

Morphometric versus genomic evidence. The systematic status of *Eryx miliaris* (PALLAS, 1773), from Sistan, East Iran (Squamata: Serpentes: Boidae)

Der taxonomische Status von *Eryx miliaris* (PALLAS, 1773) von Sistan, Ostiran
Morphometrische und genetische Hinweise im Vergleich.
(Squamata: Serpentes: Boidae)

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KURZFASSUNG

Exemplare von *Eryx miliaris* (PALLAS, 1773) aus Sistan, Ost-Iran, wurden morphologisch und genetisch untersucht. Dabei zeigte sich eine hohe Variabilität in Anordnung und Zahl bei Kopfschilden, während die DNS-Sequenzen des mitochondrialen Cytochrom *b* wenig unterschiedlich waren. Der Vergleich der Sequenzen mit denen von Nordost-Iranischen *E. tataricus* (LICHTENSTEIN, 1823) aus GenBank spricht für die artliche Identität von *E. tataricus* (LICHTENSTEIN, 1823) mit *E. miliaris* und in der Folge die Bewertung von *E. tataricus* als subjektives Juniorsynonym von *E. miliaris*. Die Autoren sprechen sich dagegen aus, morphometrische Merkmale als alleinige Informationsquelle für systematische Zuordnungen zu verwenden.

ABSTRACT

Specimens of *Eryx miliaris* (PALLAS, 1773) from Sistan, East Iran, were investigated morphologically and genetically. The results demonstrated a high variation in topology and number of head scales, while mtDNA Cytochrome *b* sequences showed little differences. Comparison with sequences from NE-Iranian *E. tataricus* (LICHTENSTEIN, 1823) taken from GenBank provides support for the identity at species level of *E. tataricus* (LICHTENSTEIN, 1823) with *E. miliaris*. As a consequence, *E. tataricus* is identified a subjective junior synonym of *E. miliaris*. Moreover, the authors strongly suggest not to use morphometric characters as the only source of information for systematic decisions.

KEY WORDS

Reptilia: Squamata: Serpentes: Boidae; *Eryx miliaris*, *Eryx tataricus*, systematics, taxonomy, cytochrome *b*, molecular genetics, morphology, pholidosis, intraspecific plasticity, Sistan, Iran

INTRODUCTION

In parallel with the scientific advances based on comparative genomics, morphological data are still being widely used to determine the systematic position of many taxa. Conflicts between molecular and morphological studies can arise from morphological characters which show homoplastic evolution. In such cases, morphological data may mislead phylogenetic and systematic interpretations (HEDGES & MAXSON 1996; WIENS et al. 2003).

The main distribution of the genus *Eryx* DAUDIN, 1803, includes a wide range from southeastern Europe, North and East Africa, over southwestern Asia as far as

western China in the East (LANZA & NISTRI 2005). According to the literature, the genus *Eryx* is represented by six species in Iran: *E. jaculus* (LINNAEUS, 1758), *E. miliaris* (PALLAS, 1773), *E. johnii* (RUSSELL, 1801), *E. tataricus* (LICHTENSTEIN, 1823), *E. elegans* (GRAY, 1849) and *E. jayakari* BOULENGER, 1888 (e.g., LATIFI 2000; RASTEGAR-POUYANI et al. 2008; SAFAEI-MAHROO et al. 2015).

However, there is disagreement on the correct identification of some specimens or systematic status of some nominal taxa of the genus. Morphological and molecular studies of *Eryx* specimens in NE Iran independently, but in confirmation of each other

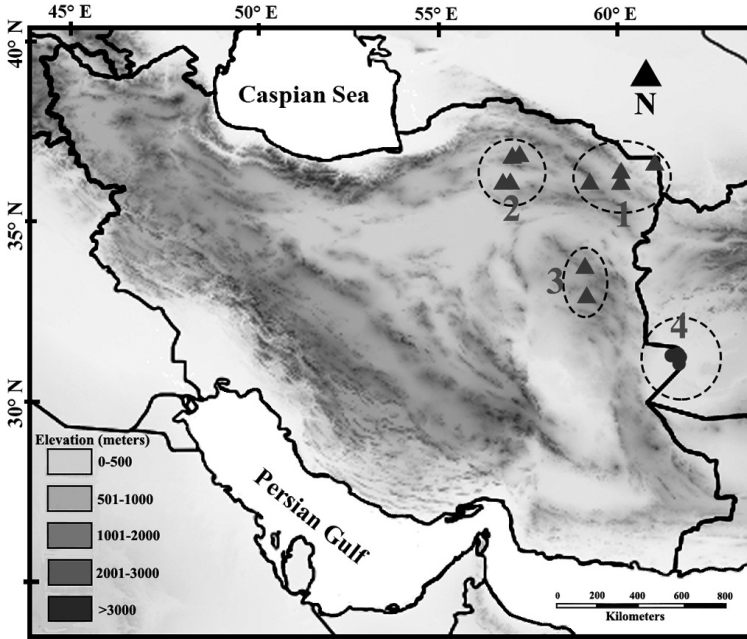


Fig. 1: Sampling areas of sand boas morphologically classified as *Eryx miliaris* (PALLAS, 1773) and *E. tataricus* (LICHTENSTEIN, 1823) included in this study. 1 – Fariman, Sarakhs and Torbate-Jam (Razavi Khorasan Province), 2 – Sabzevar, Neyshabour, Parvand and Joghtay (Razavi Khorasan Province), 3 – Birjand and Shaskooh (South Khorasan Province), 4 – Various locations in Sistan (Sistan & Blochestan Province). Details are presented in Tables 1 and 2.

Abb. 1: Fundgebiete von Sandboas, die morphologisch *Eryx miliaris* (PALLAS, 1773) und *E. tataricus* (LICHTENSTEIN, 1823) zugeordnet waren und in der vorliegenden Arbeit untersucht wurden. 1 – Fariman, Sarakhs und Torbate-Jam (Provinz Razavi Khorasan), 2 – Sabzevar, Neyshabour, Parvand und Joghtay (Provinz Razavi Khorasan), 3 – Birjand und Shaskooh (Provinz Süd-Khorasan), 4 – verschiedene Orte in Sistan (Provinz Sistan & Belutschistan). Zu Einzelheiten siehe Tabellen 1 und 2.

(ESKANDARZADEH et al. 2013; RASTEGAR-POUYANI et al. 2014), suggested that snakes previously assigned to *E. elegans* are conspecific with *E. jaculus*, and populations earlier identified as *E. tataricus* cannot be differentiated from *E. miliaris*. These findings are in disagreement with several studies (e.g., TERENT'EV & CHERNOV 1965; SØRENSEN 1988; LATIFI 2000; REYNOLDS et al. 2014; ZARRINTAB et al. 2017). A recent review of the genus *Eryx* in Iran exclusively based on external morphological traits (ZARRINTAB et al. 2017) was controversial, at least as to the *E. tataricus/miliaris* complex (sens. TOKAR 1990). According to this study, several Iranian specimens were assigned to *E. tataricus*, whereas the occurrence of *E. miliaris* was classified as ques-

tionable. The systematic status of *E. tataricus* and *E. miliaris* was already debated in preceding molecular studies, which considered the relationship between *E. tataricus* and *E. miliaris* to be on a subspecific level (PYRON et al. 2013), while the nominal subspecies *E. tataricus vittatus* (ČERNOV, 1959), was found to be more distant from these (REYNOLDS et al. 2014).

Since *E. miliaris* was reported from Sistan (LATIFI 2000), the purpose of the present study was to (i) add information on the systematic status and phylogenetic affinities of *E. miliaris* from Sistan using mtDNA Cytochrome *b* sequences and (ii) describe the degree of intraspecific variation at the external morphology level in these specimens.

MATERIALS AND METHODS

Morphological analyses.- Material for the morphological examinations included 22 alcohol-preserved specimens of *E. miliaris* from Sistan, Eastern Iran (Fig. 1). This sample belongs to the herpetological collection of Shahrekord University, Shahrekord, Iran. Details of the localities, museum and GenBank accession numbers of sequences are given in Tables 1 and 2. Specimens were identified morphologically based on TZAREWSKY (1916), TERENT'EV & CHERNOV (1965) and TOKAR (1991). The following morphological characters were measured using Vernier calipers with an accuracy to the nearest 0.01 mm: snout-vent length (SVL) from tip of snout to vent; tail length (TL) from vent to tip of tail; interorbital width (IOW); head width (HW) at the widest point; and distance between posterior edge of eye and corner of mouth (DEM). In addition to the metric variables, the following meristic variables (pholidosis counts) were determined using a stereomicroscope: numbers of loreal scales right and left (LS, R/L), supralabial scales right and left (SL, R/L), infralabial scales right and left (IL, R/L), longitudinal dorsal scale rows around the middle of the body (SM), ventral scales (VEN), subcaudal scales (SCD), pre-ventral scales (PVEN), scales between eye and nasal (BEN), scales posterior to internasal as far as to the eye (PIN), scales between eyes counted along a straight line (BES), scales around eye right and left (SAE, R/L), longitudinal dorsal scales rows, counted right behind the vent (DSV).

Molecular analyses.- Twelve specimens were selected for molecular analysis (Fig. 1, Table 1). Selection was based on the intention to include at least one individual from each discovered morph in the molecular and morphological analyses. Genomic DNA was extracted from ethanol-preserved tissue samples using the Tissue DNA Extraction kit (Aron-Gene, Isfahan, Iran). The mitochondrial cytochrome *b* (*cyt-b*) gene was amplified using the primers L14724 (5'-TGA CTTGAAGAAC-CACCGTTG-3') and H16064 (5'-CTTTG-GTTTACAAGAACAATGCTTTA-3') (BURBRINK et al. 2000). Polymerase chain reaction (PCR) amplifications were carried

out in a total volume of 20 μ l, containing 10 μ l ready to use Prime Taq premix (Ampliqon, Cat Number: A180301), 1 μ l of genomic template DNA (approximately 100 ng), 1 μ l of each primer (10 pm/ml) and 7 μ l distilled deionized water with the following conditions: an initial cycle at 94 °C for 4 minutes, followed by 36 cycles at 94 °C for 30 seconds, 58 °C for 40 seconds and 72 °C for 90 seconds, and a final cycle at 72 °C for 10 minutes. Sequences were derived from these products in an ABI 3700 automated sequencer at Macrogen, Inc. (Seoul, Korea).

Aligning and editing the chromatographs was done manually using BioEdit v. 7.0.5 (HALL 1999). To avoid amplification of pseudogenes, sequences were translated into amino-acids and checked for stop-codons. The *cyt-b* fragments were aligned using MAFFT v. 7 (KATO & STANDLEY 2013) applying default parameters (auto strategy, gap opening penalty: 1.53, offset value: 0.0). Uncorrected *p*-distances with complete deletion of positions with ambiguities and alignment gaps were calculated using Mega7 v. 7.0 (KUMAR et al. 2016). Additional sequences representing individuals of *E. miliaris* and *E. tataricus* were downloaded from GenBank and added to the dataset (Table 2). *Eryx elegans* (KJ841063 and KJ841061) were included in the analysis as an outgroup taxon, since it was revealed as sister taxon of the *E. miliaris*/*E. tataricus* complex (RASTEGAR-POUYANI et al. 2014).

The authors used maximum likelihood (ML) and Bayesian inference (BI) to derive phylogenetic relationships from a dataset containing 666 bp including 38 specimens (2 outgroup and 36 in-group taxa). The best-fitting model of nucleotide substitution was inferred using JModeltest v. 2.1.4 (DARRIBA et al. 2012) according to the Akaike Information Criterion (AKAIKE, 1973). Maximum likelihood analyses were performed in PAUP* v. 4.0 (SWOFFORD 2002) with reliability assessed using nonparametric bootstrapping with 1,000 replicates. Bayesian inference analyses were conducted in MrBayes v. 3.2.2 (RONQUIST et al. 2012), with two independent runs of eight million generations with four Markov

Table 1: List of *Eryx* specimens from Sistan, Iran, used in the present morphological ($N = 22$) and molecular ($N = 12$) studies, along with locality data, museum and GenBank accession numbers. Morphological species identification according to TZAREWSKY (1916), TERENT'EV & CHERNOV (1965) and TOKAR (1991). S & B – Sistan & Blochestan Province, HAC – Herpetological Collection in the Zoological Museum of the Shahrekord University.

Tab. 1: Liste der in den vorliegenden morphologischen ($N = 22$) und molekularen ($N = 12$) Untersuchungen verwendeten *Eryx* Exemplare aus Sistan, Iran, mit ihren Fundorten, Museums- und GenBank Zugangsnummern. Die morphologische Artbestimmung erfolgte nach TZAREWSKY (1916), TERENT'EV & CHERNOV (1965) und TOKAR (1991). S & B – Provinz Sistan & Belutschistan, HAC – Herpetologische Sammlung im Zoologischen Museum der Shahrekord Universität.

Sub-clade / Haplotype Subklade / Haplotype	Species Art	Name in dendro- gram (Fig. 4) Name im Dendro- gramm (Abb. 4)	Locality Fundort	Geographical coordinates Geographische Koordinaten	GenBank numbers GenBank- Nummern
--- / ---		HAC 46	near Milak, S & B	30.969916 N 61.796252 E	---
--- / ---		HAC 47	near Milak, S & B	30.969916 N 61.796252 E	---
Sistan / H1		HAC 48	near Milak, S & B	30.969916 N 61.796252 E	MG948422
--- / ---		HAC 65	near Milak, S & B	30.969585 N 61.805404 E	---
--- / ---		HAC 78	near Milak, S & B	30.960402 N 61.805639 E	---
Sistan / H2		HAC 89	Between Milak and Shah- Jaane Bameri, S & B	30.967967 N 61.797808 E	MG948423
--- / ---		HAC 139	Hirmand, near the Maleki Village, S & B	31.092306 N 61.708559 E	---
--- / ---		HAC 140	Hirmand, near Jahan- Tigh Village, S & B	31.083543 N 61.677341 E	---
Sistan / H6		HAC 150	near Milak, S & B	30.976328 N 61.800443 E	MG948432
Sistan / H3		HAC 169	near Zahak, S & B	30.899991 N 61.671809 E	MG948424
--- / ---		HAC 498	near Deh-No Village, S & B	30.978897 N 61.777375 E	---
Sistan / H3		HAC 499	Between Milak and Shah-Jaane Bameri, S & B	30.964107 N 61.800448 E	MG948425
--- / ---		HAC 500	near Milak, S & B	30.973522 N 61.806304 E	---
Sistan / H4		HAC 501	Zahak, near Hasanki Village, S & B	30.924783 N 61.691794 E	MG948426
Sistan / H1		HAC 502	near Milak, S & B	30.980502 N 61.803298 E	MG948427
Sistan / H5		HAC 503	Zahak, near the Khajeh- Ahmad Village, S & B	30.861207 N 61.734136 E	MG948428
Sistan / H1		HAC 504	Hirmand, near the Sanjar Viillage, S & B	30.984633 N 61.815386 E	MG948429
Sistan / H7		HAC 505	near Milak, S & B	30.977388 N 61.797564 E	MG948433
--- / ---		HAC 506	near Milak, S & B	30.978897 N 61.777375 E	---
Sistan / H1		HAC 507	near Milak, S & B	30.987029 N 61.794056 E	MG948430
Sistan / H4		HAC 508	near Milak, S & B	30.978897 N 61.777375 E	MG948431
--- / ---		HAC 548	near Milak, S & B	30.978615 N 61.798255 E	---

chains (one cold, three heated), sampling every 1,000 generations. The first 25 % of trees in each run were discarded as burn-in, and a majority-rule consensus tree was gen-

erated from remaining trees. A network of haplotypes was generated using the median-joining algorithm (BANDELTT et al. 1999) in Network 5.0.0.0 (FORSTER 2018).

Table 2: Information on additional specimens of *Eryx miliaris* (PALLAS, 1773) and *E. tataricus* (LICHTENSTEIN, 1823) from NE Iran, the GenBank sequences of which were used in the phylogenetic analyses. RKP – Razavi Khorasan Province, SKP – South Khorasan Province.

Tab. 2: Angaben zu weiteren Exemplaren von *Eryx miliaris* (PALLAS, 1773) und *E. tataricus* (LICHTENSTEIN, 1823) aus dem Nordwestiran, deren GenBank-Sequenzen in der phylogenetischen Analyse verwendet wurden. RKP – Provinz Razavi Khorasan, SKP – Provinz Süd-Khorasan.

Sub-clade/ haplotype Subklade/ Haplotyp	Species Art	Name in dendro- gram (Fig. 4) Name im Dendro- gramm (Abb. 4)	Locality Fundort	Geographical coordinates Geographische Koordinaten	GenBank Ac- cession numbers GenBank-Zu- gangsnummern
1 / ---	<i>Eryx tataricus</i>	UMFS 11688	unknown	---	KF811117.1
1 / ---	<i>Eryx miliaris nogajorum</i>	U69826	unknown	---	U69826.1
1 / ---	<i>Eryx miliaris</i>	U69824	unknown	---	U69824.1
1 / H14	<i>Eryx miliaris</i>	ERP_906	Sarakhs, RKP	36°31'N 61°09'E	KJ841071.2
1 / H12	<i>Eryx tataricus</i>	ERP_1051	Torbate-Jam, RKP	35°59'N 60°15'E	KJ841054.2
1 / H13	<i>Eryx tataricus</i>	Far_4	Fariman, RKP	35°72'N 60°11'E	KJ841064.2
1 / H13	<i>Eryx miliaris</i>	Far_5	Fariman, RKP	35°72'N 60°11'E	KJ841065.2
1 / H13	<i>Eryx tataricus</i>	Far_6	Fariman, RKP	35°72'N 60°11'E	KJ841066.2
1 / H13	<i>Eryx tataricus</i>	Far_1	Fariman, RKP	35°72'N 60°11'E	KJ841070.2
2 / H8	<i>Eryx tataricus</i>	ERP_792	Sabzevar, RKP	35°56'N 57°05'E	KJ841043.2
2 / H10	<i>Eryx tataricus</i>	ERP_875	Parvand, RKP	36°42'N 57°17'E	KJ841045.2
2 / H8	<i>Eryx miliaris</i>	ERP_1018	Sabzevar, RKP	35°56'N 57°05'E	KJ841053.2
2 / H11	<i>Eryx tataricus</i>	ERP_929	Joghtay, RKP	36°38'N 57°29'E	KJ841049.2
2 / H11	<i>Eryx miliaris</i>	ERP_976	Neyshabour, RKP	35°57'N 59°22'E	KJ841050.2
2 / H11	<i>Eryx miliaris</i>	ERP_977	Neyshabour, RKP	35°57'N 59°22'E	KJ841051.2
2 / H11	<i>Eryx miliaris</i>	Gha_10	Ghaen, SKP	33°43'N 59°11'E	KJ841069.2
2 / H9	<i>Eryx miliaris</i>	ERP_806	Sabzevar, RKP	36°06'N 57°12'E	KJ841044.2
2 / H9	<i>Eryx miliaris</i>	ERP_925	Joghtay, RKP	36°42'N 57°17'E	KJ841046.2
2 / H9	<i>Eryx miliaris</i>	ERP_926	Joghtay, RKP	36°42'N 57°17'E	KJ841047.2
2 / H9	<i>Eryx miliaris</i>	ERP_928	Joghtay, RKP	36°42'N 57°17'E	KJ841048.2
2 / H9	<i>Eryx tataricus</i>	ERP_1009	Sabzevar, RKP	36°42'N 57°17'E	KJ841052.2
3 / H14	<i>Eryx tataricus</i>	Sha_30	Shaskoh, SKP	33°43'N 59°11'E	KJ841067.2
3 / H14	<i>Eryx tataricus</i>	Bir_22	Birjand, SKP	32°52'N 59°14'E	KJ841068.2
Outgroup	<i>Eryx elegans</i>	E_elegans13	Ghaen, SKP	33°43'N 59°11'E	KJ841063.2
Outgroup	<i>Eryx elegans</i>	E_elegans12	Ghaen, SKP	33°43'N 59°11'E	KJ841061.2

RESULTS

Based on the morphological results, the individuals of *E. miliaris* differed from each other in the pholidosis character states of SM, VEN, SCD, PVEN and DSV (Table 3). Sexual dimorphism was not detected in the metric and meristic character states ($p \leq 0.05$).

In addition to the above differences in scale number, there was polymorphism in the topology of the head scales. Regarding the arrangement of subocular and supralabial scales, four types were distinguished (Fig. 2). Some specimens (~ 14 %) were characterized by separation of the subocular scales from the supralabial scales by infrasuboculars. In the remaining specimens, one subocular was in contact with supralabials in a variety of ways as follows: one subocular in

contact with (i) 4th and 5th supralabials (~ 27 %), (ii) 5th supralabial (~ 18 %) and (iii) 5th and 6th supralabials (~ 41%). Also, there was intraspecific variation in the topology of the internasal and postinternasal scales (Fig. 3). In two individuals (~ 9 %), the internasals were in full contact with three postinternasal scales present. In most individuals (~ 73 %), the internasal scales were in contact posteriorly, as the rostral scale extended between the internasals. In these specimens, there were three postinternasal scales. In four specimens (~ 18 %), the internasal scales were separated completely by the rostral scale with four postinternasals present.

New sequences obtained during this study were deposited in GenBank with ac-

cession numbers MG948422 – MG948433 (Table 1). The final alignment of 666 nucleotides from 36 individuals consisted of 554 invariable (monomorphic) and 112 variable (polymorphic) sites, 92 of which were parsimony informative. The selected TAMURA & NEI (1993) model of nucleotide substitution was TrN+I as the best fit model under the Akaike Information criterion. The phylogenetic results were nearly identical between ML and BI approaches. In both cases (Fig. 4), a well-supported clade was identified (BI 1; ML 100 %) consisting of specimens of *E. miliaris* and *E. tataricus* from NE Iran, *E. miliaris nogajorum* NIKOLSKY, 1910, and *E. tataricus* from unspecified localities from outside of Iran, along with specimens from Sistan. The clear separation between *Eryx jaculus* and the clade comprising *E. miliaris/E. tataricus* was supported by more than 10 % interspe-

cific genetic divergence (Table 4). Even though, the Sistan specimens and other populations clustered separately, their relationships remained confusing (Fig. 4). Genetic *p*-distances ranged from 2.1 to 3.3 % between the various subclades of *E. miliaris/E. tataricus* and the Sistan population (Table 4). The network of haplotypes (Fig. 5) indicated that Sistan specimens except one (H5: HAC 503), were genetically separated from all other individuals. Although the maximum within-group genetic divergence in the Sistan specimens group was 0.7 %, this subclade was formed by seven different haplotypes, which showed high haplotype diversity (Hd: 0.879). Haplotype networks of this group were star-shaped, with one most common haplotype (H1) shared mostly among individuals distributed in the Sistan basin (Fig. 5).

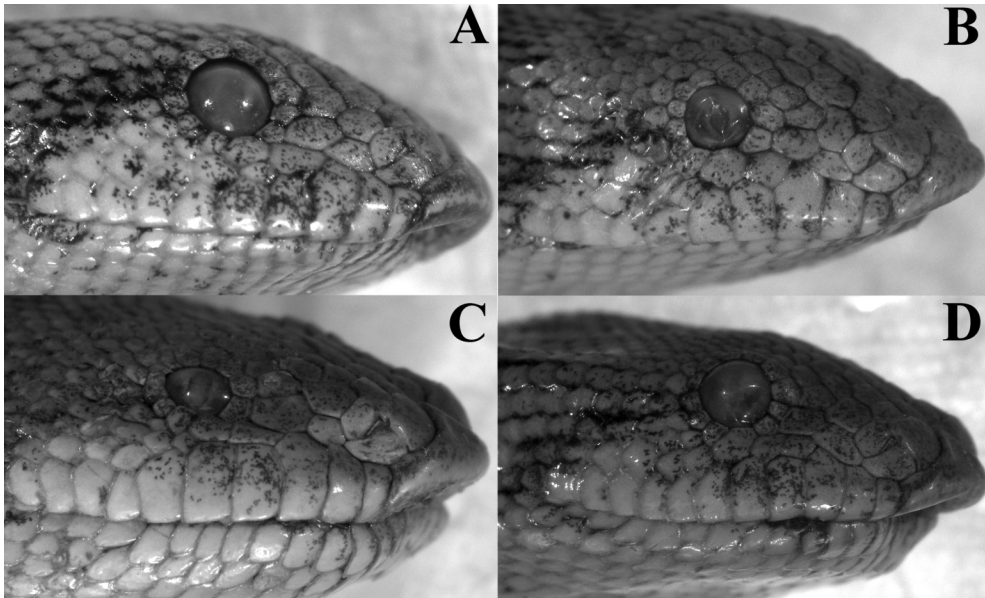


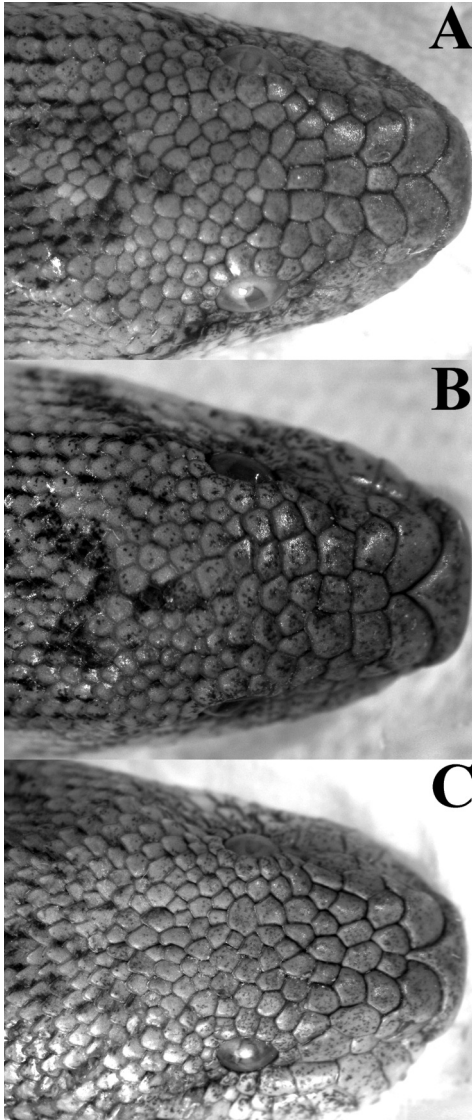
Fig. 2: Variability of subocular and supralabial scale contact in *Eryx miliaris* (PALLAS, 1773), from Sistan, Iran.

- A – Subocular scales separated from supralabials (HAC 502),
- B – one subocular in contact with 4th and 5th supralabials (HAC 504),
- C – one subocular in contact with 5th supralabial (HAC 507), and
- D – one subocular in contact with 5th and 6th supralabials (HAC 499).

Abb. 2: Variabilität in der Anordnung der Subokular- und Supralabialschilde bei *Eryx miliaris* (PALLAS, 1773) aus Sistan, Iran. A – Subokular- und Supralabialschilde voneinander getrennt (HAC 502),

- B – ein Subokularschild grenzt an Supralabialia 4 und 5 (HAC 504),
- C – ein Subokularschild grenzt an Supralabiale 5 (HAC 507),
- D – ein Subokularschild grenzt an Supralabialia 5 und 6 (HAC 499).

DISCUSSION



The present results revealed a striking intraspecific plasticity in taxonomically important traits of genetically closely related specimens of *Eryx miliaris* from Sistan (Figs. 2 and 3). Although, some variation in the constellation of subocular and supralabial scales was reported in the population of *E. jaculus* from Ahvaz (ZARRINTAB et al. 2017) such variability was not covered in *E. miliaris/E. tataricus* (see ESKANDARZADEH et al. 2013; ZARRINTAB et al. 2017). The shape of the internasal scales as a diagnostic character has been used to distinguish between *E. miliaris* and *E. tataricus* (internasal scales usually not in contact with each other in *E. miliaris* while in contact in *E. tataricus*) (TZAREWSKY 1916; SØRENSEN 1988; ZARRINTAB et al. 2017). In the present study, the clear morphological differences of systematic relevance in a classical view (Figs. 2 and 3), were not mirrored by the very low diversity for cyt-*b* sequences in 12 specimens (Fig. 4, Table 4). In the phylogenetical dendrogram, two specimen morphologically attributed to *E. miliaris nogaorum* and one of *E. tataricus* (Table 2), all from unspecified localities, clustered with samples representing *E. miliaris/E. tataricus* (Figs. 1 and 4). The genetic distance between geographically related subclades of *E. miliaris/E. tataricus* (Table 4, Figs. 1, 4 and 5) is less than the threshold for setting species boundaries (e.g., AHMADZADEH et al. 2013; JOHNS & AVISE 1998; RASTEGAR-POUYANI et al. 2012). Taxonomically, the results of the present study concur with previous proposals to synonymize *E. tataricus* with *E. miliaris* (ESKANDARZADEH et al. 2013; RASTEGAR-POUYANI et al. 2014) with emphasis, that intraspecific variation in head scalation is a fundamental feature of *Eryx miliaris*, at least in the Sistan Basin. As a

Fig. 3: Variation in internasal scales topology relative to the rostral shield and number of postinternasal scales in *Eryx miliaris* (PALLAS, 1773), from Sistan, Iran. A – three postinternasal scales, internasal scales in full contact with each other (HAC 68), B – three postinternasal scales, internasal scales in partial contact with each other, with the rostral scale partly extending between them (HAC 503), and C – four postinternasal scales, internasal scales separated completely by rostral scale (HAC 507).

Abb. 3: Variabilität in der Anordnung der Internasalia in Bezug zum Rostrale und der Anzahl der Postinternasalia bei *Eryx miliaris* (PALLAS, 1773) aus Sistan, Iran. A – drei Postinternasalia, Internasalia berühren einander vollständig (HAC 68), B – drei Postinternasalia, Internasalia berühren einander eingeschränkt, wobei das Rostrale zwischen die Internasalia hineinragt (HAC 503), C – vier Postinternasalia, Internasalia völlig durch das Rostrale voneinander getrennt (HAC 507).

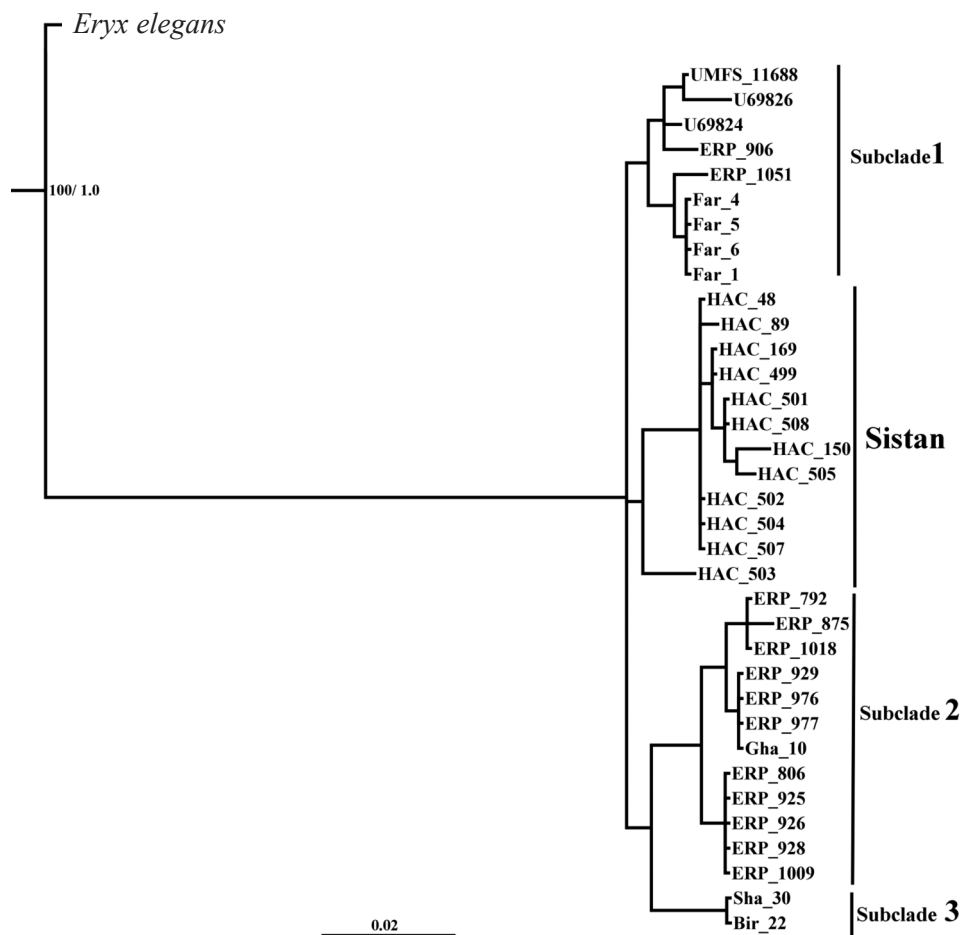


Fig. 4: The Bayesian inference tree of 36 mostly NE Iranian sand boas morphologically classified as *Eryx miliaris* (PALLAS, 1773) and *E. tataricus* (LICHTENSTEIN, 1823) was based on partial sequences (666 nucleotides) of the mitochondrial cytochrome *b* gene. Nodes solely with a posterior probability ≥ 0.90 and bootstrap values ≥ 0.80 are indicated. The tree was rooted using two specimens of *Eryx elegans* (GRAY, 1849).

Information on the specimens is given in Tables 1 and 2.

Abb. 4: Das Baumdiagramm auf Grundlage Bayesscher Inferenz ordnet 36 größtenteils nordostiranische Sandboas, die morphologisch *Eryx miliaris* (PALLAS, 1773) und *E. tataricus* (LICHTENSTEIN, 1823) zugeordnet waren, nach molekularen Ähnlichkeiten. Untersucht wurden Abschnitte des mitochondrialen Cytochrom *b* von 666 Nucleobasen Länge. Nur solche Verzweigungen sind dargestellt, die eine a-posteriori-Wahrscheinlichkeit von ≥ 0.90 und Bootstrapwerte ≥ 0.80 aufweisen. Die Outgroup ist durch zwei Exemplare von *Eryx elegans* (GRAY, 1849) repräsentiert. Weitere Angaben zum Material siehe Tabellen 1 und 2.

consequence, *E. tataricus* is identified a subjective junior synonym of *E. miliaris*.

Finally, despite the wide acceptance of intraspecific variation in traits as an axiomatic idea in biology, still a large percentage of newly introduced species has been described based on a single specimen (LIM

et al. 2012), a practice that is likely to lead to overestimation of diversity and also may confound species identification. The present investigation was constructive in that it combined morphological and molecular data to avoid misidentifications and false conclusions.

Table 3: Counts and measurements of 12 meristic and five metric characters in the 22 studied sand boas from Sistan, East Iran. SVL – snout-vent length, TL – tail length, IOW – interorbital width, HW – head width, numbers of LS – loreal scales right and left, SL – supralabial scales right and left, IL – infralabial scales right and left, SM – longitudinal dorsal scale rows around the middle of the body, VEN – ventral scales, SCD – subcaudal scales, PVEN – pre-ventral scales, BEN – scales between eye and nasal, PIN – scales posterior to internasal as far as to the eye, BES – scales between eyes counted along a straight line, SAE – scales around eye right and left, DSV – longitudinal dorsal scales rows, counted right behind the vent.

Tab. 3: Meß- und Zählwerte von 12 meristischen und fünf metrischen Merkmalen bei den 22 untersuchten Sandboas aus Sistan, Ostiran. SVL – Kopf-Rumpflänge, TL – Schwanzlänge, IOW – Interorbitalabstand, HW – Kopfbreite, Anzahlen von LS – Lorealia links und rechts, SL – Supralabialia links und rechts, IL – Infralabialia links und rechts, SM – Dorsalta-Längsreihen in Körpermitte, VEN – Ventralia, SCD – Subcaudalia, PVEN – Präventralia, BEN – Schuppen zwischen Auge und Nasale, PIN – Schuppen hinter dem Internasale bis zum Auge, BES – Schuppen zwischen den Augen, gezählt entlang einer geraden Linie, SAE – Schuppen um das Auge links und rechts, DSV – Dorsalta-Längsreihen unmittelbar hinter dem After.

Voucher Specimen	Sex	LS R/L	SL R/L	IL R/L	SM	VEN	SCD	PVEN	BEN	PIN	BES	SAE R/L	DSV	SVL	TL	SVL/ TL	IOW	HW
HAC 46	M	2	11/11	15/16	47	186	25	20	3	4	8	13/13	31	453	46	9.84	6.58	12.38
HAC 47	F	2/2	11/11	16/16	42	169	23	21	3	4	8	11/11	27	260	26	10	4.3	8.03
HAC 48	M	2/2	11/11	16/16	40	175	23	19	3	3	7	11/11	27	182	28	6.5	3.86	5.73
HAC 49	M	2/2	11/11	16/16	45	174	29	17	3	4	8	12/12	31	189	20	9.45	3.56	5.71
HAC 65	F	2/2	11/11	16/16	46	179	28	19	3	3	7	12/12	31	288	30	9.6	4.46	8.24
HAC 78	F	2/2	11/11	18/18	40	175	28	17	3	3	8	12/12	29	341	45	7.57	4.7	9.88
HAC 89	M	2/2	11/11	15/15	43	176	28	19	3	3	7	11/11	29	245	30	8.16	4.66	8.16
HAC 139	M	2/2	11/11	19/19	45	178	22	20	3	3	7	12/12	31	266	24	11.08	4.23	9.85
HAC 140	M	2/2	11/11	18/18	47	187	24	19	3	3	7	11/11	29	355	28	12.67	4.72	9.85
HAC 150	M	2/2	11/11	16/16	44	179	31	16	3	3	7	11/11	29	370	42	8.8	5.23	10.2
HAC 169	F	2/2	12/12	18/18	44	179	22	21	3	4	7	13/13	29	350	25	9.1	5.14	9.1
HAC 199	M	2/2	11/11	18/18	43	183	22	19	3	3	7	12/12	19	445	40	11.12	6.1	11.35
HAC 499	M	2/2	11/11	17/15	45	176	31	20	3	4	7	12/11	20	290	42	6.9	4.28	9.21
HAC 500	M	3/3	12/11	19/18	46	188	24	19	3	4	8	13/13	19	340	30	11.33	5.15	9.71
HAC 501	M	2/3	11/11	16/16	41	187	21	20	3	3	7	12/12	20	176	14	12.57	3.9	5.9
HAC 502	F	2/2	10/11	16/16	46	181	28	21	3	4	7	12/12	21	172	20	8.6	3.44	5.51
HAC 503	M	2/2	11/10	16/16	42	179	28	22	3	4	7	11/12	22	163	19	8.57	3.84	5.38
HAC 504	M	2/2	11/11	16/15	50	179	30	20	3	4	7	11/12	20	265	30	8.8	4.05	7.7
HAC 505	M	2/2	12/11	16/16	46	185	17	19	3	3	7	12/12	19	355	25	14.2	5.18	9.91
HAC 506	M	2/2	11/12	16/17	47	173	28	21	3	3	7	12/12	21	370	50	7.4	5.39	10.73
HAC 507	F	2/2	12/12	16/16	45	180	25	19	3	4	7	12/12	19	430	40	10.75	6.35	14.79
HAC 548	M	2/2	12/11	18/18	43	181	21	18	3	3	7	12/11	28	460	40	11.5	6.45	12.71

Table 4: Uncorrected p -distances separating the subclades of the *Eryx miliaris/tataricus* complex and *E. jaculus* as outgroup taxon.

Tab. 4: Unkorrigierte p -Distanzen, durch welche die Subkladen des *Eryx miliaris/tataricus*-Komplexes und das Outgroup-Taxon *E. jaculus* getrennt sind.

	Outgroup	Sistan	Subclade 1	Subclade 2	Subclade 3
Outgroup	---	---	---	---	---
Sistan	0.108	---	---	---	---
Subclade 1	0.106	0.021	---	---	---
Subclade 2	0.110	0.033	0.027	---	---
Subclade 3	0.107	0.031	0.027	0.028	---

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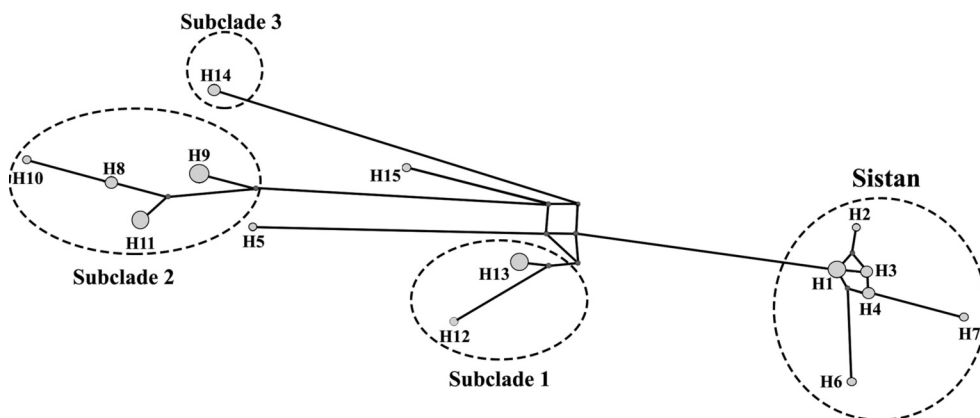


Fig. 5: A Median-joining network connecting the haplotypes of 32 NE Iranian sand boas morphologically classified as *Eryx miliaris* (PALLAS, 1773) and *E. tataricus* (LICHTENSTEIN, 1823).

The length of the branches indicates the numbers of mutational steps joining the haplotypes and the size of each circle is proportional to the number of individuals represented.

For information on the representatives of the haplotypes see Tables 1 and 2.

Abb. 5: Median-joining Netzwerk zur Darstellung der Verbindung zwischen den Haplotypen von 32 Nordost-Iranischen Sandboas, die morphologisch *Eryx miliaris* (PALLAS, 1773) und *E. tataricus* (LICHTENSTEIN, 1823) zugeordnet waren. Die Astlängen geben die Anzahl von Mutationsschritten an, welche die Haplotypen verbinden, deren Kreisgrößen die Zahl der vertretenen Individuen repräsentieren. Angaben zu den Vertretern der Haplotypen finden sich in den Tabellen 1 und 2.

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