Zoology in the Middle East

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tzme20

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**To cite this article:** Mohamed A. M. Kadry , Abdulaziz R. Al-Qahtani & Sayed A. M. Amer (2020) Morphometric and molecular differentiation between Egyptian *Stellagama stellio vulgaris* and *S. stellio salehi* (Reptilia: Agamidae), Zoology in the Middle East, 66:4, 295-301, DOI: 10.1080/09397140.2020.1826677

**To link to this article:** https://doi.org/10.1080/09397140.2020.1826677

Published online: 23 Sep 2020.

*Zoology in the Middle East,* 2020
Vol. 66, No. 4, 295–301, http://dx.doi.org/10.1080/09397140.2020.1826677

# Morphometric and molecular differentiation between Egyptian *Stellagama stellio vulgaris* and *S. stellio salehi* (Reptilia: Agamidae)

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*(Received 19 May 2020; accepted 14 September 2020; first published online 23 September 2020)*

From the seven recognised subspecies of *Stellagama stellio*, *S. s. vulgaris* is found in northwestern Egypt and *S. stellio salehi* in the south Sinai and are known to be very similar. A Principal Component Analysis was carried out for 14 morphometric features, PCA1 accounted for a little cumulative variation (87.03%) between the two subspecies with a strong canonical correlation (r = 0.999). PCA2 accounted for a high cumulative variation (98.04%) with a strong canonical correlation (r = 0.985) and head height (HH) was the only major parameter for the significant difference. Within 398 sites of 16S rRNA gene sequenced, 6 base substitutions were recorded between *S. s. vulgaris* and *S. s. salehi* and the pairwise genetic divergence was calculated as 1.5%, which is comparable to that found between some other conspecific agamids. Neither the morphometric nor the molecular data support the distinction of two different subspecies. No genetic difference was found between *S. s. salehi* and *S. s. brachydactyla* which occurs from northern Sinai over Jordan to Saudi Arabia.

**Keywords:** Morphology; mitochondrial DNA; 16S rRNA; systematics

## Introduction

*Stellagama stellio* (Linnaeus, 1758), formerly assigned to the genus *Laudakia* is distributed from western Greece, Turkey and northwestern Iraq to north Egypt (Amr et al., 2013) and inhabits a variety of Mediterranean, arid and semi-arid habitats, often also found on rocks, trees, and buildings. Seven subspecies have been distinguished: *S. s. stellio* (Linnaeus, 1758), *S. s. picea* (Parker, 1935), *S. s. brachydactyla* (Haas, 1951), *S. s. cypriaca* (Daan, 1967), *S. s. daani* (Beutler & Frör, 1980), *S. s. vulgaris* (Sonnini & Latreille, 1801) and *S. s. salehi* (Werner in Lachman et al., 2006) (Baig, Wagner, Ananjeva, & Böhme, 2012). Two of these subspecies are found in Egypt: *S. stellio vulgaris* in northwestern Egypt in Cairo and Alexandria governorates (Pierre-Andre et al., 2006) and *S. stellio salehi* in the south Sinai rocky desert (Lachman et al., 2006).

While subspecies assignment has been made mainly on the basis of morphometric assessment and colouration, a few molecular studies addressed the systematic situation of some subspecies. Using amplified fragment length polymorphism (AFLP) techniques, Brammah, Hoffman, and Amos (2010) found a high degree of genetic differentiation both between and within two subspecies *S. s. stellio* and *L. s. daani* occurring in the Greek Cyclade Islands and explained this by different colonization times. Özdemir, Gül, and Tosunoğlu (2011) analysed the 12S rRNA mitochondrial gene in six populations in Turkey and showed that not all could be attributed to *S. s. daani*, while the sta-

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tus of the population in south-eastern Turkey could not be clarified. Amer (2005), by using 1600 bp from mtDNA, addressed the molecular phylogeny of the two Egyptian subspecies without clear resolution of their systematic status. The two Egyptian subspecies need more extensive morphological and molecular research to clarify their intraspecific divergence and taxonomic relevance. The aim of this study is to contribute to the understanding of the morphometric and molecular differentiation of the two subspecies.

## Material and Methods

A total of 11 adult *S. s. vulgaris* (6 males, 5 females) were collected from the coastal desert of Burj Al-Arab, Alexandria governorate [30º49'N, 29º35'E] and 8 adults (3 males, 5 females) of *S. s. salehi* were collected from Wadi El-Arbaien, Saint Katherine (South Sinai governorate) at the southern end of Sinai [28°31'N, 33°57'E] (Figure 1). Animals were treated according to the guidelines of Cairo University Institutional Animal Care and Use Committee (CU- IACUC) with approval number CU/F/94/19.

Following Kumlutaş, Uğurtaş, Koyun, and Ilgaz (2015), the following morphometric characters were taken by using a vernier caliper: SVL (snout-vent length), MHW (maximum head width), HH (head height), HL (head length), SED (snout-eye distance), $HW_{eyes}$ (head width between eyes), TL (tail length), TR (number of tail rows), JL (jaw length) and AW (abdominal width). From morphological measurements, the following ratios and indexes were computed: TL/SVL, HL/SVL, head index (HI) [100 × HL/HW] and head length index (HLI) [100 × HL/SVL]. These 14 morphometric characters were analysed by PCA packaged in SPSS v. 26.

Liver tissues were removed from 7 individuals (5 from *S. s. vulgaris* and 2 from *S. s. salehi*) and preserved in absolute ethanol. Approximately 100 mg of liver tissue was cut into small pieces for DNA extraction using a Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The 16S rDNA gene was partially amplified using the forward primer 16SL: 5′-CGCCTGTTTATCAAAACAT-3′ and the reverse primer 16SH: 5′-CCGGTCTGAAC TCAGATCACG-3′ (Palumbi et al., 1991).

Polymerase chain reaction (PCR) was conducted as described by Amer, Ahmed, and Shobrak (2013). The amplified products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and the purified products were sequenced by Macrogen, Seoul, South Korea, according to their protocol (www.macrogen.com). The sequenced fragments were deposited in the National Center for Biotechnology Information (NCBI) GenBank with accession numbers MN641468-MN641474.

The sequenced 16S rDNA gene fragments were examined with BLAST program by NCBI (www.ncbi.nlm.nih.gov) and compared with the most similar *Stellagama*, *Paralaudakia* and *Laudakia* sequences. Accession numbers of taxa used are as follow: HQ901096, HQ901097, HQ901098, HQ901101, HQ901116, HQ901108 (Wagner, Melville, Wilms, & Schmitz, 2011); AY053765 (Pang et al., 2003); GU128464, MH047789 (Leache et al., 2009) and MH047794 (unpublished).

The pairwise alignments were conducted for these sequences according to the method of Needleman and Wunsch (1970) and modified to deal with the more flexible costs allowed by MacClade v. 4.08 (Maddison & Maddison, 2005). The aligned data were used after deleting ambiguous and gap-containing sites. The remaining sites (398 bp) were analysed by maximum-parsimony (MP), neighbor-joining (NJ) and maximum-likelihood (ML) methods with PAUP v. 4.0b10 (Swofford, 2002). For MP, 10 random stepwise-additions for the heuristic searches were conducted by tree bisection reconnection (TBR) branch swapping, while 5000 bootstrap replications were conducted by TBR and simple stepwise-additions. NJ analysis was also conducted (Saitou & Nei, 1987) using Tamura-Nei (Tamura & Nei, 1993) as a distance option and 5000 bootstrap replications. Modeltest v. 3.6 (Posada & Crandall, 1998) was used to select the best fit model (GTR + G) for ML analysis. For ML, heuristic searches by axis additions and nearest-neighbor interchange (NNI) branch-swapping with 500 bootstrap replications were adjusted. Other conditions for the ML analysis like gamma shape parameter of 0.218 and 4 rate categories were also adjusted. By using the Tamura-Nei model (Tamura & Nei, 1993), the analysis of

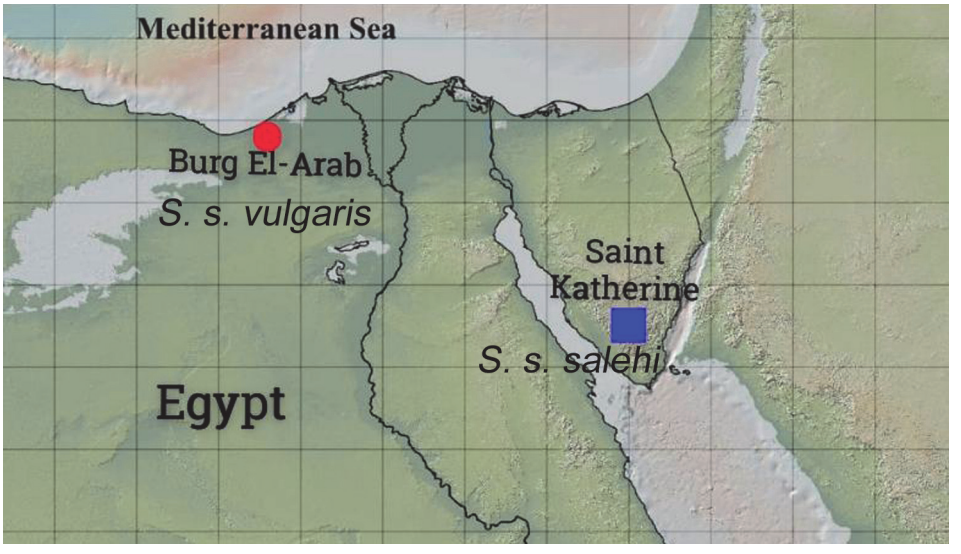


Figure 1. Map of Egypt showing site localities for *Stellagama stellio vulgaris* and *S. s. salehi*.

pairwise distance for 12 nucleotide sequences was conducted with pairwise deletion option for each sequence pair. Evolutionary analyses were done in MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

## Results

**Morphological results.** *Stellagama stellio vulgaris* has a rounded or a depressed body. The head is covered with small scales, lower eyelids are present and movable, nostril is very close to the end of the snout, tongue is broad, fleshy, and not deeply forked. The tail is not prehensile and provided with regular whorls of hard spinose scales, each tail whorl consists of two rows of scales dorsally; digits are not in opposable bundles, the 4$^{th}$ toe is longer than the 3$^{rd}$ one and dorsal scales are enlarged with yellow oval spots. *Stellagama stellio salehi* has the same morphology but is larger with heart-shaped head and strongly built body; the legs are long and slender; the ears are very large and obvious and the scales on the dorsum, head and jaws show orange spots.

Females of both subspecies are slightly larger than males in most characters (Table 1). For tail characters, TL and TR are slightly larger in male *S. s. vulgaris* than in male *S. s. salehi*. TL is slightly larger in female *S. s. salehi* than in female *S. s. vulgaris,* while female *S. s. vulgaris* is slightly larger than female *S. s. salehi* in TR.

In a Principal Component Analysis (PCA; Table 2), PCA1 accounted for a low (87.03 %) percentage of cumulative variation and a strong canonical correlation (r=0.999, eigenvalue=849.26) between both subspecies. PCA2 accounted for a high percentage of cumulative variation (98.04 %) that can be interpreted as a significant difference between both subspecies. A slightly higher canonical correlation (r=0.985) and a low eigenvalue (11.16) between the two subspecies was found for HH, the major parameter for significant difference, and SED, TR, JL, TL/SVL and SVL/HL.

**DNA sequencing results.** Approximately 398 bp of 16S rRNA gene were sequenced for *S. s. salehi* and *S. s. vulgaris*. The sequenced fragment from each subspecies was identical and 6 base substitutions between the two subspecies were recorded. The aligned data were first analysed by MP method. The parsimony analysis showed the

Table 1. Mean and Standard Deviation (SD) of morphometric measurements of *Stellagama s. vulgaris* (5♀, 6♂) and *S. s. salehi* (3♀, 5♂).

| Character | Taxon | Male | Female |
|---|---|---|---|
| Snout-vent length (SVL) | *S. s. vulgaris* | 106.10±14.50 | 106.30±7.80 |
| | *S. s. salehi* | 118.70±10.50 | 127.70±11.70 |
| Maximum head width (MHW) | *S. s. vulgaris* | 24.50±3.71 | 26.80±3.46 |
| | *S. s. salehi* | 27.30±4.02 | 33.60±3.75 |
| Head height (HH) | *S. s. vulgaris* | 12.59±1.32 | 12.95±1.50 |
| | *S. s. salehi* | 15.10±1.47 | 19.70±0.78 |
| Head length (HL) | *S. s. vulgaris* | 26.96±2.31 | 29.7±3.48 |
| | *S. s. salehi* | 31.60±3.04 | 34.50±4.10 |
| Snout-eye distance (SED) | *S. s. vulgaris* | 15.30±2.01 | 15.60±2.34 |
| | *S. s. salehi* | 15.9±1.55 | 17.4±0.58 |
| Head width between eyes ($HW_{eyes}$) | *S. s. vulgaris* | 5.07±0.39 | 5.82±0.80 |
| | *S. s. salehi* | 5.71±1.18 | 7.00±1.30 |
| Tail length (TL) | *S. s. vulgaris* | 131.30±8.39 | 128.50±26.90 |
| | *S. s. salehi* | 122.20±18.70 | 145.80±27.30 |
| Number of tail rows (TR) | *S. s. vulgaris* | 23.30±1.79 | 21.70±3.86 |
| | *S. s. salehi* | 19.80±4.17 | 20.00±6.00 |
| Jaw length (JL) | *S. s. vulgaris* | 30.10±3.27 | 32.90±3.11 |
| | *S. s. salehi* | 32.70±3.76 | 38.70±2.80 |
| Abdominal width (AW) | *S. s. vulgaris* | 29.14±2.33 | 29.90±3.51 |
| | *S. s. salehi* | 35.10±8.57 | 37.20±7.78 |
| TL/SVL | *S. s. vulgaris* | 1.17±0.22 | 1.26±0.13 |
| | *S. s. salehi* | 1.19±0.23 | 1.03±0.13 |
| SVL/HL | *S. s. vulgaris* | 3.60±0.16 | 3.95±0.17 |
| | *S. s. salehi* | 3.82±0.14 | 3.80±0.17 |
| Head index (HI) | *S. s. vulgaris* | 27.8±1.20 | 25.20±1.40 |
| | *S. s. salehi* | 26.00±0.67 | 26.40±1.30 |
| Head length index (HLI) | *S. s. vulgaris* | 522.33±37.33 | 546.78±68.91 |
| | *S. s. salehi* | 517.95±35.26 | 565.49±52.75 |

Table 2. Results of the PCA with the loading values for the two principal components.

| Character | PCA1 | PCA2 |
|---|---|---|
| Snout-vent length (SVL) | 0.999 | - |
| Maximum head width (MHW) | 0.964 | - |
| Head height (HH) | 0.692 | 0.722 |
| Head length (HL) | 0.995 | - |
| Snout-eye distance (SED) | 0.873 | 0.376 |
| Head width between eyes ($HW_{eyes}$) | 0.998 | - |
| Tail length (TL) | 0.993 | - |
| Number of tail rows (TR) | 0.949 | -0.307 |
| Jaw length (JL) | 0.931 | -0.362 |
| Abdominal width (AW) | 0.977 | - |
| Tail length/Snout-vent length | 0.730 | -0.605 |
| Snout-vent length / Head length | 0.933 | -0.359 |
| Head index (HI) | 0.966 | - |
| Head length index (HLI) | 0.992 | - |
| Eigenvalue | 849.260 | 11.162 |
| Cumulative % | 87.034 | 98.04 |
| Canonical correlation | 0.999 | 0.985 |
| Wilks' Lambda | 0.000 | 0.016 |

Table 3. Genetic distance matrix (%) between sequences of selected taxa of *Stellagama*, *Laudakia*, *Paralaudakia*, and *Trapelus* as calculated by Tamura-Nei model. Details of taxa and their accession numbers are given in Figure 2.

| No. | Taxon name | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *S. s. vulgaris* | **-** | | | | | | | | | | |
| 2 | *S. s. salehi* | **1.54** | - | | | | | | | | | |
| 3 | *S. s. daani* | 1.80 | 2.85 | - | | | | | | | | |
| 4 | *S. s. brachydactyla* | 1.54 | **0.00** | 2.85 | - | | | | | | | |
| 5 | *L. sacra* | 14.83 | 15.11 | 14.83 | 15.11 | - | | | | | | |
| 6 | *L. nupta* | 16.74 | 17.05 | 16.37 | 17.05 | 12.77 | - | | | | | |
| 7 | *P. caucasia* [AY053765] | 14.33 | 13.94 | 14.63 | 13.94 | 12.36 | 14.49 | - | | | | |
| 8 | *P. caucasia* [HQ901098] | 13.16 | 13.42 | 13.44 | 13.42 | 11.75 | 14.47 | 3.61 | - | | | |
| 9 | *P. himalayana* | 14.83 | 15.11 | 14.83 | 15.11 | 0.25 | 12.44 | 12.36 | 11.75 | - | | |
| 10 | *P. microlepis* | 12.81 | 13.74 | 13.09 | 13.74 | 12.70 | 14.46 | 6.89 | 4.69 | 12.70 | - | |
| 11 | *T. mutabilis* | 15.48 | 15.76 | 15.45 | 15.76 | 12.78 | 17.47 | 13.86 | 13.91 | 12.45 | 14.87 | - |
| 12 | *T. agilis* | 14.26 | 14.23 | 14.26 | 14.23 | 13.22 | 17.86 | 14.45 | 13.90 | 12.87 | 14.87 | 3.88 |

character-status summary of the aligned 398 bp with 289 constant positions, 28 parsimony uninformative sites and 81 informative sites. The specified substitution rate matrix of the data was as follows: R(a)= 4.74, R(b)= 8.37, R(c)=4.62, R(d)= 0.27, R(e)= 19.06 and R(f)= 1.00. A parsimony tree with a length of 183 has the following criteria: consistency index (CI) = 0.759, CI excluding uninformative sites = 0.714, homology index (HI) = 0.240, HI excluding uninformative sites = 0.286, retention index (RI) = 0.800 and rescaled consistency index (RC) = 0.607. The data were also analysed by NJ and ML (Figure 2) and the resulting NJ tree strongly supported the monophyly of *Stellagama* (100% bootstrapping for MP, NJ, and ML). The subclade containing the two Egyptian subspecies was supported by reasonable bootstrap probabilities for MP and NJ (BP= 70, 54) without resolution for ML. *Stellagama s. salehi* and *S. s. brachydactyla* exhibited a strong sister relationship (bootstrap= 100%).

The pairwise genetic distance between various *Stellagama* taxa was lowest (1.54%) between *S. s. vulgaris* and *S. s. salehi* (Table 3). *Stellagama s. vulgaris* from Burg El-Arab is differentiated from Turkish *S. s. stellio* by 1.8% while *S. s. salehi* inhabiting Sinai is differentiated from Turkish *S. s. stellio* by 2.85%. *Stellagama s. salehi* and *S. s. brachydactyla* showed zero divergence.

## Discussion

The PCA analysis of the 14 morphometric characters studied could not show significant differences between *S. s. vulgaris* and *S. s. salehi* as high correlation coefficients were found except of HH which was distributed between PCA1 and PCA2 with a reasonable percentage of cumulative variation and a low eigenvalue. In the genetic analysis, *S. s. vulgaris* proved to be sister to *S. s. salehi* being located in the same subclade. As shown in Table 3, the divergence between species belonging to different genera (*Paralaudakia himalayana* and *L. sacra*; D matrix = 0.25) and that was found between the congeneric agamids (*Trapelus mutabilis* and *Trapelus pallidus*; D matrix =1.4%) (Wagner et al., 2011) are comparable to that has been found between *S. s. vulgaris* and *S. s. salehi* (D matrix =1.54%).

No genetic distance was found between *Stellagama s. salehi* and *S. s. brachydactyla,* and they appear to be synonymous. Both subspecies are found sympatrically in Sinai (Spaneli & Lymberakis, 2014). Lachman et al. (2006) discriminated *S. s. salehi* from *S. s. brachydactyla* according to morphological features; however, Panov and Zykova (2016) did not find characters which allowed distinguishing *S. s. salehi* from *S. s. brachydactyla.*

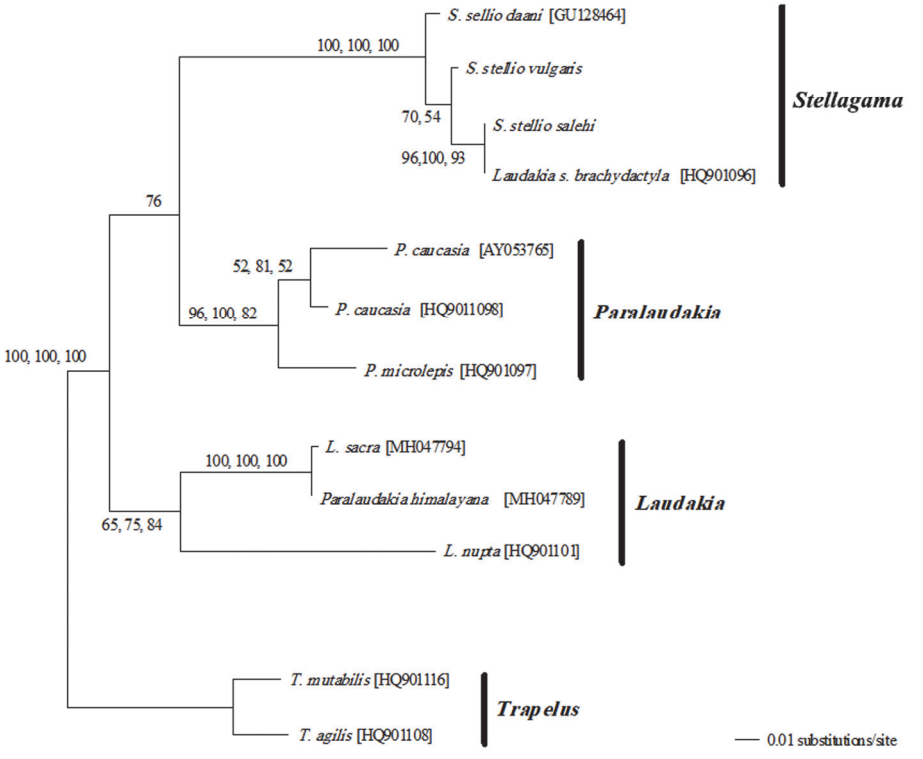


Figure 2. NJ phylogeny of *Stellagama* and other related agamas by using 398 bp of the 16S rRNA gene. Values at nodes represent bootstrap probabilities for MP, NJ and ML methods, respectively when they are above 50%.

*Stellagama stellio salehi* was described by Lachman et al. (2006) based on morphometric characters only (Panov & Zykova, 2016). The morphometric measurements of the Egyptian *S. stellio vulgaris* and *S. stellio salehi* are comparable with that of Turkish *S. stellio daani* (Kumlutaş et al., 2015). Almog et al. (2005) identified the populations of *Laudakia stellio* inhabiting Turkey as *S stellio daani* while Özdemir et al. (2011) found two molecular lineages of Turkish *S. stellio daani*.

In conclusion, among the 14 morphometric parameters, HH was the only character distinguishing *S. s. vulgaris* and *S. s. salehi* and the results of the 16S rRNA weakly support their subspecific status. Sequencing of more genes and more samples are therefore necessary to clarify the subspecific situation of the Egyptian *S. stellio*.

**Disclosure Statement**

No potential conflict of interest was reported by the authors.

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