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The first record of the genus *Microgecko* Nikolskii, 1907 for Iraq

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Abstract

The genus *Microgecko* Nikolskii, 1907 (Gekkonidae) currently includes eight species distributed from western Iran to north-western India and Pakistan. During field research in Iraq, we found a population of the genus near to the Darbandikhan Lake in the north-eastern part of the country. Because members of the genus are characterized by a higher level of morphological and genetic diversity, we investigated the population using both morphological and molecular approaches. The phylogenetic analyses based on a fragment of the mitochondrial marker cytochrome *b* and morphological characters showed that our investigated population belongs to *M. helenae fasciatus*. This is the first record of the genus and species for Iraq. Moreover, the phylogenetic structure within *M. helenae* shows divergences that suggest the elevation of *M. h. fasciatus* to species level.

Key Words

Gekkonidae, Middle East, mitochondrial DNA, phylogeography, range extension, taxonomy

Introduction

The genus *Microgecko* Nikolskii, 1907 (dwarf geckos) comprises eight known species which are distributed from western Iran to Pakistan and north-western India, with a high species diversity in the Zagros Mountains and Iranian Plateau (Sindaco and Jeremcenko 2008; Agarwal 2009; Gholamifard et al. 2019; Masroor et al. 2020; Torki 2020). The species of *Microgecko* were for a long time ranked under the genus *Tropiocolotes* Peters, 1880 that is distributed mainly in North Africa and Arabia (Sindaco and Jeremcenko 2008). However, Kluge (1983) correctly highlighted the differentiation of *Microgecko* and suggested them as a subgenus of *Tropiocolotes*. The monophyly of *Microgecko* was later supported by genetic analysis of Gamble et al. (2012) and Bauer et al. (2013) that showed it is one of the phylogenetically basal members of the family Gekkonidae. They are small, secretive nocturnal lizards, and relatively little is known of their distribution, molecular phylogeny, taxonomy, and historical biogeography (Bauer et al. 2013; Gholamifard et al. 2019; Masroor et al. 2020). Up to now, they were never reported from Iraq. Therefore, in this paper we bring the first molecular and morphological evidence of the genus *Microgecko* in Iraq.

Material and methods

During field research in Iraq (Kurdistan) conducted from 21 to 31 March 2019, we in a one-day survey investigated the vicinity of the Darbandikhan Lake, Sulaymaniyah Province in the north-eastern part of the country close to the border to Iran (Fig. 1). Here we found two specimens

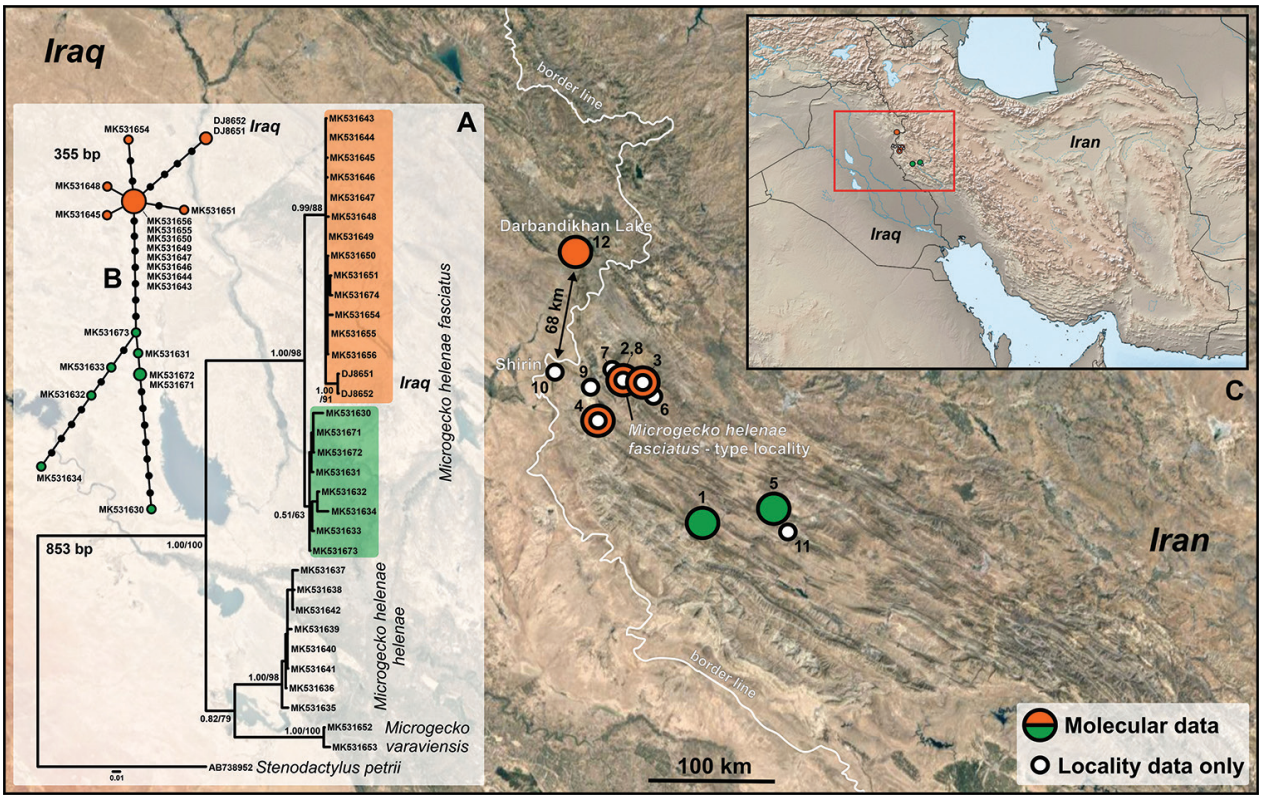

Figure 1. The position of the *Microgecko* population from Iraq in the context of the phylogenetic tree, haplotype network and geography. **A.** Bayesian tree reconstructed from the dataset of mtDNA (cyt *b*) sequences of *Microgecko helenae* complex (Gholamifard et al. 2019). Numbers above the branches show posterior probabilities/bootstrap support values resulting from BA and ML analyses respectively. Each terminal branch represents GenBank accession number; **B.** 95% parsimony haplotype network of *M. helenae fasciatus* clade sequences. Small black circles are missing node haplotypes; each line connecting two haplotypes corresponds to one mutation step; colours corresponding with the tree. The geographic position of localities and their sources are presented in Suppl. material 1: Table S1.

belonging to the genus *Microgecko*. Both specimens were collected, euthanized, preserved in 70% ethanol and deposited in the herpetological collection of the Department of Zoology, Comenius University in Bratislava, Slovakia (voucher numbers DJ8651, DJ8652). Tissue samples were either preserved in 96% ethanol or frozen and stored at -25 °C under the same codes. Blood and part of the muscle taken from the two collected specimens were used as a DNA source. We extracted DNA using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). The cytochrome *b* (cyt *b*) mitochondrial gene fragment was PCR-amplified using primers CytbF700 and CytbR700 and following conditions used by Bauer et al. (2007) and subsequently bi-directionally sequenced. PCR products were purified with ExoSAP-IT PCR Product Cleanup 132 Reagent (USB Europe GmbH, Staufen, Germany). The sequencing was performed by Macrogen Inc. (Amsterdam, The Netherlands; http://www.macrogen.com). For phylogenetic comparison, we combined our newly obtained sequences (GenBank accession numbers MT731967 and MT731968) with those published by Gholamifard et al. (2019). For the final phylogenetic dataset (853 bp), we used 29 sequences of *Microgecko helenae* Nikolskii, 1907 (MK531630–MK531651, MK531654–MK531656, MK531671–MK531674), two sequences of *M. varaviensis* Gholamifard, Rastegar-Pouyani, Rastegar-Pouyani, 2019 (MK531652, MK531653), and one sequence of *Stenodactylus petrii* Anderson, 1896 (AB738952) as outgroup.

DNA sequences were manually checked, aligned, and inspected using BioEdit 7.0.9.0 (Hall 1999). No stop codons were detected when the sequences were translated using the vertebrate mitochondrial genetic code in the program DnaSP 5.10 (Librado and Rozas 2009). The same program was used to calculate inter- and intraspecific uncorrected *p*-distances and the number of haplotypes (*h*), haplotype diversity (*Hd*), and nucleotide diversity (π). The best-fit codon-partitioning schemes and the best-fit substitution models were selected using PartitionFinder v1.1.1 (search algorithm: all, branchlengths = linked; Lanfear et al. 2012), according to the Bayesian information criterion (BIC). Phylogenetic trees were inferred using the Bayesian approach (BA) and maximum likelihood (ML) by MrBayes 3.2 (Ronquist et al. 2012) and RAxML 8.0. (Stamatakis 2014), respectively. The best-fit substitution model with each codon position was as follows: K80+I (1st), HKY+G (2nd), HKY (3rd). For the ML analysis, GTR+G model in each codon position was used. The ML clade support was assessed by 1,000 bootstrap pseudoreplicates. MrBayes analysis was set as

follows: two separate runs with four chains for each run, 10 million generations with samples saved every 100th generation. The convergence of the two runs was confirmed by the convergence diagnostics. The first 20% of trees were discarded as the burn-in after inspection for stationarity of log-likelihood scores of sampled trees in Tracer 1.6 (Rambaut et al. 2013).

We also constructed a mitochondrial haplotype network for in-group (*M. helenae fasciatus*), shortened sequence dataset (355 bp; without "N" positions) using the 95% limit of parsimony (TCS algorithm; Clement et al. 2000) as implemented and visualized in the software PopArt (http://popart.otago.ac.nz).

For the morphological examination, we compared published morphological characters mostly following Gholamifard et al. (2015): snout-vent length (SVL), tail length (TL), head length (HL), head height (HH), head width (HW), eye diameter (ED), the distance from anterior eye margin to the posterior edge of the nostril (NED), the distance from posterior eye margin to ear (EED), interorbital distance (IOD), length of forelimb (LFL), length of hind limb (LHL), distance between forelimb and hind limb (DFH), number of supralabials (SL), number of infralabials (IL), number of scales separating the first pair of postmentals (SSPM), number of scales connected to the first infralabial scale (SCIL), number of scales in a row just below the postmental shields (SBIL), number of interorbital scales (IOS), number of ventral scales from behind the postmentals (GVA), number of dorsal scales in the midline from axilla to groin (AGS). Coloration in life was estimated by examination in the field and using colour digital photographs. The collected specimens were photographed under a Zeiss Axio-Zoom V-16 stereomicroscope using diffuse LED lighting and a Canon 5D Mark IV camera attached. The distribution data on the *Microgecko* from the nearest localities of Iran were taken from Gholamifard et al. (2015, 2019) and were visualized using QGIS (2020).

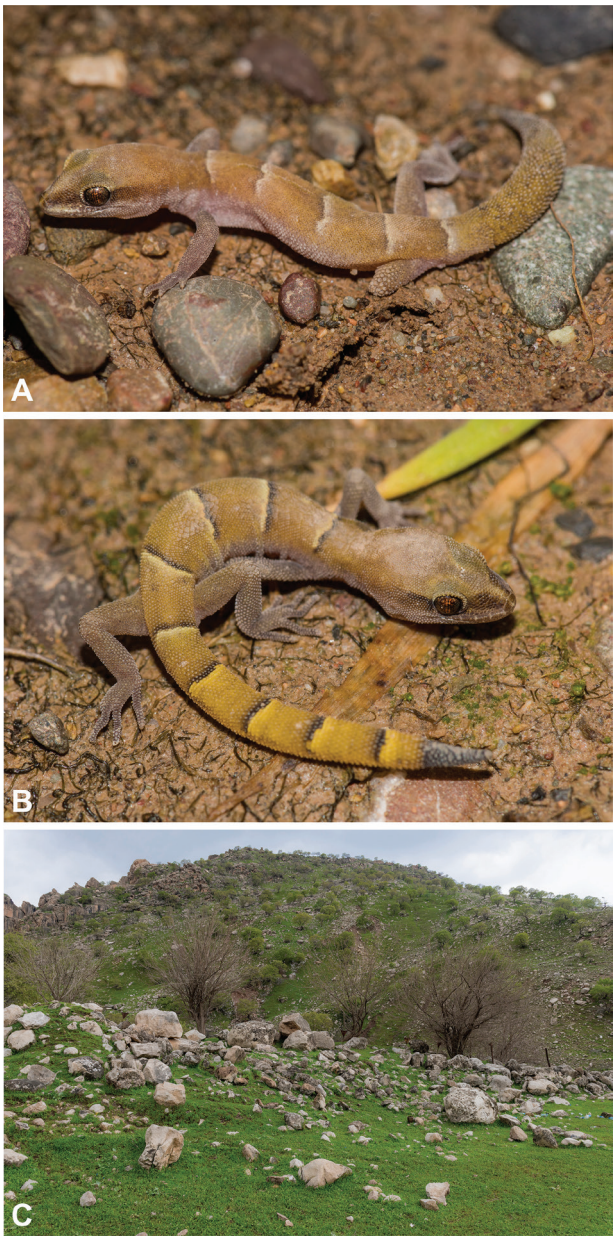

Figure 2. Individuals DJ8651 (**A**) and DJ8652 (**B**) of *Microgecko helenae fasciatus* from Iraq in life and their habitat around the Darbandikhan Lake (**C**).

Results

Two individuals of *Microgecko* (Fig. 2A, B) were found on 29 March at around 11 am south of the Darbandikhan Lake (35°06'32.4"N, 45°44'49.2"E, WGS84, 536 m elevation). The habitat consisted of rocks, lower grassy vegetation, oak trees and shrubs near to a small stream (Fig. 2C). Both individuals were found under rocks where they were hidden. Due to heavy rains during the period of research, the soil was wet also under rocks.

The results of the phylogenetic analyses (BA/ML; 853 bp, both with essentially identical topology of trees) place the specimens from Iraq to *M. helenae fasciatus* (Schmidtler & Schmidtler, 1972) clade showing high statistical support (BA/ML = 1.00/98; Fig. 1A). This clade is formed by two, only partly supported lineages (green and orange in Fig. 1; 0.51/63, 0.99/88, respectively) with average uncorrected *p*-distances 3.4% between them. The two sequences from Iraq form distinct haplotypes given to other sequences within the orange lineage originated from Iran (*p*-distances 1.3%). The phylogenetic position of the Iraqi haplotypes and their divergence from Iranian individuals is also supported by haplotype network analysis on a shortened 355 bp length dataset of *M. h. fasciatus* clade, where the Iraqi sequences are distant ≥ 5 mutation steps from other haplotypes of the orange lineage (Fig. 1B). The number of mutations' steps between the two recognized lineages of *M. h. fasciatus* is ≥ 8 with average *p*-distances of 3.6%. In the short dataset, we recognized overall six haplotypes (*Hd* = 0.68; π = 0.65%) in the orange lineage with Iraqi and Iran sequences, and seven haplotypes (*Hd* = 0.96; π = 1.6%) in the green lineage, including sequences only from Iran. The average uncorrected *p*-distance between *M. h. fasciatus* and

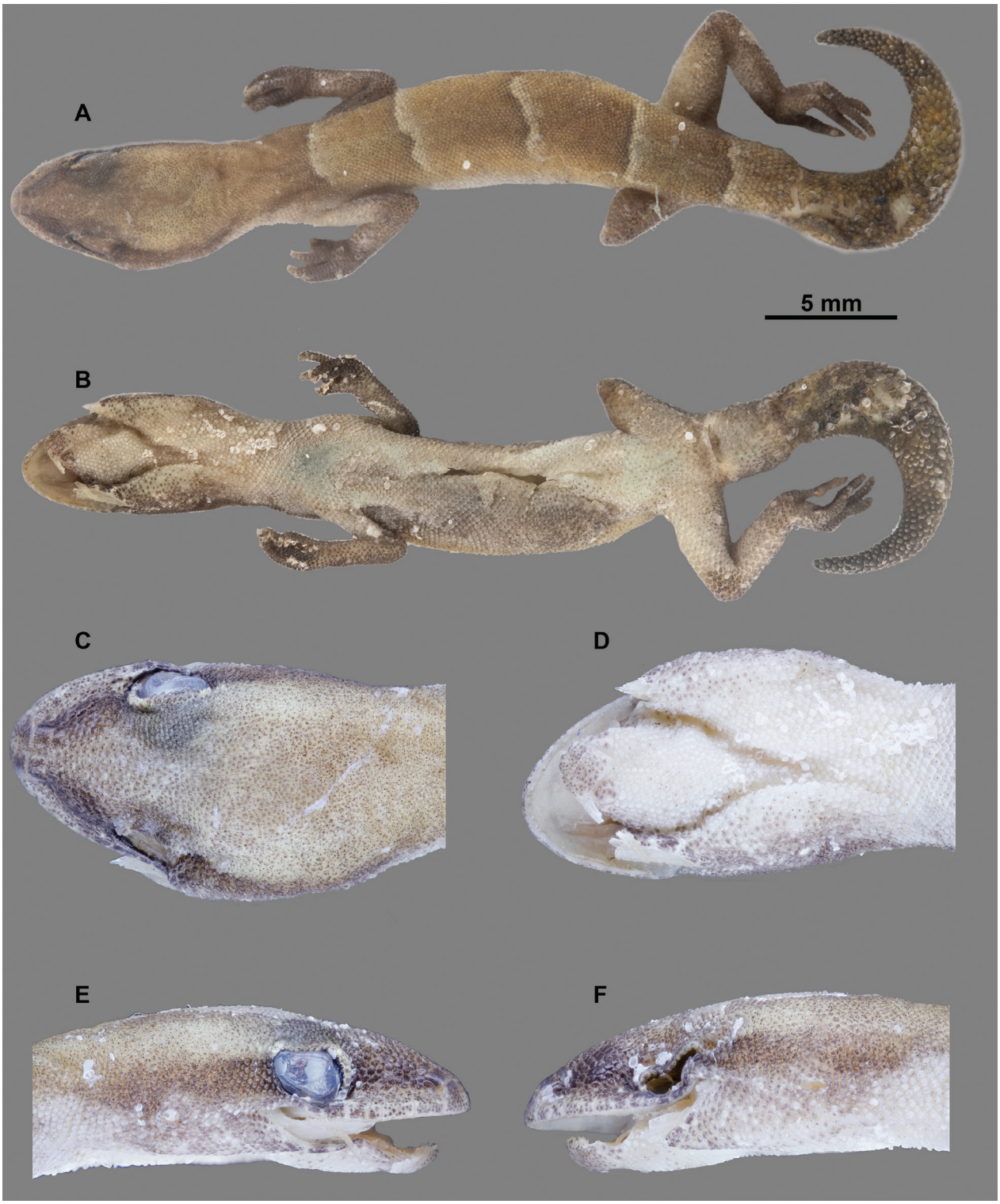

Figure 3. The preserved specimen DJ8651 from Iraq; **A.** In dorsal view; **B.** Ventral view; **C–F.** Details of the head.

M. h. helenae in the full dataset is 17.8%, and within each of these clades 2.0% and 0.8%, respectively. The genetic distance between *M. varaviensis* and *M. h. fasciatus* is 18.7%, and 15.1% between *M. varaviensis* and *M. h. helenae*.

Also, morphological data (i.e. coloration, morphometry, and meristic) correspond with those of *M. helenae fasciatus*. The specimen DJ8651 (male) had an overall brown body coloration with five grey transverse bars that are bordered by white margins. The regenerated tail has no bars and had a brown-yellowish coloration in life. The lateral part between the rostrum and the end of the head was darker than the rest of the head. The ventral part of the body is lighter than the dorsal side (Figs 2A, 3). The specimen DJ8652 (female) had a brown-yellow coloration with five transverse black bars bordered by yellow margins. The lateral part between the rostrum and the end of the head was darker than the rest of the head with a clear space in the middle of the dorsal part of the rostrum.

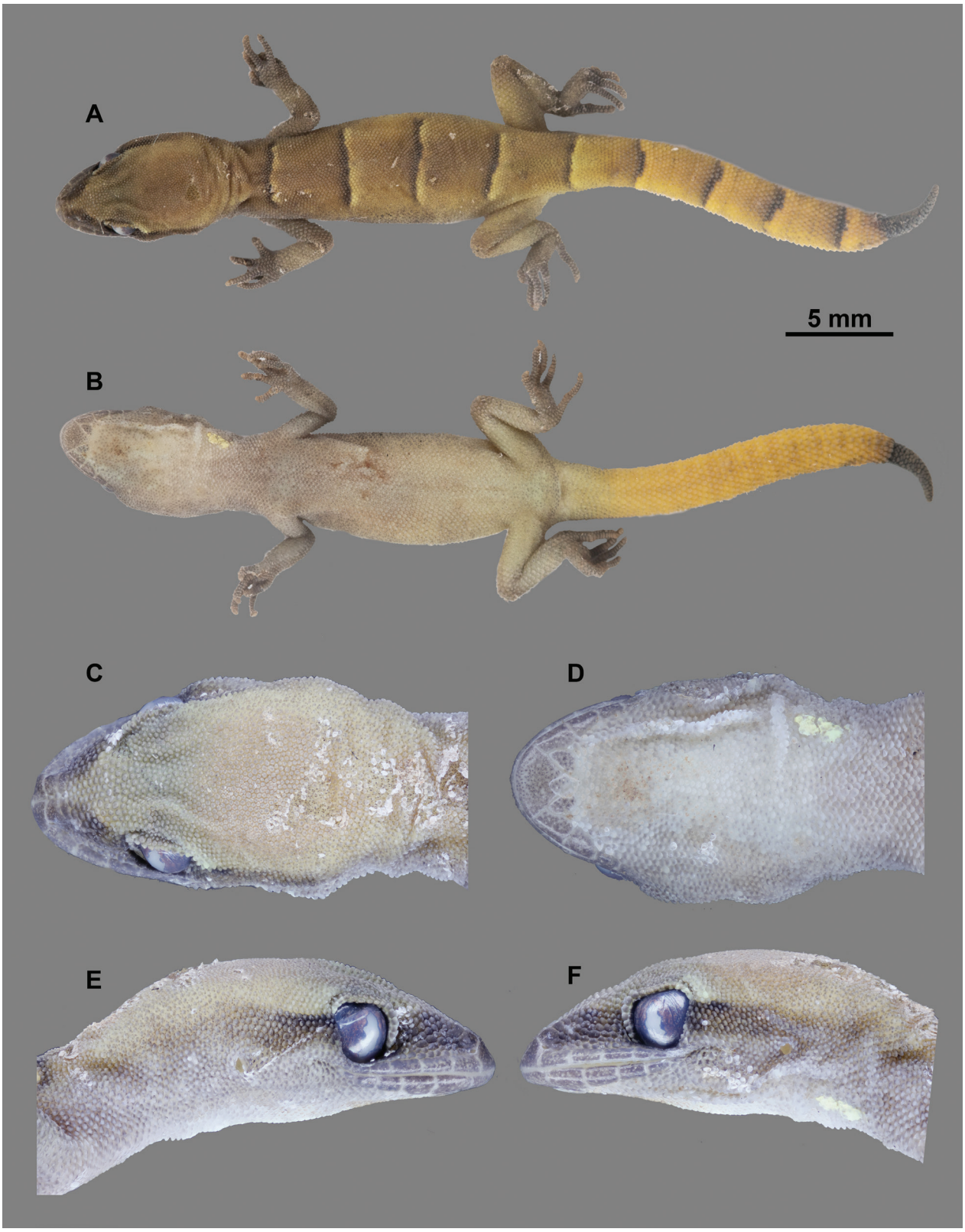

Figure 4. The preserved specimen DJ8652 from Iraq; **A.** In dorsal view; **B.** Ventral view; **C–F.** Details of the head.

The yellow tail shows four transverse bars. The tip of the tail was regenerated. The ventral part of the body was lighter than the dorsal side, whereas the yellow colour of the ventral part of the tail corresponds well to the dorsal part (Figs 2B, 4). The snout-vent length of specimens DJ8651 and DJ8652 was 23.72 and 19.60 mm, respectively. Other morphometric and meristic characters are shown in Table 1.

Eleven published localities of *M. h. fasciatus* known from Iran (Gholamifard et al. 2015, 2019; Suppl. material 1: Table S1) are distanced 68 (Shirin; loc. 10 on the Fig. 1C) and more kilometers from the Darbandikhan Lake in Iraq.

Table 1. Morphometric (mm) and meristic characters of two specimens of *Microgecko* from Iraq (for details see Material and methods).

	DJ8651	DJ8652
Morphometry		
SVL	23.72	19.60
TL	14.92	16.83
HL	5.75	4.96
HH	2.37	2.30
HW	4.07	3.61
ED	0.86	1.27
NED	1.63	1.63
EED	1.89	1.94
IOD	2.66	2.66
LFL	8.80	7.76
LHL	11.40	9.36
DFH	12.57	10.01
Meristic		
SL	7	6
IL	5	5
SSPM	3	2
SCIL	n/a	3
SBIL	11	12
IOS	25	27
GVA	122	120
AGS	92	96

Discussion

The results of our molecular analyses and the morphological examination of two *Microgecko* specimens from Iraq were congruent and support the identification as *M. helenae fasciatus* belonging to the *M. helenae* complex (Gholamifard et al. 2019). Although we examined only two individuals, morphological and molecular data range within the variation of this taxon (Gholamifard et al. 2015, 2019). This is thus the 26th genus and 52nd species of lizard and 14th species of the family Gekkonidae for Iraq. The family now comprises six genera in the country (*Bunopus*, *Cyrtopodion*, *Hemidactylus*, *Mediodactylus*, *Microgecko*, and *Stenodactylus*; Mohammed et al. 2017; Midtgaard 2019)

The amphibian and reptile fauna of Iraq is less studied than herpetofauna in the Iranian part of the Zagros Mountains. However, one gekkonid species was also recently added to the herpetofauna of Iraq (Hosseinzadeh et al. 2018), *Hemidactylus romeshkanicus* Torki, 2011. This species is surprisingly conspecific with *Hemidactylus kurdicus* Safaei-Mahroo, Ghaffari, Ghafoor & Amini, 2017 that was originally described as an endemic species for the Qara-Dagh Mountains in Iraq (Safaei-Mahroo et al. 2017). Similarly, other reptiles were relatively recently discovered in the country, e.g. *Zamenis hohenackeri* (Strauch, 1873) or *Dolichophis andreanus* (Werner, 1917), suggesting that Iraq is still not well explored (Afrasiab and Mohamad 2011; Auer et al. 2016). Especially the record of *D. andreanus* represents a range extension to the western parts of the Zagros, reminiscent of the case described here on *Microgecko*. Even though the research on reptiles of Iraq has a long history (e.g. Werner 1895; Khalaf 1959), due to political instability, conflicts, and the inaccessibility of some regions, many areas are still pending an evaluation of their biodiversity.

Considering previously published data (Gholamifard et al. 2019) and our results including distributional, molecular and morphological data, *M. h. fasciatus* should be elevated to species rank, *M. fasciatus* (Schmidtler & Schmidtler, 1972), with two diverged mitochondrial lineages of comparable DNA polymorphism, endemic to western Zagros Mts. The cyt *b* data of the northernmost known *Microgecko* population also present a slight divergence from other available sequences of *M. fasciatus* (Fig. 1A, B). The phylogeographic structure within these populations suggests historical divergences related probably to the terrain of the Zagros Mts. that is known as an effective barrier in other reptiles (e.g. Javanbakht et al. 2017; Sanchooli et al. 2018). Our data suggest that the population in Iraq is autochthonous, forming the putatively northern-westernmost range of the whole genus *Microgecko*. Our study could also have conservation implications as the taxon most probably has only a very small distribution area overall. However, to perform a comprehensive assessment of the population status in Iraq and Iran, additional research is needed.

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References

Afrasiab SR, Mohamad SI (2011) First record of the rat snake, *Zamenis hohenackeri* (Strauch, 1873), from north-eastern Iraq with notes on other colubrid snakes. Zoology in the Middle East 54: 19–22. https://doi.org/10.1080/09397140.2011.10648877

Agarwal I (2009) First record of *Tropiocolotes persicus euphorbiacola* Minton, Anderson and Anderson 1970 (Sauria: Gekkonidae) from the Republic of India, with notes on its habitat and natural history. Russian Journal of Herpetology 16: 83–87.

Auer M, Khudur FA, Ararat K, Hussei RH, Auer S, Zönnchen F (2016) First record of *Hierophis andreanus* from Iraq. Sauria 38: 49–51.

Bauer AM, de Silva A, Greenbaum E, Jackman T (2007) A new species of day gecko from high elevation in Sri Lanka, with a preliminary phylogeny of Sri Lankan *Cnemaspis* (Reptilia, Squamata, Gekkonidae). Zoosystematics and Evolution 83: 22–32. https://doi.org/10.1002/mmnz.200600022

Bauer AM, Masroor R, Titus-McQuillan JT, Heinicke MP, Daza JD, Jackam TR (2013) A preliminary phylogeny of the Palearctic naked-toed geckos (Reptilia: Squamata: Gekkonidae) with taxonomic implications. Zootaxa 3599: 301–324. https://doi.org/10.11646/zootaxa.3599.4.1

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Gamble T, Greenbaum E, Russell AP, Jackman TR, Bauer AM (2012) Repeated origin and loss of toepads in geckos. PLoS ONE 7: e39429. https://doi.org/10.1371/journal.pone.0039429

Gholamifard A, Rastegar-Pouyani N, Rastegar-Pouyani E (2015) Rediscovery of *Microgecko helenae fasciatus* (Schmidtler & Schmidtler, 1972) from Kermanshah Province, Western Iran with notes on taxonomy, morphology, and habitat. Asian Herpetological Research 6: 339–346.

Gholamifard A, Rastegar-Pouyani N, Rastegar-Pouyani E (2019) A new species of the genus Microgecko Nikolsky, 1907 (Sauria: Gekkonidae) from the southern Zagros Mountains, Iran. Zootaxa 4648: 435–454. https://doi.org/10.11646/zootaxa.4648.3.2

Hosseinzadeh MS, Aliabadian M, Rastehar-Pouyani E (2018) Deep mitochondrial and morphological differentiation of *Hemidactylus persicus* Anderson, 1872 (Squamata: Gekkonidae) in Iran. Herpetological Journal 28: 101–109.

Javanbakht H, Ihlow F, Jablonski D, Široký P, Fritz U, Rödder D, Sharifi M, Mikulíček P (2017) Genetic diversity and Quaternary range dynamics in Iranian and Transcaucasian tortoises. Biological Journal of the Linnean Society 121: 627–640. https://doi.org/10.1093/biolinnean/blx001

Khalaf KT (1959) Reptiles of Iraq with some notes on the Amphibians. Ar-Rabitta Press, Baghdad.

Kluge AG (1983) Cladistic relationships among gekkonid lizards. Copeia 1983: 465–475. https://doi.org/10.2307/1444392

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Masroor R, Khisroon M, Khan MA, Jablonski D (2020) A new species of *Microgecko* Nikolsky, 1907 (Sauria: Gekkonidae) from Pakistan. Zootaxa 4780: 147–164. https://doi.org/10.11646/zootaxa.4780.1.7

Midtgaard R (2019) RepFocus – A survey of the Reptiles of the world. [E-book available at] www.repfocus.dk

Mohammed RG, Rhadi FA, Rastegar-Pouyani N, Rastegar-Pouyani E, Hosseinian Yousefkhani SS (2017) Zoogeography of lizards fauna from central and southern Iraq with a checklist of Iraqi lizard′s fauna. Russian Journal of Herpetology 24: 193–201. https://doi.org/10.30906/1026-2296-2019-24-3-193-201

QGIS Development Team (2020) QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

Rambaut A, Suchard MA, Xie W, Drummond A (2013) Tracer 1.6. http://beast.bio.ed.ac.uk/Tracer

Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Hulsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Safaei-Mahroo B, Ghaffari H, Ghafoor A, Amini S (2017) A new species of *Hemidactylus* (Squamata: Gekkota: Gekkonidae) from Qara Dagh Mountains, Kurdistan Region, with a key to the genus in Iraq. Zootaxa 4363: 377–392. https://doi.org/10.11646/zootaxa.4363.3.4

Sanchooli N, Rastegar-Pouyani E, Hosseinian S (2018) Genetic variability among *Paralaudakia microlepis* (Blanford, 1874) (Sauria: Agamidae) populations in the Iranian Plateau. Zootaxa 4526: 245–250. https://doi.org/10.11646/zootaxa.4526.2.10

Schmidtler JJ, Schmidtler JF (1972) Zwerggeckos aus dem Zagros-Gebirge (Iran). Salamandra 8: 59–66.

Sindaco R, Jeremčenko VK (2008) The reptiles of the Western Palearctic. Volume 1. Edizioni Belvedere, Latina, IT.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Torki F (2020) A new species of dwarf gecko of the genus *Microgecko* (Squamata: Gekkonidae) from Iran. Sauria 42: 41–54.

Werner F (1895) Ueber eine Sammlung von Reptilien aus Persien, Mesopotamien und Arabien. Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien 45: 13–22. https://doi.org/10.5962/bhl.part.26910

Supplementary material 1

Table S1. The published genetic and faunistic data used in this study

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Data type: species data

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