



A new species of *Pseudotrapelus* (Reptilia: Squamata: Agamidae) from Central Arabia

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Abstract

A recent molecular phylogeny of the agamid genus *Pseudotrapelus*, distributed in the rocky areas of North Africa and the Arabian Peninsula, revealed the presence of a genetically distinct lineage around the city of Riyadh in central Saudi Arabia. With the inclusion of additional specimens, we were able to describe this lineage as a new species, *P. tuwaiqensis* **sp. nov.**, confined to the Tuwaiq Escarpment, thus endemic to central Saudi Arabia. Our results of morphological examinations and molecular analyses, using three mitochondrial (COI, 16S, ND4-tRNAs) and two nuclear (c-mos, MC1R) gene fragments, show the new species is genetically differentiated and phylogenetically close to *P. sinaitus* and *P. chlodnickii*.

Keywords

Acrodonta, biogeography, DNA barcoding, Middle East, multilocus phylogeny, reptiles, Saudi Arabia

Introduction

The Arabian Peninsula is comprised of diverse landscapes with massive deserts spanning across most of the interior, enclosed by mountain ranges on the margins of the peninsula (Edgell 2006), and is renowned for its harsh, hot, and arid climate. These diverse landscapes are often inhabited by specialized species/lineages displaying a wide array of unique adaptations to arid conditions. As the dominant inhabitants of arid areas, squamates became

exemplary models to investigate biodiversity patterns, phylogeographic assessments, and ecological and evolutionary studies (Camargo et al. 2010). Squamate reptiles from the arid areas of Arabia have been recently shown to harbor more diversity than previously thought. In the past two decades, the growing number of taxonomic, biogeographic, and phylogenetic studies have drastically broadened our knowledge of the Arabian squamate fauna,

often presenting many specialized deep lineages, as well as newly formed species. Some studies included revisions of geographically widely distributed genera (e.g., Carranza and Arnold 2012; Metallinou et al. 2012, 2015; de Pous et al. 2016; Tamar et al. 2016a, 2016b, 2018; Šmíd et al. 2013, 2015a; Simó-Riudalbas et al. 2019; Machado et al. 2021). Other studies focused on species complexes or identification and description of new taxa (e.g., Busais and Joger 2011; Metallinou and Carranza 2013; Vasconcelos and Carranza 2014; Machado et al. 2019; Tamar et al. 2019a, 2019b, 2019c; Šmíd et al. 2015b, 2017, 2023; Burriel-Carranza et al. 2023).

Within the agamid fauna of Arabia, the genus *Pseudotrappelus* Fitzinger, 1843 is restricted to the hilly and mountainous areas enclosing the Arabian Peninsula and the Red Sea (Fig. 1; Arnold 1980; Disi et al. 2001; Baha El Din 2006; Gardner 2013; Tamar et al. 2016b; Bar et al. 2021; Carranza et al. 2021; Šmíd et al. 2021). In previous years, taxonomic work on *Pseudotrappelus* has been hampered by the conservative and homogeneous morphology of its members. For many years authors followed a conservative approach treating most variations as a single species, *P. sinaitus* (Heyden, 1827), with several mor-

phological forms or as a species complex (e.g., Anderson 1896, 1901; Arnold 1980; Fritz and Schütte 1988; Schätti and Gasperetti 1994; Baha El Din 2006; Sindaco and Jeremčenko 2008). Within the past decade, four new species have been described and one was resurrected (Melnikov and Pierson 2012; Melnikov et al. 2012, 2013a, 2015; Uetz et al. 2023). Currently *Pseudotrappelus* consists of six recognized species (Uetz et al. 2023): (i) *P. aqabensis* Melnikov, Nazarov, Ananjeva & Disi, 2012, from north western Saudi Arabia, through southern Jordan westwards to the Sinai Peninsula; (ii) *P. chlodnickii* Melnikov et al., 2015, from Sudan and Egypt, including the Sinai Peninsula; (iii) *P. dhofarensis* Melnikov & Pierson, 2012, from the mountains of southern Oman and southern Yemen; (iv) *P. jensvindumi* Melnikov, Ananjeva & Papenfuss, 2013, endemic to the Hajar Mountains of Oman and the United Arab Emirates (UAE); (v) *P. neumanni* (Tornier, 1905), from the mountains of southern Yemen and south-western Saudi Arabia; and (vi) *P. sinaitus* from the Sinai Peninsula in Egypt, eastwards through most of Jordan to southern Syria and northwestern Saudi Arabia (Fig. 1).

Unfortunately, the recent descriptions of four species were based on a small number of specimens, with no

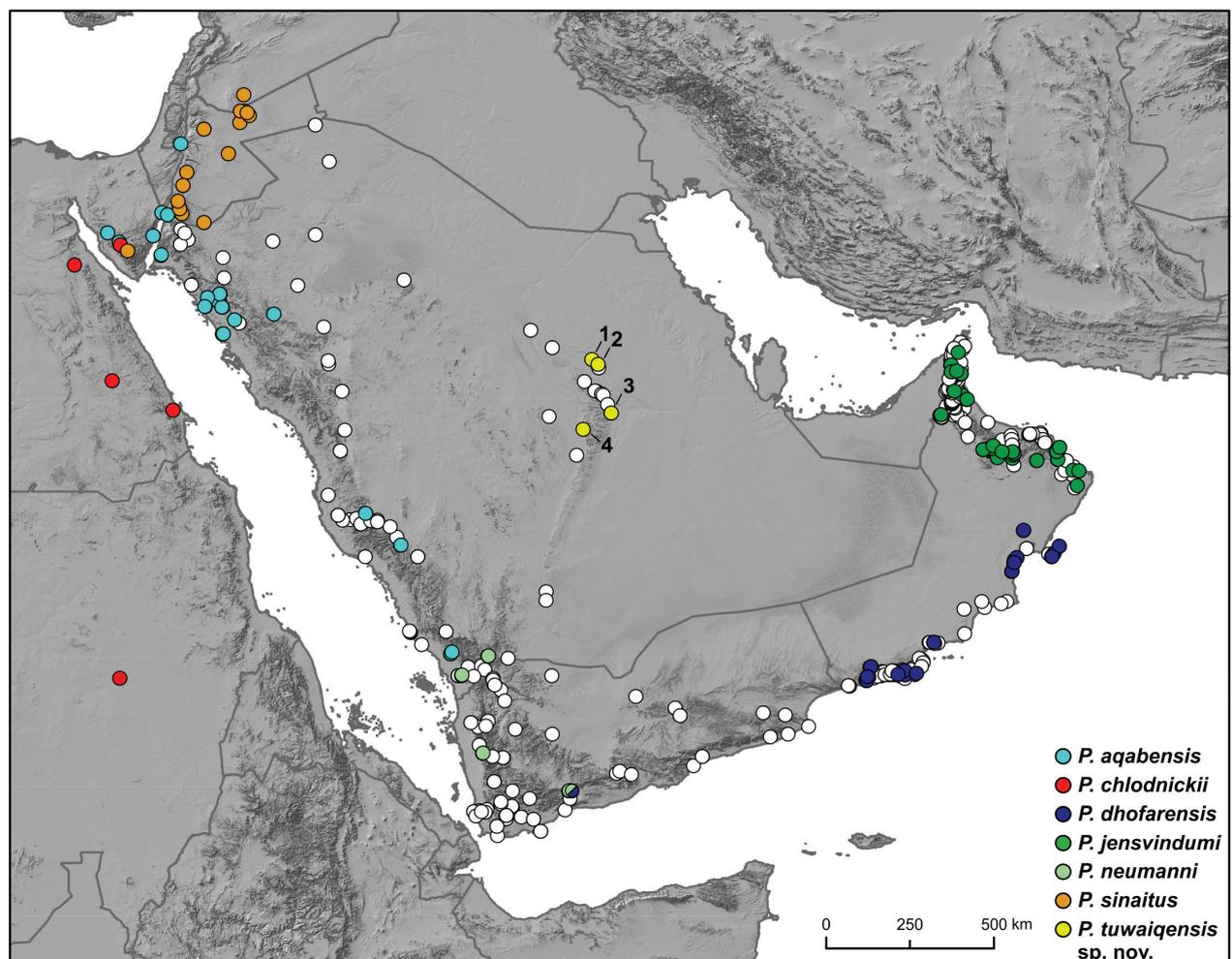


Figure 1. Geographic distribution of the genus *Pseudotrappelus* in Arabia and adjacent territories. The circles show distribution records, those in color have been confirmed genetically to belong to the respective species. The white circles can be identified with confidence only to the genus level. All known records for Saudi Arabia, Yemen, Oman, and the UAE are shown. The map is not exhaustive in terms of records of *P. aqabensis*, *P. chlodnickii*, and *P. sinaitus* from Jordan west to Africa. Locality details of the numbered localities of *P. tuwaiqensis* **sp. nov.** are given in Appendices 1–2.

comprehensive comparisons among species, and genetic data that were based solely on the mitochondrial COI gene (Melnikov and Pierson 2012; Melnikov et al. 2012, 2013a, 2013b, 2015). This resulted in insufficient diagnostic characters to identify specimens or describe new species (see Tamar et al. 2016b and references therein). These studies thus created much biogeographic uncertainty and taxonomic confusion within *Pseudotrapelus*.

The comprehensive molecular phylogenetic study of *Pseudotrapelus* by Tamar et al. (2016b) provided much needed information on the genetic structure of the genus, and the phylogenetic relationships among its members using multiple loci, as well as on the distribution ranges of its species. Tamar et al. (2019a) later revealed cryptic diversity within the genus, with an unnamed lineage from central Saudi Arabia that is distinct in all genetic analyses performed, including both mitochondrial and nuclear species delimitation methods, and is phylogenetically close to *P. sinaitus* and *P. chlodnickii*. The absence of detailed morphological comparisons among *Pseudotrapelus* members, caused by the lack of specimens of this cryptic lineage, hindered its taxonomic evaluation at that time. In this study, we aim to provide an integrative taxonomic assessment of this lineage, including the addition of new specimens from central Saudi Arabia.

Materials and Methods

In addition to the two specimens reported in Tamar et al. (2019a), we collected two additional *Pseudotrapelus* individuals from the vicinity of Riyadh in Saudi Arabia (Fig. 1) during a targeted fieldwork in 2019. The specimens were deposited in the National History Museum Prague, Czech Republic (NMP-P6V; see voucher codes in the taxonomic section below and in Appendices 1–3).

Molecular dataset and analyses

We assembled two *Pseudotrapelus* datasets for this study: (i) a concatenated dataset for investigating phylogenetic relationships, including sequences of mitochondrial and nuclear gene fragments for 40 specimens (Appendix 1); (ii) a barcoding dataset for comparison to type specimens of the genus, including sequences for 29 specimens (Appendix 2). Specimens of *Acanthocercus*, phylogenetically close to *Pseudotrapelus* (Pyron et al. 2013), were used as an outgroup. Sample codes, localities, and GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>)/BOLD (www.barcodinglife.org) accession numbers are in Appendices 1–2.

We extracted DNA from ethanol-preserved tissue samples using the Geneaid extraction kit following manufacturer's instructions. The concatenated dataset comprised four gene fragments (2,375 bp): two mitochondrial, the ribosomal 16S rRNA (16S; ~500 bp) and the NADH dehydrogenase subunit 4 (ND4; 681 bp) with the adja-

cent histidine, serine, and leucine tRNA genes (tRNA; ~158 bp), and two nuclear, the oocyte maturation factor Mos (*c-mos*; 372 bp) and the melano-cortin 1 receptor (MC1R; 663 bp). These markers were used in previous phylogenetic studies on *Pseudotrapelus* (Tamar et al. 2016b, 2019a), thus providing reliable and consistent comparison of phylogenetic relationships. Primers and Polymerase Chain Reaction (PCR) conditions used for the amplification of these markers are as detailed in Tamar et al. (2016b). We additionally amplified the barcoding mitochondrial gene fragment Cytochrome *c* oxidase subunit I (COI; 645 bp) to compare with the holotypes, paratypes, and topotypes of most species (following Melnikov et al. 2015; Melnikova et al. 2015). We amplified the barcoding region using RepCOI-F and RepCOI-R primers as detailed in Nagy et al. (2012). In all amplifications, we sequenced both strands of the PCR products at Macrogen (the Netherlands). We checked, assembled, and edited chromatographs using Geneious v.7.1.9 (Biomatter Ltd.). We aligned the sequences for each marker using MAFFT v.7.3 (Katoh and Standley 2013). To remove difficult-to-align regions and poorly aligned positions of the 16S and tRNA fragments we used Gblocks (Castresana 2000) with low stringency options (Talavera and Castresana 2007). We translated the protein-coding genes into amino acids, and we detected no stop codons, suggesting that they were not pseudogenes. For the nuclear markers, we identified heterozygous positions and coded them according to the standard IUPAC ambiguity codes and resolved these sites, for each gene independently, by using the PHASE 2.1.1 algorithm (Stephens et al. 2001; Stephens and Donnelly 2003) implemented in DNASP v.6 (Rozas et al. 2017) with probability threshold=0.9. We tested the occurrence of recombination for the two phased nuclear-gene alignments using the Pairwise Homoplasmy Index (PhiTest; Bruen et al. 2006) implemented in SplitsTree v.4.14.5 (Huson and Bryant 2006), and we detected no evidence of recombination.

For the phylogenetic analyses of both the concatenated dataset (16S, ND4, tRNA, *c-mos*, MC1R) and the COI dataset, we partitioned by gene and selected substitution models for each marker using JModelTest v.2.1.7 (Darriba et al. 2012; Guindon and Gascuel 2003). The best models were as follows: HKY+G for 16S, tRNA and MC1R, TrN+I+G for ND4 and COI, and JC for *c-mos*. We analyzed both datasets under Maximum Likelihood (ML) and Bayesian Inference (BI) frameworks. We treated alignment gaps as missing data and the nuclear gene sequences were not phased. We performed the ML analyses using IQ-TREE v. 1.6 (Nguyen et al. 2015) through the web interface (Trifinopoulos et al. 2016). Branch support was assessed with the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT; Guindon et al. 2010) and the ultrafast bootstrap (UFBoot; Hoang et al. 2018), both with 1,000 replicates. We conducted BI analyses using MrBayes v.3.2.7 (Ronquist et al. 2012) with nucleotide substitution model parameters unlinked across partitions. The different partitions were allowed to evolve at different rates. Two simultaneous parallel runs were performed with four chains per run for 10⁶ genera-

tions with sampling every 100 generations. We examined the standard deviation of the split frequencies between the two runs and the potential scale reduction factor (PSRF) diagnostic; convergence was assessed by confirming that all parameters had reached stationarity and had sufficient effective sample sizes (>200) using Tracer v.1.6 (Rambaut et al. 2014). We conservatively discarded the first 25% of trees as burn-in. We explored patterns of intraspecific diversity and nuclear allele sharing within *Pseudotrappelus* by inferring statistical parsimony networks for the two individual nuclear phased genes with the program TCS v.1.21 (Clement et al. 2000; connection limit of 95%), consisting of all sampled specimens for

each marker. We used tcsBU (Múrias dos Santos et al. 2016) for visualization of the nuclear networks. We calculated inter- and intraspecific uncorrected p-distance for 16S, ND4, and COI with pairwise deletion in MEGA11 v.11.0.11 (Tamura et al. 2021).

Morphological data and analysis

We examined the morphology of the four specimens from central Saudi Arabia and compared it with (i) data published in the descriptions of the recently described species and (ii) additional vouchered and photographs of

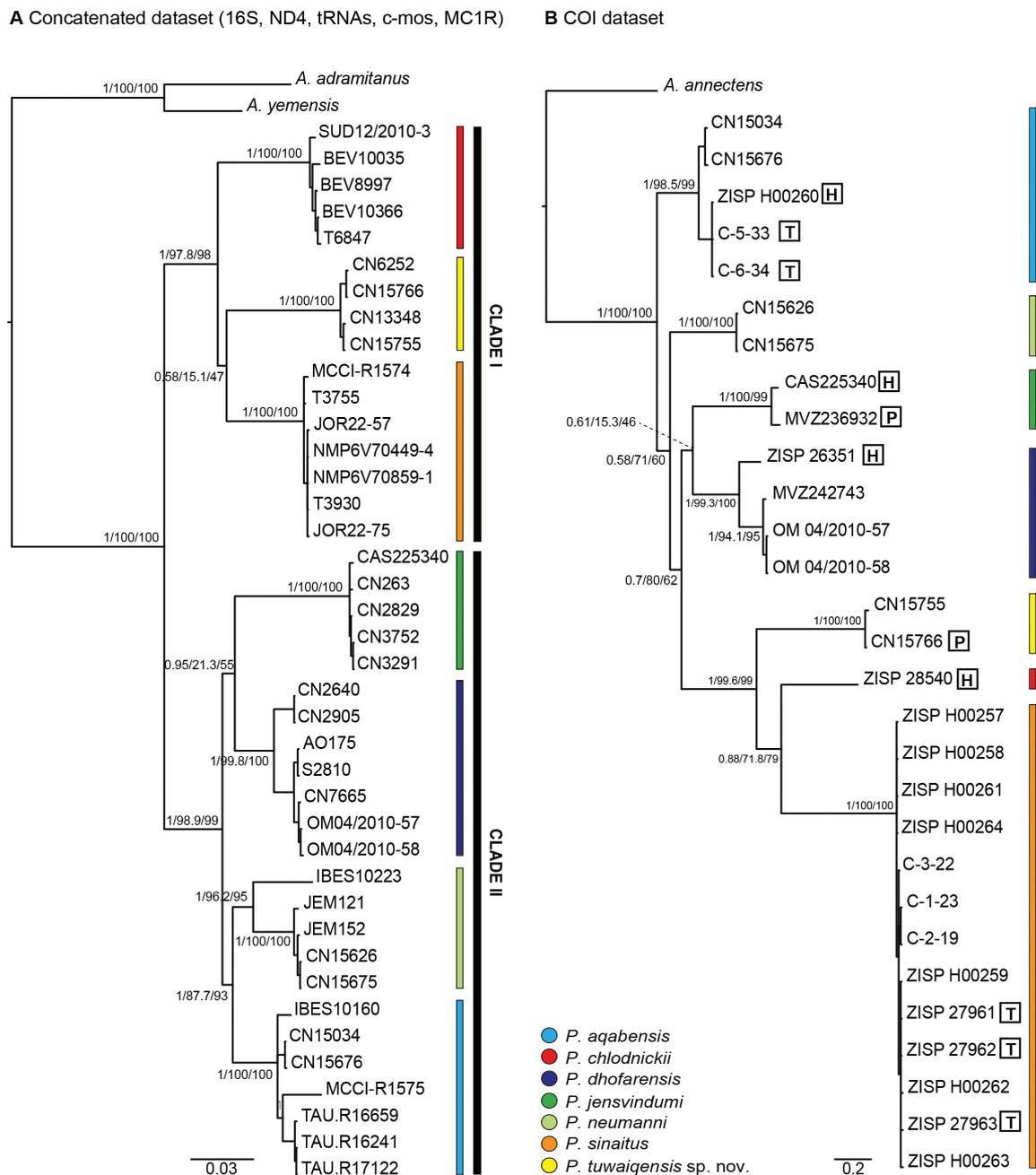


Figure 2. Bayesian inference phylogenetic trees of *Pseudotrappelus*. The trees were reconstructed based on **A** the concatenated dataset (16S, ND4, tRNA, c-mos, MC1R) and **B** the barcoding COI marker dataset. Support values are indicated near the nodes (Bayesian posterior probabilities/SH-aLRT/UFBoot). Sample codes correspond to specimens in Appendices 1, 2. Letters in rectangles in the COI tree indicate type specimens – H: holotype, P: paratype, T: topotype.

unvouchered specimens of all the other *Pseudotrapelus* species. The examined specimens included four specimens of the new species described below, three specimens of *P. aqabensis*, two specimens of *P. chlodnickii*, three specimens of *P. dhofarensis*, one specimen of *P. jensvindumi*, two specimens of *P. neumanni*, and eight specimens of *P. sinaitus*. High quality photographs (479 in total) of all specimens examined morphologically have been deposited in the public database MorphoBank (<http://www.morphobank.org>) in their original resolution where they are freely available for download (project number 4714). The MorphoBank accessions along with locality details are provided in Appendix 3.

We recorded the following morphological data: total length; snout-vent length (SVL), measured from the tip of the snout to the anterior margin of the cloaca; number of upper and lower labials; shape and size of the ear opening; position and arrangement of head scales; the presence of enlarged scales in the occipital area of the head; character of dorsal lateral scales on the body and tail (homogeneous versus heterogeneous, keeled versus unkeeled); the length of fingers and toes; number of pre-cloacal pores and their arrangement; body and tail coloration and patterning.

Results

The phylogenetic analyses of *Pseudotrapelus* using BI and ML methods based on the concatenated dataset yielded similar topologies, with most nodes well supported and all recognized species monophyletic (Fig. 2). The phylogenetic structure within *Pseudotrapelus* recovered two major clades. Clade I (Bayesian posterior probabilities/SH-aLRT/UFBoot support values 1/97.8/98; support

values are given in the same order hereafter), though with unsupported internal topology in both the ML or BI analyses, included the recognized species *P. chlodnickii* and *P. sinaitus*, and the four samples from the vicinity of Riyadh as the new species described below. Clade II (support 1/98.9/99) included the four remaining species with a sister species relationship between *P. jensvindumi* and *P. dhofarensis* (support 0.95/21.3/55), and between *P. neumanni* and *P. aqabensis* (support 1/87.7/93), though the topological structure was not supported for the former relationship. In contrast to the concatenated dataset, the phylogenetic tree based on the single barcoding COI marker recovered mostly weak support for the topological structure, apart from the distinction of clade I with strong support (support 1/99.6/99). The BI and ML analyses of the COI dataset recovered similar unsupported topology, though in both methods all recognized species were monophyletic, as well as the new species described herein. The paratype of the new species was recovered in a distinct lineage and did not cluster with any type specimens of the other recognized species. The nuclear networks inferred for the phased c-mos and MC1R alignments (Fig. 3) exhibited no allele sharing between any of the species in the MC1R network, contrasting to the c-mos network, in which allele sharing was present among the phylogenetically close Arabian species of clade II, which may indicate incomplete lineage sorting rather than gene flow among species. The two allele networks yielded a similar pattern of distinct haplotypes for the species of clade I, including the new species described below. Mitochondrial uncorrected interspecific genetic distances among *Pseudotrapelus* species (Appendix 4) ranged between 2 and 8.6% in 16S and between 11–19.8% in ND4. The lowest distances are between *P. aqabensis* and *P. neumanni* (2.2% in 16S and 11% in ND4). The genetic distances between the new species described herein and the other recognized species of clade

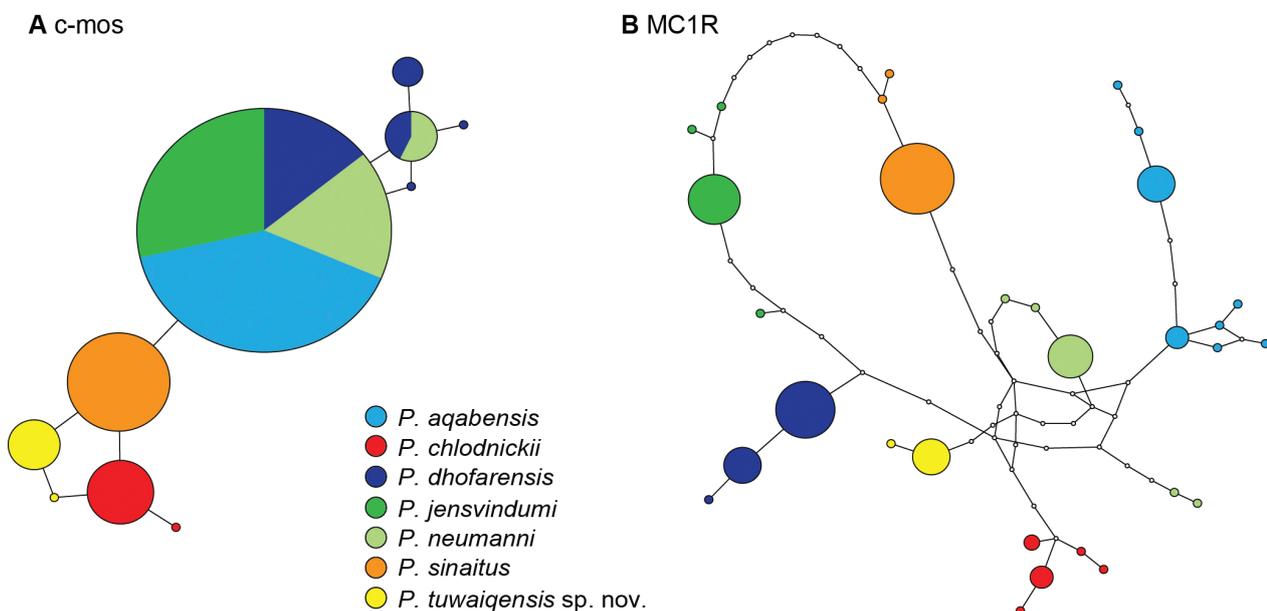


Figure 3. Allele networks of *Pseudotrapelus* for the two nuclear markers. **A** c-mos and **B** MC1R. Circle sizes are proportional to the number of alleles. White circles represent mutational steps.

II ranged between 7.9 and 8.6% in 16S and between 18.2 and 19.8% in ND4, while the distance to the species of clade I, to which it belongs, ranged between 6.3 and 7.0% in 16S and between 15.3 and 15.6% in ND4. The uncorrected interspecific genetic distances among the species in the COI marker indicate that the new species described below presents mostly high distances from all other species (between 13.6% and 15.9%).

Taxonomic account

Based on the degree of genetic differentiation of the new lineage from central Saudi Arabia from all other *Pseudotrapelus* species at both mitochondrial and nuclear levels, we recognize this lineage as a new species that we formally describe herein.

Pseudotrapelus tuwaiqensis sp. nov.

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MorphoBank, project 4714, M862812–M863071

Figures 1–6, Table 1, Appendices 1–6

English name: Tuwaiq Agama

Arabic name: عطاءة طويق

Chresonymy.

Agama (*Pseudotrapelus* [sic]) *sinaita* in Al-Sadoon (1988)

Agama (*Pseudotrapelus*) *sinaita* in Al-Sadoon et al. (1991)

Pseudotrapelus sinaitus in Kordges (1998)

Pseudotrapelus sp. in Tamar et al. (2019b)

'*Pseudotrapelus* sp Riyadh' in Šmíd et al. (2021)

Holotype. NMP-P6V 76634 (sample CN6252), adult male, a hill in a wadi, north-west to Thumamah, Saudi Arabia (25.592°N, 46.401°E; 670 m elevation), collected by Laurent Chirio on 25th March 2016 (Fig. 4; MorphoBank accessions M862812–M862870).

Paratypes. NMP-P6V 76635 (sample CN13348), adult female, foothill of Jebel Baloum, Saudi Arabia (23.699°N, 46.173°E; 800 m elevation), collected by Laurent Chirio on 27th April 2018 (Fig. 5A; MorphoBank accessions M862871–M862941). NMP-P6V 76636 (sample CN15766), adult female, north west of King Khalid Royal Reserve, north of Riyadh, Saudi Arabia (25.45933°N, 46.56276°E, 630 m elevation), collected by Salvador Carranza, Jiří Šmíd, and Mohammed Shobrak on 14th June 2019 (Fig. 5B; MorphoBank accessions M862992–M863071).

Other examined specimen. NMP-P6V 76637 (sample CN15755), juvenile, west of Al-Kharj, south of Riyadh, Saudi Arabia (24.15093°N, 46.9317°E, 530 m elevation), collected by Salvador Carranza, Jiří Šmíd, and Mohammed Shobrak on 15th June 2019 (MorphoBank accessions M862942–M862990).

Etymology. The species epithet *tuwaiqensis* is derived from the geographic feature the species is associated with, the Tuwaiq Escarpment, that cuts through central Saudi Arabia from the southwest of the country to slightly north and northwest of the city of Riyadh.

Diagnosis. A *Pseudotrapelus* species forming a clade together with *P. sinaitus* and *P. chlodnickii*, with the follow-

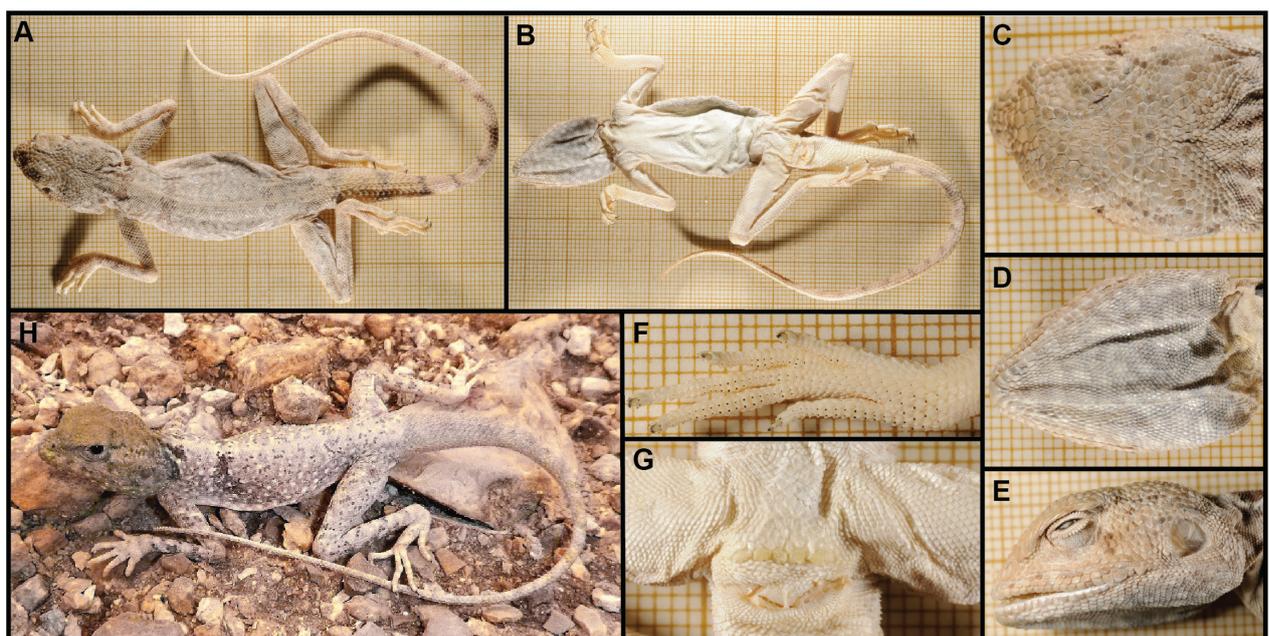


Figure 4. General appearance of *Pseudotrapelus tuwaiqensis* sp. nov. holotype (NMP-P6V 76634), adult male. **A** Habitus, dorsal view; **B** Habitus, ventral view; **C** Head, dorsal view; **D** Head, ventral view; **E** Head, lateral view; **F** Hind foot, ventral view; **G** Preloacal area, ventral view; **H** In life. Photos by Salvador Carranza (A–G) and Laurent Chirio (H).

ing combination of morphological and genetic characters: (1) large size with a total length of 200–203 mm and SVL 70.7–76.6 mm; (2) 14–19 upper and 14–18 lower labial scales; (3) ear opening very large, oval, rimmed anterodorsally by conical scales of different sizes that give it a serrated appearance; (4) scales in the occipital area predominantly not enlarged; (5) heterogeneous dorsal scales with the mid-dorsals being distinctly keeled and larger than the scales on the flanks; (6) one continuous row of 4–7 precloacal pores in both sexes; (7) 3rd toe longer than the 4th; (8) tail scales not arranged in whorls; (9) body and tail beige-brown in life with dark brown or orange transverse bars, the first on the nape, the second and the most prominent one in the scapular region, the third at midbody, the fourth in front of the insertion of the hind limbs, the fifth at the tail base; and the tail with regular dark bars down its length; (10) three unique mutations in the MC1R alignment: position 264 C instead of T, position 508 G instead of A, position 562 G instead of C; (11) one unique mutation in the c-mos alignment in position 202 C instead of G (see Appendices 5, 6).

Differential diagnosis. The genus *Pseudotrapelus* is morphologically very conservative, and it is virtually impossible to phenotypically distinguish one species from another without knowing precise locality data. *Pseudotrapelus tuwaiqensis* **sp. nov.** is no exception to this. While available literature and recent descriptions of new species in-

dicating some key features that allow species identification, these do not hold when confronted with additional material (Table 1). For example, one of the diagnostic characters of *P. jensvindumi* was a small gap in the middle of the precloacal pores row (Melnikov et al. 2013a). However, similar state is present in some other species including *P. aqabensis* (specimen CN15112, MorphoBank accessions: M862611–M862613), *P. dhofarensis* (Melnikov and Pierson 2012, fig. 7 therein; JIR107, MorphoBank accession: M862670), and *P. sinaitus* (JOR22_75, MorphoBank accession: M862793; JOR22_85, MorphoBank accession: M862799). Similarly, *P. neumanni* is supposedly the only species with enlarged scales in the occipital area of the head (Melnikov et al. 2013a), but our examination of specimens of the other species revealed that in fact all but *P. aqabensis* and *P. sinaitus* have these enlarged scales present, at least in some specimens. In line with the above-said, our morphological examinations of *P. tuwaiqensis* **sp. nov.** did not reveal any characters that would be unique for the species and allowed its unambiguous identification (Table 1). As a result, we advise caution and prudence when trying to key out *Pseudotrapelus* specimens on the basis of morphology alone.

With the currently available evidence, the safest and perhaps the only way to differentiate the individual *Pseudotrapelus* species is either based on the origin of the specimens, or by using DNA barcoding. The ranges of most species are allopatric or parapatric, with *P.*

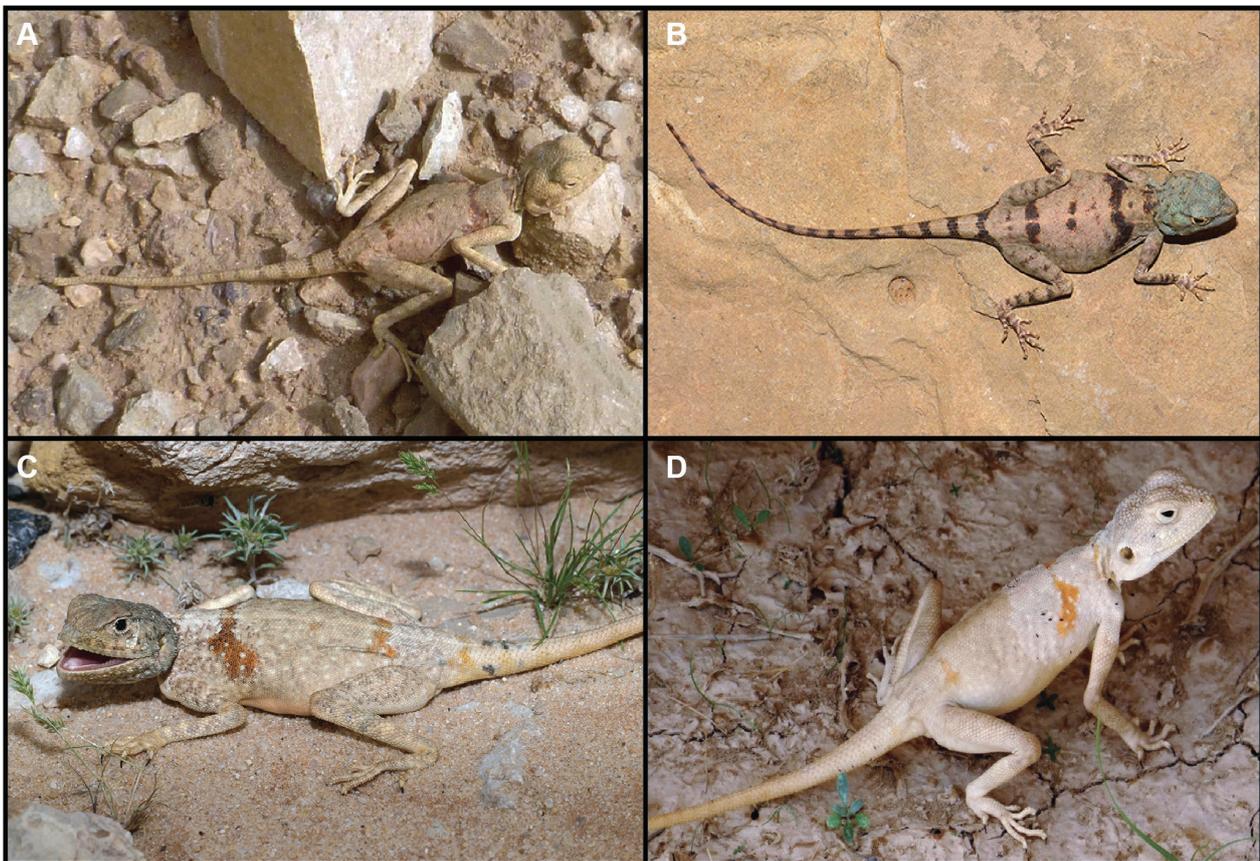


Figure 5. General appearance of *Pseudotrapelus tuwaiqensis* **sp. nov.** **A** paratype NMP-P6V 76635, adult female. Photo by Laurent Chirio; **B** paratype NMP-P6V 76636, adult female. Photo by Jiří Šmíd; **C** Uncollected specimen from locality 25.45933°N, 46.56276°E. Photo by Marius Burger; **D** Uncollected specimen, locality Shaib-Luha, Saudi Arabia. Photo by Laurent Chirio.

Table 1. Morphological comparisons of *Pseudotrapelus* species. The comparisons show traits that were traditionally considered key for individual species identification. Taxon names correspond to changes proposed in this paper. Superscript letters refer to the original references from which the data was obtained as follows: 1 – this study; 2 – Tornier (1905); 3 – Melnikov et al. (2012); 4 – Melnikov and Pierson (2012); 5 – Melnikov et al. (2013a); 6 – Melnikov et al. (2015); 7 – Moravec (2002).

Species	3 rd toe longer than 4 th	Preanal pores	Enlarged occipital scales
<i>P. aqabensis</i>	YES ^{1,3-7}	4–7 ^{1,3-7}	NO ^{1,3-6}
<i>P. chlodnickii</i>	YES ^{1,6}	6–7 ^{1,6}	YES ¹ ; YES/NO ⁶
<i>P. dhofarensis</i>	YES ^{1,4-6}	5–8 ^{1,4-6}	YES ¹ ; NO ⁴⁻⁶
<i>P. jensvindumi</i>	YES ^{1,5,6}	4 ^{5,6}	YES ¹ ; YES/NO ⁵ ; NO ⁶
<i>P. neumanni</i>	YES ^{1,2,4-6}	4 ²⁻⁶	YES ¹⁻⁶
<i>P. sinaitus</i>	NO ^{1,3-7}	4–10 ¹⁻⁷	NO ^{1,3-6}
<i>P. tuwaiqensis</i> sp. nov.	YES ¹	4–7 ¹	YES/NO ¹

tuwaiqensis sp. nov. being the only species that occurs in central Saudi Arabia around the city of Riyadh and further to the north and south along the Tuwaiq Escarpment. The other species are distributed in the peripheral mountain ranges of Arabia and some of them overlap to a certain extent (e.g., *P. neumanni* and *P. dhofarensis* in Yemen, *P. neumanni* and *P. aqabensis* in southwestern Saudi Arabia, *P. aqabensis* and *P. sinaitus* in northwestern Arabia; Fig. 1). The most reliable species identification tool in *Pseudotrapelus* is thus DNA barcoding. All presently recognized species have multiple specimens sequenced for three mitochondrial genes, including the COI marker that is the most commonly used barcode for animal identification (Vences et al. 2012) and show marked genetic differentiation in all these markers across species (Appendix 4). *Pseudotrapelus tuwaiqensis* sp. nov. can be clearly differentiated from its congeners at the genetic level by p-distances of 6.3–8.6% in 16S, 15.3–19.8% in ND4, and 13.6–15.9% in COI (Appendix 4). In addition, all *Pseudotrapelus* species have been sequenced for two nuclear genes, of which the MC1R has unique alleles for each species and the c-mos has unique alleles for *P. tuwaiqensis* sp. nov., *P. sinaitus*, and *P. chlodnickii* (Fig. 3; Appendices 5, 6). *Pseudotrapelus aqabensis*, *P. dhofarensis*, *P. jensvindumi*, and *P. neumanni* are genetically indistinguishable in the c-mos sequences.

Description of the holotype. Total length 203.9 mm (SVL 70.7 mm; original tail 133.2 mm). Head and body depressed (Fig. 4A, B). Three internasal scales, the middle one is triangular and contacts the rostral dorsally. Nostril tubular directed laterally and slightly posterodorsally, pierced in the posterior part of a large convex, smooth, pear-shaped nasal scale, which is situated on the anterior edge of the canthus rostralis. The nasal scale is partially visible from above and directly in contact with the first canthal scale. The first six canthal scales not in contact with the eye. Scales on the head smooth, somewhat polyhedral, interorbital scales as large or larger than the supraorbital scales; imbrications of temporal scales directed ventrally. Occipital pierced by a visible pineal foramen posteriorly, surrounded by irregular scales. Occipital scales are somewhat smaller than other head scales (Fig. 4C). 19 (left)–17 (right) upper and 18 (left)–17 (right) lower labial scales (Fig. 4D, E). Ear opening as large as

the eye, surrounded at its border by several single short conical scales of different sizes, with one single conical scale at the lower anterior edge of ear opening (Fig. 4E). Gular fold absent. Dorsal scales heterogeneous with a medial longitudinal rows of enlarged scales present. Medial dorsal scales diamond shaped and distinctly keeled, with the keel extending along the entire scale and not mucronate. Lateral scales not keeled. Gular and ventral scales smooth, becoming feebly keeled toward the lateral side of the body. Dorsal scales on limbs keeled and imbricate. Hind limbs long with the 3rd toe reaching to the nostril level when adpressed. The 3rd toe is the longest, reaching 9.3 mm (Fig. 4F). Ten lamellae under the left 4th finger, 17 lamellae under the left 4th toe. Forelimbs long with digits reaching to the cloaca when adpressed. The 3rd finger is the longest, reaching 6.5 mm. One row of six continuous preloacal pores each about the size of two to three other preloacal scales (Fig. 4G). Tail depressed at its base, with a small pit after the cloaca. Large hemipenial pockets are absent, but two small bulges on either side of the pit are present. Dorsal tail scales strongly keeled, slightly mucronate, somewhat larger than the body scales. Ventral tail scales keeled and slightly mucronate. Tail scales not arranged in whorls.

Coloration (ethanol preservation). Upper parts of the body and head brownish, a pale vertebral stripe from just behind the occiput along the body and extending on the tail is visible (Fig. 4A). The neck and anterior part of the dorsum are a dark brownish-gray color. A dark transverse band posteriorly to the forelimbs is present. Tail with dark brown, transversal, hourglass-shaped (when viewed dorsally) stripes starting just behind the cloaca and crossing the tail in regular intervals; the stripes do not extend onto the ventral side; the first and second stripe separated by seven light scales and the distance between the stripes diminishes posteriorly; the hourglass shape clear for the first four stripes, from the fifth on they become uniformly broad. Throat dark with a pattern of regular white spots or ocelli (Fig. 4D). Belly and the underside of the limbs and tail white (Fig. 4B).

Variation. All specimens resemble the holotype in overall coloration, morphology, and head scalation, except for the following variation: larger SVL (76.6 mm for NMP-

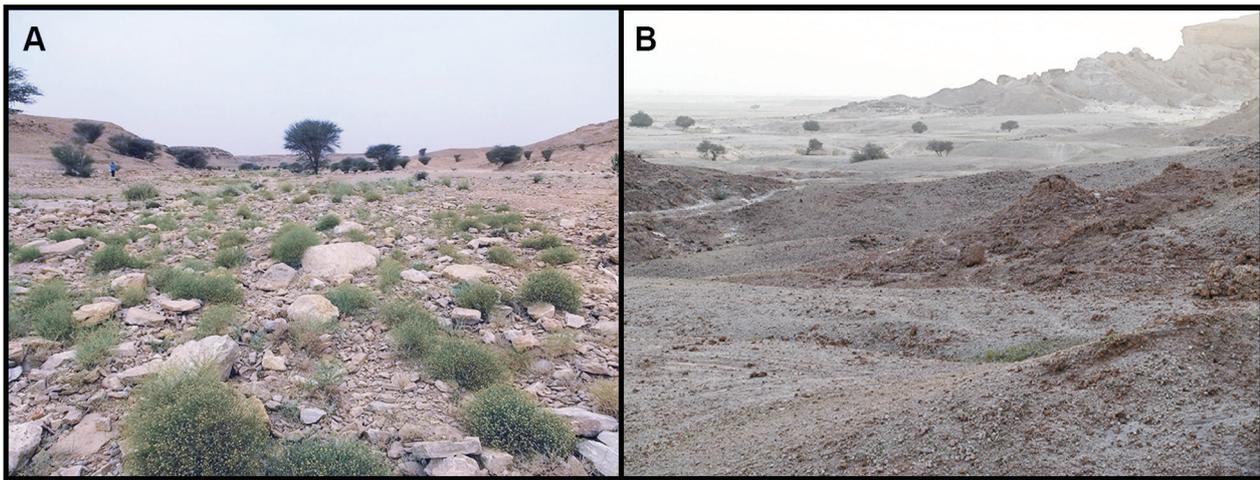


Figure 6. Habitat of *Pseudotrapelus tuwaiqensis* sp. nov. **A** Locality of specimen NMP-P6V 76637 (24.15093°N, 46.9317°E). Photo by Jiří Šmid; **B** Locality of paratype NMP-P6V 76636 (25.45933°N, 46.56276°E). Photo by Marius Burger.

P6V 76635; 78.8 mm for NMP-P6V 76636); shorter tail (122.1 mm for NMP-P6V 76636); lower number of upper and lower labials for NMP-P6V 76635 (14–15 in both); number of precloacal pores (4 for NMP-P6V 76635; 7 for NMP-P6V 76637).

Coloration in life. All specimens share the general coloration pattern (Figs 4H, 5). Body is light brown with faint light speckles scattered randomly on the body dorsum. Head is slightly darker than body and has a blue tint in females. The nape is dark. There are prominent dark brown transverse bars across the body, the first is behind the insertion of forelimbs and runs to about the middle of the flank. Another, shorter and less conspicuous dark band is situated before the hind limbs. In some specimens, another small transverse stripe is in the middle of the body, sometimes marked only as a small darker patch. The dark dorsal stripes are orange or have reddish outlines in females. All body parts are white from the ventral side except for the throat, which has a dark background with regular, white, and evenly spaced ocelli. In some specimens, the throat pattern is less discernible and the dark and light scales form a reticulated pattern.

Habitat and Distribution. *Pseudotrapelus tuwaiqensis* sp. nov. is a rock-dwelling species inhabiting rocky areas, outcrops, isolated rock mounds and even dry riverbeds with large boulders (Fig. 6). Like all other *Pseudotrapelus* species, it perches on top of stones and rocks during the day. Individuals were found sleeping at night tucked in rock crevices or laying on the ground in the open.

Currently available distribution data indicate that the species is endemic to Saudi Arabia, where it is confined to central Saudi Arabia around the city of Riyadh (Fig. 1). Most records come from the Tuwaiq Escarpment that runs from southwestern Arabia northeastwards to Riyadh where it bends to the northwest. The escarpment is bordered by massive sand seas: Rub al Khali from the southeast, Ad Dahna from the east, and An Nafud from the north, which seem to form a barrier to dispersal for these rock-dwelling agamas. There are several records west of

Tuwaiq, suggesting that the species' range extends more to the west and is not confined to the escarpment. More field data are however needed to delineate the geographic limits of its distribution and its possible contact with *P. aqabensis* in the west and north-west, *P. sinaitus* in the northwest, and possibly *P. neumanni* in the south-west. Other reptile species observed to live in syntopy with *P. tuwaiqensis* sp. nov. were *Bunopus tuberculatus*, *Stenodactylus doriae*, *Tropicolotes wolfgangboehmei* (all Gekkonidae), *Ptyodactylus hasselquistii* (Phyllodactylidae), *Acanthodactylus boskianus*, *Mesalina* lineage 4 (sensu Sindaco et al. 2018; Lacertidae), and *Echis coloratus* (Viperidae).

Discussion

A previous phylogenetic study of the agamid genus *Pseudotrapelus* disclosed an old cryptic lineage in the Arabian Peninsula (Tamar et al. 2019a). That study, using species delimitation analyses and phylogenetic relationships among currently recognized species, highlighted the presence of genetic differentiation and existence of a yet undescribed taxon within this genus. In this study, we investigated this old diversification event that has resulted in the description of a new species within *Pseudotrapelus*. We used an integrative approach applying phylogenetic analyses with morphological comparisons to assess the interspecific diversity of these agamid species distributed in the mountainous regions of Arabia and northeast Africa. The inferred topology of the concatenated dataset was congruent across analyses and supports the current taxonomy (Tamar et al. 2016b, 2019a). We support the differentiation of a lineage occurring in the mountains of central Saudi Arabia described herein as *P. tuwaiqensis* sp. nov., phylogenetically close to *P. sinaitus* and *P. chlodnickii*. According to our phylogenetic analyses and genetic distances, the genetic distinctiveness of *P. tuwaiqensis* sp. nov. is evident at both the mitochon-

drial and nuclear levels, not clustering with any samples of other members of the genus, as well as owning private alleles in the two nuclear genes analyzed, suggesting no signal of gene flow. Tamar et al. (2019a) included two samples of *P. tuwaiqensis* **sp. nov.** (the holotype and one paratype) and showed their distinctiveness using concatenated and species tree analyses, and mitochondrial- and nuclear based species delimitation analyses. These results further support the species status of *P. tuwaiqensis* **sp. nov.**

The current distribution of *P. tuwaiqensis* **sp. nov.** (specimens genetically identified) is known from around the city of Riyadh and is confined to the Tuwaiq Escarpment in Central Saudi Arabia (sightings are also known from the Ibex Reserve Protected Area located ca. 150 km south of Riyadh). The divergence time estimates of the clade comprising *P. tuwaiqensis* **sp. nov.**, *P. sinaitus*, and *P. chlodnickii* was approximately from the late Miocene to the Early Pliocene, around 6 million years ago (Tamar et al. 2019a). The aridification of Arabia, fluctuating climate, and the progression of sandy areas in the Arabian region during the Late Miocene (Edgell 2006; Preusser 2009) might have created distributional restrictions for the ancestral *Pseudotrapelus* populations, limiting them to the remaining rocky habitats. Although the current geographic distribution of *P. tuwaiqensis* **sp. nov.** seems to have been influenced by the formation of the sand seas of Arabia (Edgell 2006), it is not possible to draw any firm conclusions about the biogeographic history of the species, especially given the unsupported phylogenetic relationships and the complex and dynamic geological history of the area.

Thanks to the extensive DNA barcoding efforts across Arabia, we have fairly good knowledge on the distribution limits of the individual *Pseudotrapelus* species across the peninsula. Nonetheless, there are still regions that would require more attention both in terms of fieldwork and genotyping. One such region is eastern Yemen where *P. dhofarensis* and *P. neumanni* probably connect. Other such areas are the geographically intermediate, and somewhat isolated, populations in Hail Province in northwestern central Saudi Arabia and near Wadi ad-Dawasir close to the ‘Uruq Bani Ma’ arid protected area in the southwest of the country. Both are part of the Arabian Shield rocky desert that stretches from the western coast inland to the Arabian interior and is typical for rugged, rocky outcrops and isolated inselbergs (Edgell 2006), all of which represent an ideal habitat for *Pseudotrapelus*. It therefore seems likely that they belong to some of the western mountain species, probably *P. aqabensis*. However, the area around Wadi ad-Dawasir lies at the southern edge of the Tuwaiq Escarpment and it cannot be ruled out that *P. tuwaiqensis* **sp. nov.** occurs this far south along the ridge of the escarpment. These are, however, mere speculations that will need to be verified by means of DNA barcoding.

Interestingly, in the phylogenetic study of *Pseudotrapelus* by Melnikova et al. (2015), the authors hinted at the potential existence of an unidentified population in northern and central Saudi Arabia, that should be further taxonomically investigated. Their hypothetical pop-

ulation from Saudi Arabia around Riyadh most likely represents *P. tuwaiqensis* **sp. nov.** They also implied a possible hybridization between populations of *P. sinaitus* with that of the central Arabian species in the area of Al Mudawwara in southern Jordan. Our findings indicate no evidence of previous hybridization events between the two species, and propose complete reproductive isolation between *P. tuwaiqensis* **sp. nov.** and its geographically neighboring species. However, we must bear in mind that both our and Melnikova et al. (2015) results were based on a rather limited number of specimens, and thus more data and denser sampling of specimens and loci is needed to properly assess the distribution ranges and phylogenetic relationships within clade I of *Pseudotrapelus*.

Regarding conservation implications, considering the probable endemicity of the new species to central Saudi Arabia, its conservation status should be evaluated at national and worldwide levels, taking into account its limited distribution range, population density and trends, potential hybridization with other species, and future urbanization that is planned in the area of its occurrence. Interestingly, our field observations indicate that population densities of *P. tuwaiqensis* **sp. nov.** are much lower than those of other *Pseudotrapelus* species (e.g., *P. dhofarensis* in southern Oman, *P. sinaitus* in Jordan). In March 2023, a comprehensive field survey was conducted for three weeks by a team of four herpetologists actively searching for reptiles. The survey took place in habitats suitable for *Pseudotrapelus* within the known range of *P. tuwaiqensis* **sp. nov.** in the area north of Riyadh. Despite the efforts, the survey yielded the observation of only two individuals.

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Supplementary Material 1

Appendices 1–6

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Data type: .docx

Explanation note: **Appendix 1.** Information on the specimens used in this study for the concatenated dataset (16S, ND4, tRNA, MC1R, c-mos) and related GenBank accession numbers. — **Appendix 2.** Information on the specimens used in this study for the COI dataset and related GenBank/BOLD accession numbers — **Appendix 3.** Locality details and MorphoBank accession numbers of specimens whose high-resolution photographs have been deposited in the publicly accessible MorphoBank repository (<http://www.morphobank.org>; project number 4714). — **Appendix 4.** Mean uncorrected genetic distances (percentage) between (below diagonal) and within (diagonal) *Pseudotrapelus* species based on the mitochondrial markers used in this study. — **Appendix 5.** Diagnostic differences in the nuclear alignments (see Appendix 6) within *Pseudotrapelus* used in this study. — **Appendix 6.** Alignments (in fasta format) of the phased nuclear gene sequences of *Pseudotrapelus* for the molecular diagnostic characters used in this study.

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