Studbook breeding programme

Testudo graeca

(Spur thighed tortoise)

Photo: Lutz Geiszler

Annual report 2014

Henk Zwartepoorte, studbook keeper
Peter Bulsing, co studbook keeper

European Studbook Foundation
KvK nr. 41136106
www.studbooks.eu

Contents:

1. **Introduction and activities 2014**
2. **Studbook population**
3. **Living population**
4. **Locations**
5. **Births**
6. **Imports**
7. **Deaths**
8. **Transfers**
9. **Discussion**
10. **Acknowledgements**

1. Introduction and activities 2014.

In the latest studbook annual report 2010/2011 the taxonomy of the *Testudo graeca* complex was discussed. Drawings of the scute formations on the front legs were showed in relation to subspecies on in particular the northern African subspecies.
During 2013 and 2014 DNA sampling on a number of studbook specimens was investigated but also executed. Also within this period a number of so called ESF frontdoor animals were offered to the studbook. They were DNA sampled and reported to the Dutch CITES bureau and accepted as studbook specimens and as such they received a legal status. However the studbook population significantly increased we still can not speak of a genetically healthy population. Too many animals died during the past few decades and also the offspring was not always viable.
The species is legally protected by CITES appendix I, EU appendix A. Internationally law enforcement however fails preventing large scale international illegal trade. Large numbers are exported from different northern African countries (Marocco, Tunesia, Algeria, Libya) and via trade routes ranging from the ferries to Europe (Spain, France, Italy) and via the mainland via the Middle East they are further transported to mainly Asia.
Asia and in particular China has set eyes on African wildlife ranging from ivory and rhino horn to tortoises.
During 2014 DNA sampling improved. 25 Studbook animals, including some frontdoor animals, were analyzed by the Gendika company. Interesting results were those of a TSA Europe imported shipment of 32 Hong Kong confiscated animals of unknown origin. 9 Out of 10 sampled animals belong to the lineage C (Fritz et al, 2009) meaning that the animals concerned *Testudo graeca cyrenaica* from Libya. The DNA results could also very well benefit international law enforcement.

2. Studbook population:
December 31, 2014 the total registered studbook population counts 149.142.224 (515) specimens, meaning an increase of 12.12.54 (78) studbook specimens compared to the studbook of 2011.

3.1 Living population:
December 31, 2014 the total living population counts 48.48.87 (183) specimens. Due the 332 historical deaths and Lost To Follow up (LTF) animals a decrease of the registered living animals.

3.2 Lost to Follow up (LTF):
Over the past few decades a number of animals was registered as LTF. Some participants did no longer participate and got lost for the studbook and so got their animals. Sometimes participants simply did no longer respond to the inquiries done at the beginning of each year. After a few years of silence the participants were removed from the studbook and their animals kept were registered as LTF.
In 2014 8 animals at 4 locations had to be removed from the studbook as LTF.

3.3 ESF front door animals:
Sometimes animals are brought to the attention of either ESF board members or to the CITES authorities directly. These animals are either found on the streets in cities or in fields in the countryside. But also some animals are handed over by relatives who no longer can take care of the animals. Between the CITES bureau and the ESF board a long time good communication exists about these animals. In order to give them a legal status the animals have to be registered in the studbook at a location where breeding possibilities exist or can be created. This can be at the location who reported the animals to the CITES bureau or to the ESF board. The animals become property of ESF and are placed on a breeding loan basis at a studbook location. The species- and subspecies status have to be assessed first; if necessary with help of a DNA test. The animals are accompanied by an ESF breeding loan contract. There is an annual inquiry executed by the ESF board on the status of the animal(s).

3.4 ESF animals:
During the past few years the Rotterdam Zoo transferred some animals to ESF privates. This often concerned animals found on the streets in Rotterdam or simply unanimously donated to the zoo. In 2014 it was decided by the zoo to consider all past transferred animals and future transfers as transfer to the ESF. This change of policy was discussed and agreed with the ESF board. So all these

animals became property of ESF and were registered as a breeding loan animal to the studbook participant.
Historically 185 ESF animals died.
At present 38 animals are still alive. All keepers not having received an ESF contract for these animals will receive a contract soon.

3.5 IBG animals:

IBG stands for "**I**n **B**eslag genomen **G**oederen" in English confiscated goods. Animals confiscated at the Dutch borders or elsewhere in the country are sometimes offered to the ESF with the request to place them at a studbook location. These animals are placed under the same conditions as those for the so called front door animals. Sometimes also for these animals a DNA test is executed.

4. Locations:

December 31, 2014 42 participants in 6 European countries were registered. The majority originating from The Netherlands (32); furthermore there are participants in Belgium (1), United Kingdom (4), Germany (2) Spain (1) and Italy(2).
In 2011 the ESF board decided that publication on the ESF-website of the studbook is no longer required. Names of participants will remain only known within the *Testudo graeca* studbook community and the studbooks will be sent to the participants only by email and on request. The annual reports will be sent to all studbook participants and will be published on the ESF website www.studbooks.eu

5. Births:

In 2014 8 births were reported at 1 location.

6. Imports:

The Sparks software programme considers new entries into the studbook as imports and as such these imports will be reported and discussed here.
In 2014 54 new arrivals(imports) are reported. These concerned several "front door animals" and 32 animals were imported by TSA Europe. These 32 animals were confiscated in Hong Kong and temporarily homed at the Kadoorie Farms and Botanical Gardens. After the juridical process they were offered to the TSA Europe. Shortly after arrival at the Rotterdam Zoo 28 animals were handed over to ARCO Spain and transported to Tabernas/Spain in October 2014. 4 animals did shortly after arrival at the Rotterdam Zoo.

7. Deaths:

In 2014 16 deaths were reported at 10 locations.

In general the cause of death is very unclear. No autopsy reports were received by the studbook.
For confiscated dead tortoises in general there might be a suspected reason. Between the moment of capture, mainly occurring in North African countries such as Morocco and Tunisia and the moment of arrival at the studbook participants collections lies often a long period.
The time between collecting from the wild and the arrival at Dutch airports or borders is unknown but one can assume that this will often take weeks or months.
The cause of death of a significant number of confiscated animals is not always clear, but what we do see in the dead animals is that post mortem is impossible in a large number of cases because of the autolytic state of the carcasses.
This situation makes it very difficult to assess a cause of death.

8. Transfers:

In 2014 4 animals were transferred from one studbook location to another.

9. Discussion:

9.1 Introduction:

In the annual report of 2008 a chapter was published on DNA research and the establishment of so called haplotypes within the studbook population. It is good to repeat this chapter in this annual report 2014, however with some additional recently published results in blue.
"*Between 1997 and 2007 it became more and more clear that the North African "subspecies" Testudo graeca graeca was not a clear subspecies based on the whole Northern African population. Earlier, the subspecies Testudo graeca ibera was classified by a number of taxonomists as a separate species Testudo ibera. During the last 10 years on a significant number of studbook specimens a DNA research was carried out. All participants voluntarily financed this research which was carried out by the AMC faculty in Amsterdam by ms. Tonja van der Kuyl (Van der Kuyl, 2000).*
During 2008 the University of Ghent in Belgium was asked to proceed this research in particular important with respect to proper homing of the confiscated tortoises in the Netherlands. Prof. Dr. Frank Pasmans responded positively to this request and he and his team did their very best to successfully test several dozens of samples of confiscated Testudo graeca.
March 2009 the studbook received the first results making it possible to compare these results with the morphological identification and transfer these animals to ESF studbook participants in the course of 2009. The ESF board as well as the Testudo graeca studbook are Prof. Dr. Frank Pasmans and his team extremely grateful for the cooperation.
The taxonomy of Testudo graeca, in the Northern African region as well as the Middle East and western Asia is still under discussion.

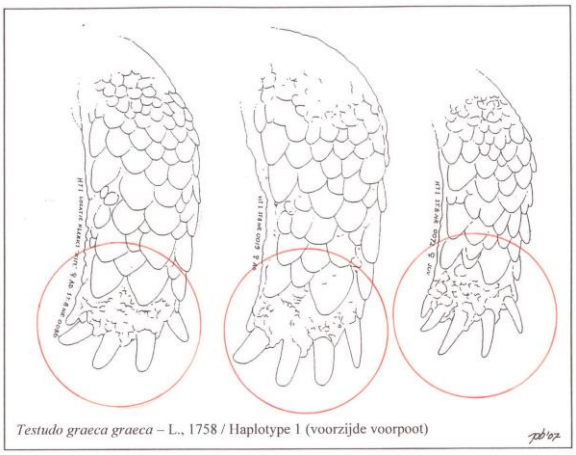
As communicated earlier elsewhere the ESF Testudo graeca studbook now focuses only on the North African population.
Currently within the wild population in Morocco, Algeria, and Libya 6 subspecies are described by Fritz et al and Pieh and Perala:
Testudo graeca graeca (Fritz et al, 2009),
Testudo graeca cyrenaica (Pieh and Perala, 2002),
Testudo graeca soussensis (Pieh, 2000),
Testudo graeca lamberti (Pieh and Perala, 2004),
Testudo graeca marrokensis (Pieh and Perala, 2004).
From Tunesia by Highfield Testudo nabeulensis is described (Highfield, 1990). The validity of this newly described species was not internationally accepted and still is not, although in 2002 Pieh and Perala consider the species nabeulensis as a subspecies of T. graeca. Now in 2015 this subspecies is more or less generally accepted and as such can be accepted as valid, the more DNA results show that this is a distinct DNA lineage.

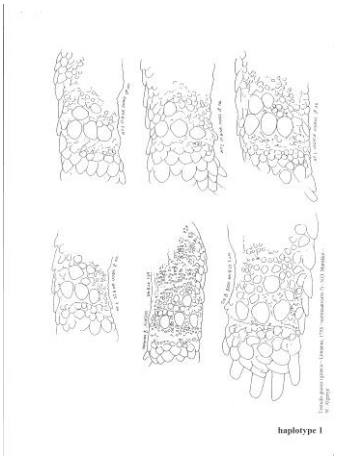
The originally described subspecies T.g.graeca still is valid for a specific type in Morocco; the number of subspecies is now 6.
Furthermore the types Testudo whitei BENNETT, 1836 and Testudo flavominimaralis HIGHFIELD & Martin, 1989 are still discussed and by some people considered as a valid species or subspecies. Pieh and Perala however consider these taxa as nomina dubia.
Over the last few years all studbook participants were asked to send photo's of their animals. From these photos collected a large number of specimens with an emphasis on head shape, colour and shape of both carapace and plastron and scute formations on both front and backside of the front legs a significant number could be determined. And they now partly correlate with the recent received DNA results. Bulsing already earlier started to make line drawings of these scute formations which is published by Bulsing and Zwartepoorte in Trionyx (2007)."

9.2: current status of DNA progress:
In 2014 the Gendika BV company continued with the DNA sampling and proceeded with the Gent university work. The first sampling was done October 2014 on 10 samples of animals at the Rotterdam Zoo; among these 10 one *T.ibera* and 1 *T.hermanni boettgeri* and two ealier Gent university analized *T.graeca*. The results were very promising. The *T.hermanni boettgeri*, the *T.ibera* and the two *T.graeca* were correctly sampled and assessed. The DNA work developed and published by Fritz et al (2009) was the basis for taxonomic determination. So we now no longer write about haplotype numbers but DNA lineages A, B1, B2, C and D. See below under Distribution in the wild.

Some precautious determinations of subspecies/lineage to scute formation on front legs found in captive specimens and where ever possible on specimens in the wild.

***Testudo graeca graeca* – DNA lineage B1(former Haplotype 1):**



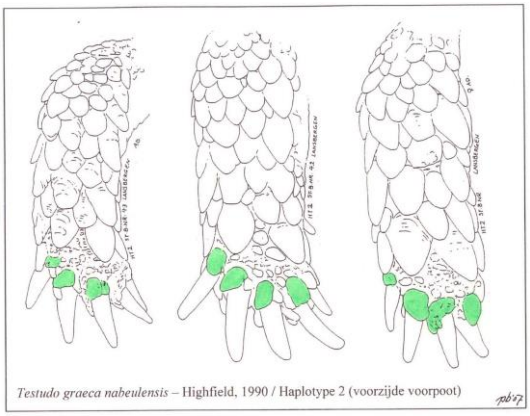


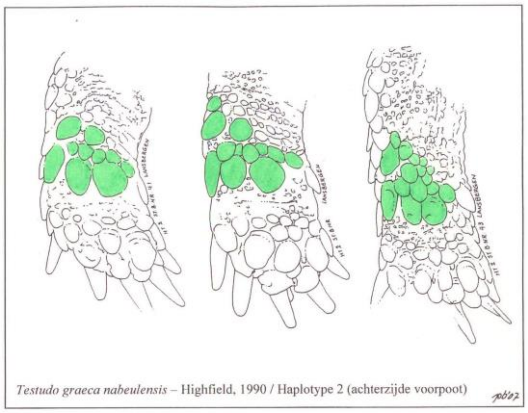
Front- and backside of a front leg of *Testudo graeca graeca* Lineage B1 (former Haplotype 1).

***Testudo graeca graeca* DNA Lineage B1 (former Haplotype 1) at location Zwartepoorte.**

A long term captive specimen in the United Kingdom of *Testudo graeca graeca* Lineage B1 (former Haplotype 1). The origin of the animal is unknown.

***Testudo graeca nabeulensis* – DNA lineage A (former Haplotype 2).**

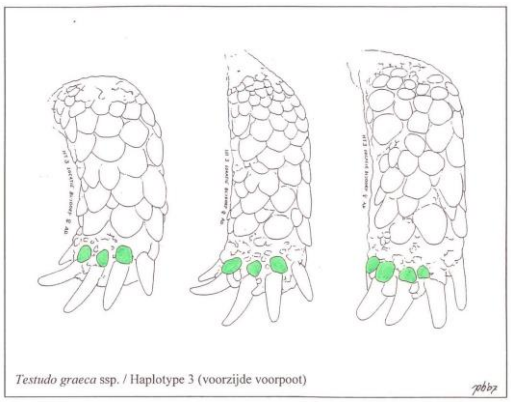


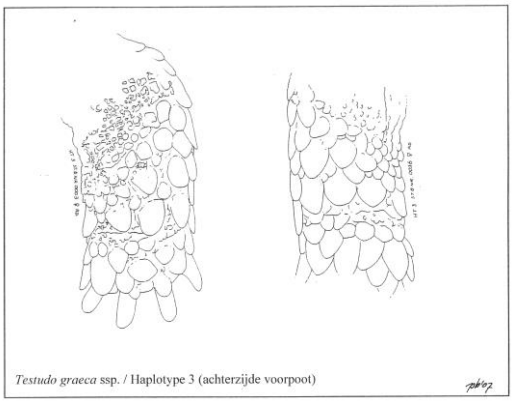


***Testudo graeca nabeulensis* DNA Haplotype 2.**
Front- and backside of a front leg.

Scutes on the frontleg of a captive *Testudo graeca nabeulensis*. Lineage A(former Haplotype 2). The origin of the animal is unknown.

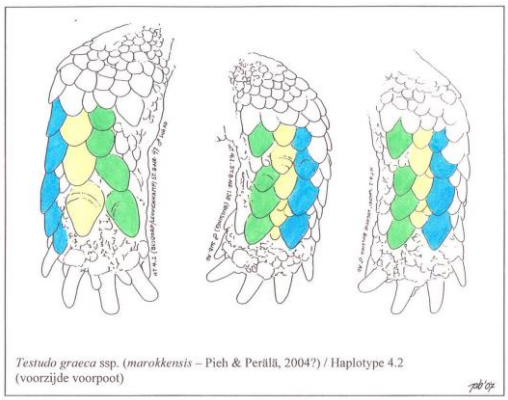
Testudo graeca ssp DNA lineage not yet assessed (former Haplotype 3).





Testudo graeca ssp DNA Haplotype 3.
Front- and backside of a front leg.

Testudo graeca marokkensis DNA Lineage B2 (former Haplotype 4.2)



Front side of a front leg.

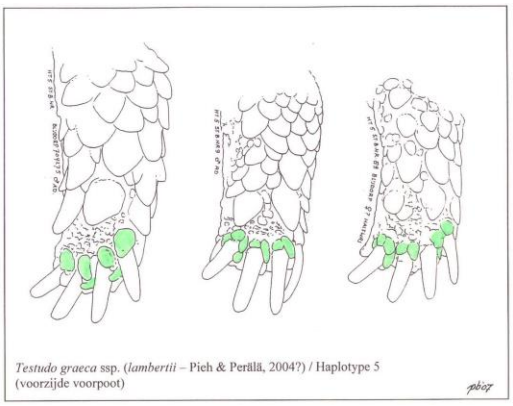
***Testudo graeca marokkensis* DNA Lineage B2 (former Haplotype 4.2) Juvenile specimen at the Rotterdam Zoo.**

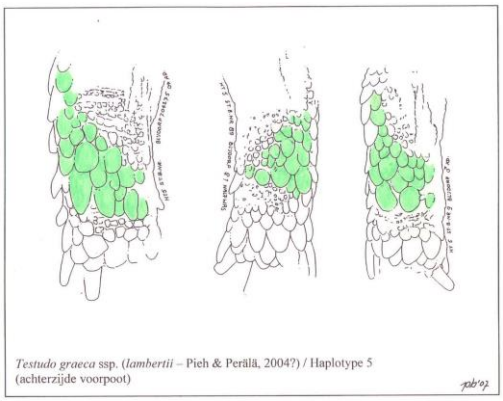
***Testudo graeca marokkensis* DNA Lineage B2 (former Haplotype 4.2) Male specimen at the Rotterdam Zoo.**

Scutes on the front leg of a captive *Testudo graeca marokkensis* Lineage B (former Haplotype 4.2). The location of the animal is unknown.

Testudo graeca lamberti DNA Haplotype 5.

According to Fritz et al (2009) the subspecies *T.graeca lamberti* and *T.g.marokkenis* not only share the same mitochondrial lineage but also the same individual haplotype. However the sub species is described by Pieh and Perela (2004) and also in captive animals differences in carapace shape and front foot scute formations have been assessed.





Front- and backside of a front leg of a captive specimens of unknown location.

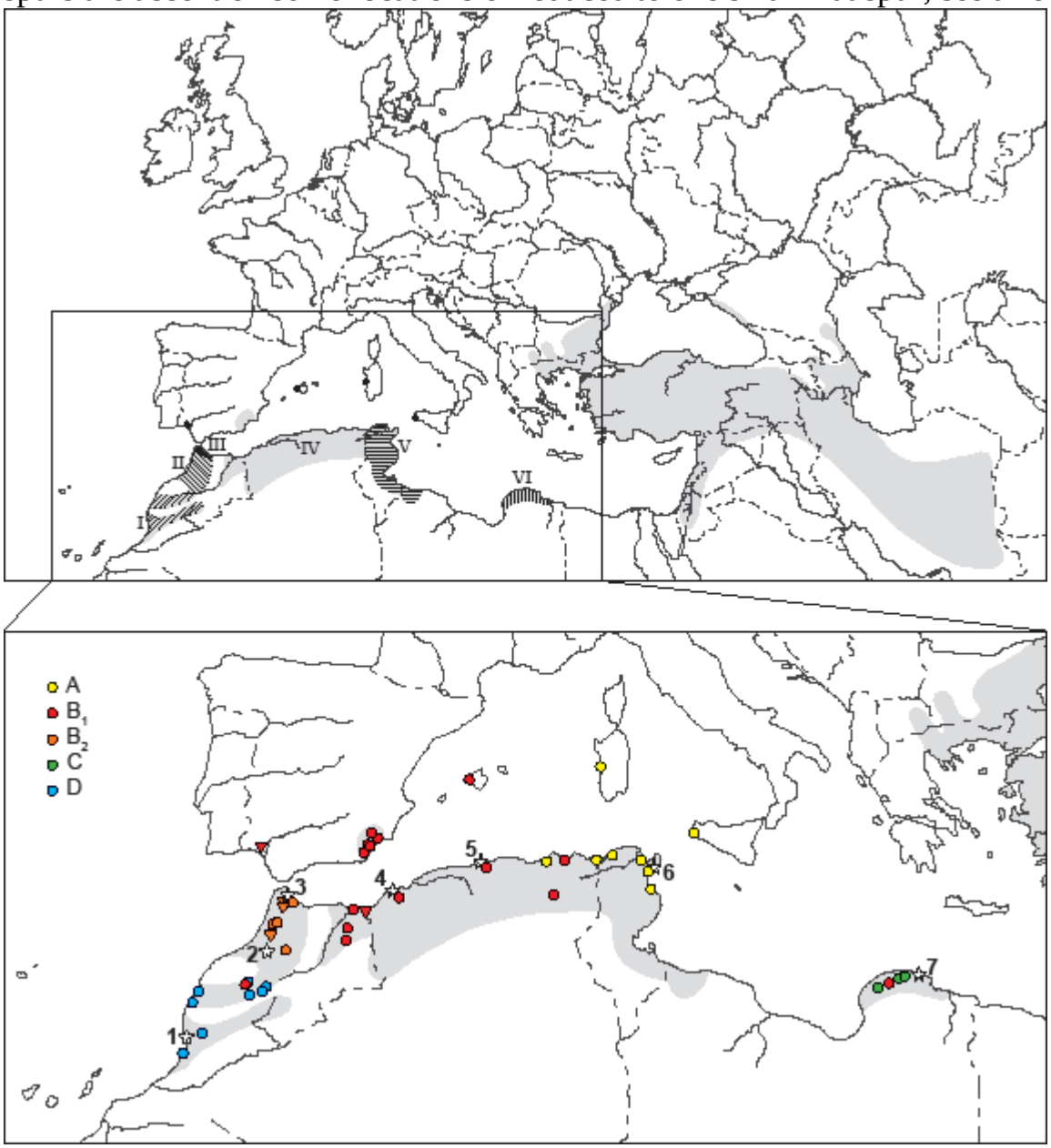
Testudo graeca soussensis DNA Lineage D

A wild specimen at Taraudant in Marocco.
Photo: Lutz Geiszler.

***Testudo graeca soussensis* Lineage Photo: A.Pieh.**

***Testudo graeca soussensis* – Lineage C Photo: A.Pieh.**

Testudo graeca soussensis is described by Pieh (2004). In this subspecies the spurs are absent on some locations or reduced to one small flat spur; see arrows.



Distribution of *Testudo graeca* (shaded; combined from Loveridge and Williams, 1957; Bannikov et al., 1977; Anderson, 1979; Iverson, 1992; Bons and Geniez, 1996; Buskirk et al., 2001; Disi et al., 2001).
Ranges of currently recognized Western Mediterranean subspecies indicated on the top, type localities (asterisks) and sampling sites (circles) on the bottom.
Subspecies ranges: I – *Testudo graeca soussensis* Pieh, 2001; II – *T.g.marokkensis* Pieh and Perala, 2004; III *T.g.lamberti* Pieh and Perala, 2004; IV – *T.g.graeca* Linnaeus, 1758; V – *T.g.nabeulensis* (Highfield, 1990); VI – *T.g.cyrenaica* Pieh and Perala, 2002.
Type localities: 1 – *T.g.soussensis*; 2 – *T.g.marokkensis*; 3 – *T.g.lamberti*; 4 – *T.g.graeca*; 5 – *T.g.whitei* Bennett in White, 1836 (designated by Highfield and Martin, 1989); 6 *T.g.nabeulensis*; 7 – *T.g.cyrenaica*.
Colours correspond to figs 2,3 and indicate Western Mediterranean clades of mtDNA haplotypes. Triangles indicate haplotypes identified by Alvarez ert al. (2000).

Important progress over the past few years was the assessment of lineages and subspecies of a growing number of studbook animals. See above for this the leg scutes drawings by Bulsing and the distribution map and publication by Fritz et al (2009) was very helpful.
Much progress is made regarding the photo gallery of the studbook specimens. The online registration from January 1, 2016 has the option entering photos of individual animals and put them online.
Photos of head, plastron, carapace and scutes on front- and backside of the front legs are required and those participants who did not deliver photos so far will be reminded. This photo identification tool in combination with DNA tests is vital for proper assessment of the DNA lineages/subspecies. Costs for DNA-sampling are now assessed at €50 per sample. Co operation will be voluntarily but a contribution into the costs will be asked. A genetically healthy studbook population is desperately needed acquiring an ex situ assurance population from which future re-introduction into the wild can be carried out. This of course under strict IUCN guidelines regarding this and after thorough health checks and quarantine periods.
Further evaluating of the studbook show some remarkable topics.
The total 149.142.224 (515) historically reported studbook animals show a positive image. However the current living population of 48.48.87 (183) shows the death of 332 and LTF animals. This really is a serious matter of concern. The fact that almost no autopsy is carried out on these deaths the cause of these early deaths is impossible to assess.
The number of births is rather low. The subspecies *T.g.nabeulensis* proves to be best bred. Recommendations for reproduction will be communicated soon.

At present the studbook counts 42 participants. This decrease is caused by the LTF-participants. Aim for the next few years is to increase this number again to around 50 and between 300 and 400 living animals which seem reasonably to be managed numbers.
At present within the ESF studbook of the now accepted DNA lineages the following numbers are assessed with certainty:
Testudo graeca cyrenaica: 29
Testudo graeca graeca: 18
Testudo graeca lamberti: 1
Testudo graeca marokkensis: 4
Testudo graeca nabeulensis: 42
Testudo graeca soussensis: 1

9.3 Conservation status:
Still very unwanted and illegal are the ongoing imports of North African tortoises from in particular Marocco. This has consequences for the private turtle keeping sector. Although this illegal trade is mainly focussing on export to Asia often private people in Europe buy illegally exported/imported tortoises. This is a matter of great concern within both the private ESF and EAZA studbook population. Participation within the ESF studbook is only possible with proven legal animals. Cooperation and participation with EAZA institutions (European Zoos) is vital and highly demanded.
During 2014 the studbook got in touch with Marie Petretto of the Marwell Zoo in the UK. Marie is leading a safe haven and reintroduction project in Tunesia for *Testudo graeca nabeulensis*. Confiscated animals from the region are rehabilitated in order to reintroduce the Tunesian animals into the wild in protected areas. In the near future with this project ESF is planning to intensify the cooperation and exchange of vital information already took place.
Current participation with zoos is highly appreciated. Both EAZA and ESF are supposed to participate as equal Turtle Survival Alliance (TSA) partners building the Turtle Ark.
A viable ex situ population of this very attractive species must belong to the possibilities and the studbook keeper calls upon the studbook participants to achieve this goal.

10. Acknowledgements:
Our thanks and appreciation go to Uwe Fritz at the Dresden Museum in Germany for the use of the distribution map.
Also thanks to Peter Bulsing for using his great drawings of the front legs of the different haplotypes and some photos.
Alexander Pieh and Lutz Geiszler for using photos and information.
Unless differently mentioned above other photos are by Henk Zwartepoorte.
The Gendika BV company for DNA sampling and quick good communication.

Marie Petretto of the Marwell Zoo for good communication and co operation.

11.References:

Bulsing P. (2000): De Noord Afrikaanse moorse landschildpad (Testudo g. graeca) toch een probleemloze schildpad?. Special ter gelegenheid van het 125-jarig bestaan van de Nederlandse Schildpadden Vereniging: 27-31.
Bulsing P. en H.A. Zwartepoorte (2007): Herkenning van enkele types van Testudo graeca uit Marocco, Algerije en Tunesie: Trionyx ( ).
Ballasina D.L.Ph., A.C. van der Kuyl, J.T. Dekker, J. Maas, R.E. Willemsen en J. Goudsmit (2001):
Phylogenetic relationships among the species of the genus Testudo (Testudines: Testudinidae) inferred from mitochondrial 12S rRNA Gene sequences, Congres International Sur le genre Testudo –
7 au 10 mars 2001 Hyeres – France / Actes du Congres.
Fritz U, D.James Harris, Soumia Fahd, Rachid Rouag, Eva Gracia Martinez, Andres Gimenez Casalduero, Pavel Siroky, Mohsen Kalboussi, Tareek B. Jdeidi, Anna K. Hundsdorfer (2009) :
Amphibia-Reptilia 30 (2009) : 63 :80.
Harris D.J., M. Znari, J.C. Mace, M.A. Carretero (2003) : Genetic variation in Testudo graeca from Morocco estimated using 12S rRNA DNA sequensing. Revista Espagnola de Herpetologia, 17: 15-9.
Highfield, A.C. & Martin J. (1989): Description of a miniature tortoise Testudo flavominimaralis n. species from North Africa. The Tortoise Trust, London; 4 S.
Pieh A. (2002): Testudo graeca soussensis, eine neue Unterart der Maurischen Landschildkrote aus dem Sousstal (Sudwest-Marokko). Salamandra 36(4) : 209-222.
Pieh A., J. Perala (2004) : Variabilitat de Maurischen Landschildkroten (Testudo graeca – LINNEUS, 1758 – Komplex) im Zentralen und nordwestlichen Marokko mit Beschreibung zwei neuer Taxa. Herpetozoa 17 (1/2): 19-47.
Fritz U., A.K. Hundsdorfer, P. Siroky, M. Auer, H. Kami, J. Lehmann, L.F. Mazanaeva, O. Turkozan, M. Wink (2007): Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western Palaearctic tortoises (Testudo graeca complex; Testudines, Testudinidae). Amphibia-Reptilia 28 (2007): 97-121
Hufer H. & Buddenfeld V. (2000): Haltung und Zucht der Tunesischen Landschildkrote. Radiata, Rheinbach; 9 (20): 3-14.
Pieh A. & Jarmo Perala (2002): Variabilitat von Testudo graeca LINNAEUS, 1758 im ostlichen Nordafrika mit Beschreibung eines neuen Taxons von der Cyrenaika (Nordostlibyen). Herpetozoa 15 (1/2): 3-28.
Zwartepoorte H.A. (2000): Project DNA-typering moorse landschildpad (Testudo graeca). Special ter gelegenheid van het 125-jarig bestaan van de Nederlandse Schildpadden Vereniging: 24-26.

Henk Zwartepoorte, Studbook coordinator and studbook keeper.
Peter Bulsing, co-studbook keeper.
September 2015.
