hybrid1:0.00,*Testudo horsfieldii*:1.00) requires a total of 7.000:

between	and	length
1	2	3.00
2	*Testudo hermanni*	3.00
2	Hybrid 2	0.00
1	Hybrid 1	0.00
1	*Testudo horsfieldii*	1.00

The analysis shows two clearly differentiated clusters, one with hybrid 1 and *Testudo horsfieldii* (red box) and the other with hybrid 2 and *Testudo hermanni* (blue box) (Figure 2).

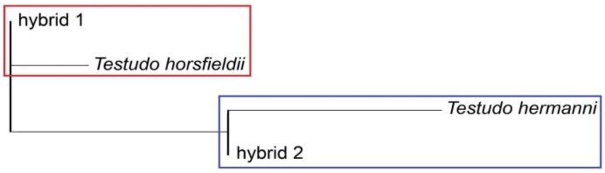

Fig. 2: One Most Parsimonious Tree Found by Discrete Character Parsimony Algorithm with PARS ©, Version 3.695 by Joseph Felsenstein (University of Washington) and Treeview © 2000 by Roderic Page.

Measurements of specimen #17

CL med	CL	CB	CH	PLT	PLW	AnN	PBax	PBing	BrLsin	BrLdex	BI	L-B-I	L-H-I
113	115	94	61	105	102,15	2,85	57,23	76,93	53,3	53,32	2,12	1,21	1,85
CerL	CerW	C1L	C1W	C2L	C2W	C3L	C3W	C4L	C4W	C5L	C5W	CauL	CauW
7,97	3,61	24,59	29,1	20,97	32,33	22,04	33,4	23,36	29,79	24,81	30,43	21,93	27,46
GuL	GuW	PeL	PeW	HuL	HuW	AbdL	AbdW	FeL	FeW	AnL	AnW		
17,98	25,47	13,65	50,3	9,63	79,26	41,81	60,83	8,73	45,59	8,78	34,91		

4. Discussion

Fossil remains of tortoises can be found in many old and new world sites from the end of the Paleocene. Unfortunately, the fragmentary state of preservation usually does not allow any deeper conclusions to be drawn about their relationships to one another. The characteristics of the complete tank finds are so discreet that clear delimitations are only possible in optimal cases. The bases for addressing the Palearctic Cenozoic Testudinidae are the morphological and genetic relations of the recent European-Mediterranean species. A further requirement would be a comprehensive biometric analysis of the *Testudo antiqua* form the Miocene. Quantitative biometric methods have already been used by Gmira (1995) and Schleich (1981). Another problem with the assessment arises with the large range of variation of the characteristics that can be used for taxonomy purposes. This phenomenon has been known since the early days of paleochelo-niology and has been published regularly by Karl (1996), Karl &Tichy (2004), Meyer (1867) and above all Staesche (1961). For the species from the so-called *Testudo hermanni* group, Lapparent de Broin et al. (2006) the genus *Eurotestudo* n. gen., as this genus within the genus *Testudo* sensu 1ato, (in the broader sense) as a Palearctic genus can be consistently delimited both from *Testudo* sensu stricto (in the narrower sense) and from *Agrionemys*. That preliminary cladistic analysis of the osteological features including the limited evidence of fossil "species" shows the clear division into three lines, probably since the Oligocene, but certainly since the Upper Miocene. The diagnosis of the newly interpreted genus is based on the combination of several characteristics. The authors point out the main stages in the change during the evolution of these three putative lines. On the one hand, the authors describe the expressive features with diagnostic value of the fossil species. However, they point out that the phylogenetic relationships between the supposed generic divisions are not yet clear, which actually contradicts the justification of a new generic name. The comparative taxon *Agrionemys* mentioned here, which Karl &Tichy (2002) reported on, is just as problematic. Also, Kirsche (1984) intruduced 2.0 hybrids *Testudo horsfieldii* x *Testudo hermanni* and one of these present here, which was used by Gmira (1993) to combine both species in the genus / subgenus *Agrionemys* Khosatzky&Mlynarski, 1966. Karl &Tichy (2002) pointed out, however, that the only two individuals known to date have died of infertility. This fact shows that a shift from *Testudo hermanni* to the genus / subgenus *Agrionemys* was not justified. The taxonomic status of *Agrionemys* has also not yet been established with certainty. The circumstances of the first publication are also problematic. The most important features such as four-clawed, undivided caudal or the fame of the ento-plastron through the humeropectoral sulcus also occur in the variation spectrum of the other European tortoises. *Agrionemys* can therefore not be used as a reference taxon without this itself having been thoroughly revised.

Based on mitochondrial gene sequences, Parham et al. (2006) earlier recognized conditions, whereby within the Testudinidae the genus *Manouria* forms the sister taxon to the other tortoises. *Geochelone pardalis* (= *Stigmochelys*) is the sister taxon of a group called Testudona. This is divided into two groups, with one, *Testudo*, in turn divided into two pairs of clades, on the one hand the Asian and African populations of *Testudo graeca* and on the other hand the species *Testudo kleinmanni* and *Testudo marginata* included under *Chersus*. *Testudo hermanni* forms a species pair with *Testudo horsfieldi*, which forms a group with the genera *Malacochersus* and *Indolestudo*. The constellation of the two *Testudo graeca* populations alone shows that analogous to this, no specific or generic consequences can be proven. See also Soler et al. (2011).

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