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Morphological Variability of the *Eremias persica* Complex (Reptilia: Sauria: Lacertidae) on the Iranian plateau

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Abstract
Eremias persica is a complex and polymorphic species of lacertid lizards, distributed across Iran, Afghanistan, and Pakistan. Despite the recognition of some intraspecific variation as separate species, the morphological boundaries between the newly proposed species and other phylogenetic clades remain ambiguous. In the present study, we aim to identify the boundaries between populations and phylogenetic clades by evaluating the morphological characters of different populations within this species complex. Our morphological analyses of populations from 12 regions of the Central Iranian plateau revealed significant divergence within this lineage. While, sexual dimorphism was limited to minor variations in the ratio of tail length to snout-vent length and the number of collar scales. Our results identified six distinct morphological clusters within *E. persica*, which differed significantly from each other in characters such as head length, eye diameter, eye-to-tympanum distance, and abdominal width. Furthermore, a more limited morphological differentiation was traced among local populations in characters such as eye-to-tympanum distance, tympanum diameter, and abdominal width. In contrast to *E. nigrolateralis*, which differs from *E. persica* solely in dorsal coloration, *E. fahimii* and *E. rafiqi* were clearly distinguished from *E. persica* based on both morphology and dorsal coloration patterns. We also identified dorsal coloration patterns as a reliable diagnostic tool and a complement to morphological characters in this species complex.

Keywords: Lizard, Eremiadini, Sexual dimorphism, Biometry, Diagnostic characters, Discrimination.

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INTRODUCTION

The genus *Eremias* Fitzinger, 1834, comprising about 44 species and 12 subspecies, is one of the most diverse genera within the tribe Eremiadini in Eurasia, exhibiting a wide distribution range including Korea, eastern and central Asia, southeastern Europe, the Iranian plateau, Afghanistan, and Pakistan (Sindaco et al., 2008, Rastegar Pouyani et al., 2010, Uetz, 2025). The Persian racerunner, *Eremias persica* Blanford, 1875, is considered one of the most diverse species within the genus *Eremias*. Since its original description from a site near Isfahan, Iran, the species has been shown to occupy an extensive range across the Iranian plateau (Boulenger, 1921, Terentiev & Chernov, 1965, Anderson, 1999, Rastegar Pouyani et al., 2010). Recent findings indicate that prolonged isolation has led to significant morphological variation among *E. persica* populations across the Iranian plateau, giving rise to a complex of distinct species (Rastegar Pouyani & Nilson, 1997, Rastegar Pouyani & Rastegar Pouyani, 2001, Mozaffari et al., 2020). Phylogenetic studies have validated the existence of multiple geographical lineages, which have emerged as a result of the group's diversification across the Iranian plateau to Afghanistan and Pakistan (Rastegar

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Pouyani, 2007, Masroor et al., 2022). Nevertheless, to date no comprehensive studies have been carried out on the morphology of the populations within this species complex. Morphological data from certain described species in this group also remain unreliable due to number of reasons, including descriptions based on rare morphotypes (Rastegar Pouyani & Nilson, 1997), without molecular validation (Rastegar Pouyani et al., 2010), or taxonomic delineation relying on subadult specimens despite molecular evidence (Mozaffari et al., 2020).

The ambiguous morphological boundaries led to the genetic clades of *Eremias persica*, *E. rafiqi*, and *E. fahimii* being collectively considered as *E. persica* (sensu lato) in present study (Rastegar Pouyani et al., 2010, Mozaffari et al., 2020, Masroor et al., 2022). The distribution of *E. persica* (s. l.) in the Middle East is limited by the Zagros and Alborz Mountain ranges in western, southwestern and northern regions of Iran (Anderson, 1999, Szczerbak, 2003), suggesting that lineage emerged after the Alpine orogeny (about 9–11 Mya) in Iran, and has not crossed these mountain ranges since then (Rastegar Pouyani et al., 2010, Mohajjel & Fergusson, 2014). The habitat of *Eremias persica* (s. l.) typically consists of open plains and slopes, and areas with poor vegetation. However, it is absent from the central deserts Dasht-e Lut and Dasht-e Kavir, as well as the Registan Desert in southwestern Afghanistan (Rastegar Pouyani, 2007, Wagner et al., 2016). The distribution of this species across the Iranian plateau is characterized by a fragmented pattern, resulting from the presence of various elevations and desert regions in the central and eastern parts of the plateau (Rastegar Pouyani, 2007). This fragmentation has given rise to distinct geographical isolates, which have played a significant role in shaping the species' evolutionary trajectory (Rastegar Pouyani, 2007, Rastegar Pouyani et al., 2010).

The genetic variation within this complex species has been topic of considerable interest among herpetologists (Rastegar Pouyani et al., 2010, Mozaffari et al., 2011, Rastegar Pouyani et al., 2016, Ahmadzadeh et al., 2017, Mozaffari et al., 2020, Masroor et al., 2022). However, the predominant focus of studies on the molecular diversity of *E. persica* has overshadowed the contribution of morphological variations as the primary raw material shaped by natural selection (Lande, 1976, Wake, 2012).

The phenotypic and morphological variation of *Eremias persica* populations on the Iranian plateau, similar to that of other *Eremias* species (Hosseinian Yousefkhani et al., 2016, Chirikova et al., 2019), is strongly influenced by habitat type (Rastegar Pouyani & Nilson, 1997, Riki et al., 2015). Hence, habitat diversity and the resulting wide morphological variation within *E. persica* (s. l.) can be one of the reasons for preferring molecular studies over morphology (Rastegar Pouyani, 2007). While the status of phylogenetic clades of this lacertid lizard lineage has been substantially clarified after many studies on the Iranian plateau (Rastegar Pouyani et al., 2010, Khan et al., 2021), persistent discrepancies in morphological characterizations continue to impede the operational delimitation of putative cryptic species (Rastegar Pouyani & Nilson, 1997, Rastegar Pouyani & Rastegar Pouyani, 2001, 2005). The present study seeks to resolve the existing ambiguities in this lineage by addressing the morphological variations among populations and phylogenetic clades of *Eremis persica* (s. l.).

MATERIAL AND METHODS

Sampling and study system

Following the recommendations of Soulsbury et al. (2020), we prioritized the use of museum specimens to minimize anthropogenic impacts on populations of this species. The specimens utilized in this study, acquired on loan from the Zoology Laboratory of Hakim Sabzevari University, had been collected between 2002 and 2023 during both government-funded research projects (Iranian Ministry of Environment) and independent field campaigns. However, sampling of the gaps was unavoidable. Therefore, sampling was conducted to fill the existing gaps during June to July 2024. A total of 78 adult specimens (48 males and 30 females) of the target species were collected, covering almost the entire distribution range of the species in Iran (Figure 1, Appendix table 1). Here, samples from 12 sites in the central plateau of Iran were collected. Among these specimens, according to Rastegar Pouyani et al. (2010), six phylogenetic clades with significant genetic distance from the type locality near Isfahan province are present among the samples (Figure 1).

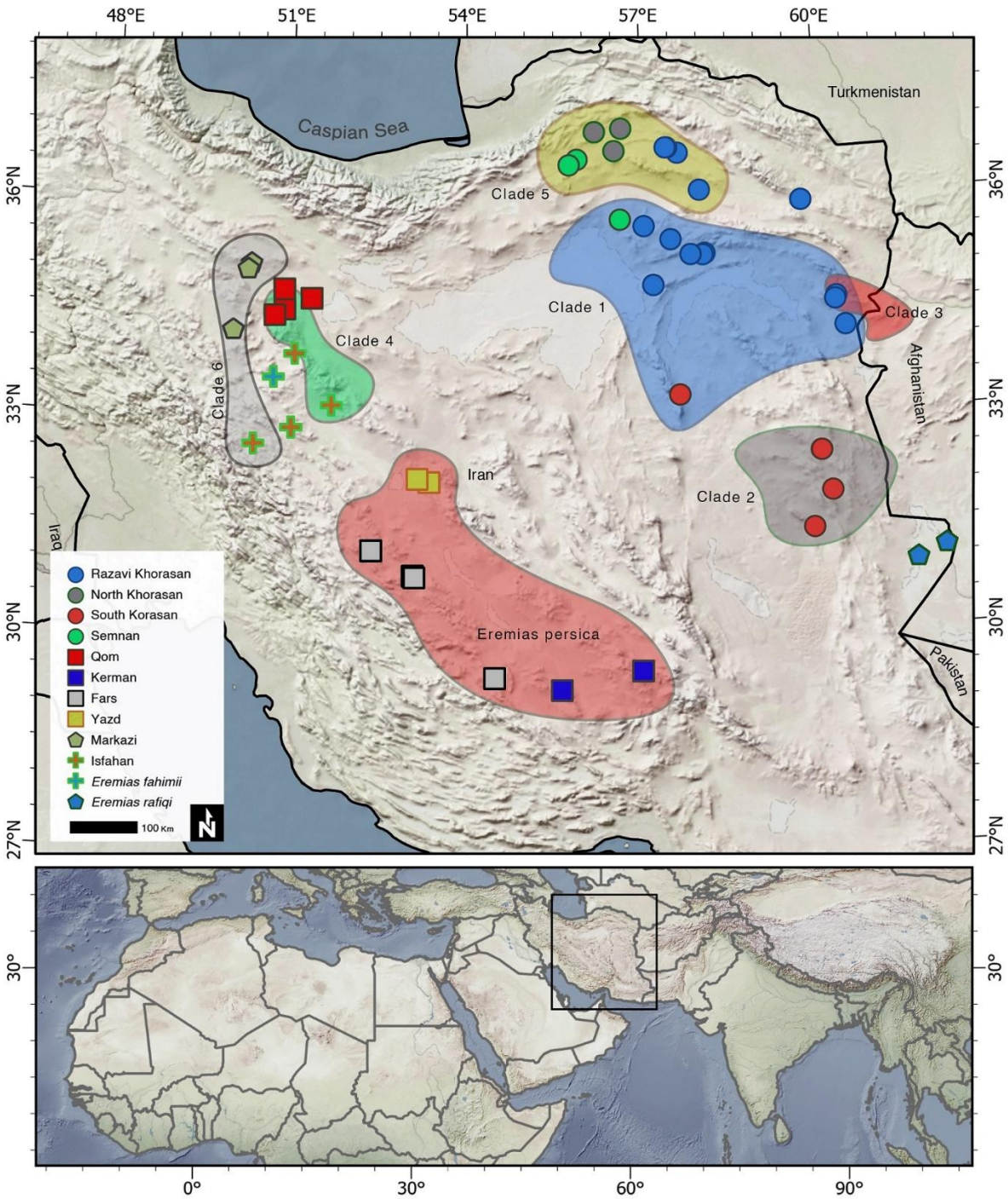

FIGURE 1. Sampling locations of the *Eremias persica* complex used in the present study, divided by phylogenetic clades and geographic location.

Specimens were euthanized using a conventional and approved method by inducing cooling followed by freezing (Shine et al., 2015), after recording the coordinates of the location and taking photographs. After humane killing, specimens were deposited in the Sabzevar University Herpetological Collection (SUHC). The entire research process was conducted in accordance with the approval of the Ethics Committee of Razi University of Kermanshah (Approval ID: IR.RAZI.AEC.1403.056).

The measurement of metric characters carried out using a digital caliper with 0.01 mm accuracy, enabling for precise quantification of metric traits. Additionally, meristic characters were examined and counted under a stereo microscope, and sex was determined by cloacal probing using a smooth, blunt instrument. The color patterns and other descriptive characters were also recorded and documented. Finally, a schematic of the dorsal color patterns was created based on the current dataset and observations extracted from iNaturalist.org (Van Horn et al., 2018), providing a visual summary of the observed patterns.

Overall, a total of 19 metric and 9 meristic characters were chosen from previous studies (Anderson, 1999, Rastegar Pouyani et al., 2016, Orlova et al., 2023), of which, six ratio characters were derived and used in the morphological analyses (Table 1).

Statistical analysis

Considering the sample size of the present study (<100), it is necessary to check the normality of the data (Mishra et al., 2019). Consequently, the data distribution was examined using SPSS Statistics V. 29.0.2.0 software (SPSS, 2023), using a combination of graphical and numerical methods, and the Kolmogorov-Smirnov test (Mishra et al., 2019). Outliers were identified using Mahalanobis distance test and box plots, and re-measured to verify the accuracy of the outlier identification (Kannan & Manoj, 2015). The presence of Sexual dimorphism was tested using Independent Sample t-test (2-tailed) (Kim, 2015), and Mann-Whitney U test (Mann & Whitney, 1947, Wilcoxon, 1992), for parametric and non-parametric data, respectively. Characters with significant sex-based differences (*P*-Value ≤ 0.05) were excluded from Operational Taxonomic Units (OTUs) variance analysis. In addition, Principal Component Analysis (PCA) was performed separately for all characters on gender. Following the exclusion of sex-dependent characters, the metric characters were adjusted using the *GroupStruct* R package, to remove the effect of body size variations (Thorpe, 1983, Chan & Grismer, 2021, 2022).

A pairwise comparison of characters between populations and phylogenetic clades were performed using parametric One-way ANOVA and non-parametric Kruskal-Wallis tests (Armstrong & Hilton, 2010). Multivariate analyses for all traits were performed using Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) based on the two considered OTUs. PCA reduces morphometric data redundancy, extracting independent variables for population differentiation. The DFA adopts a similar methodology as to the PCA, but its objective is to determine a linear function that accentuates the differences between groups, rather than simply reducing dimensionality (Davis, 1986).

RESULTS

Sexual dimorphism

We identified a weak sexual dimorphism in two characters of NCS (*P*-Value = 0.04) and the ratio of TL to SVL (*P*-Value = 0.03) (Figure 2, Table 2). Among these, TL/SVL can be a better indicator of sexual size dimorphism (SSD) (Braña, 1996, Liang et al., 2022, Candan et al., 2024), which is commonly observed in lacertids. However, the results of the PCA test show a high overlap of traits based on sex (Figure 2). We did not include these two characters in the analysis between OTUs in order to prevent the generation of error signals in our calculations.

Intergroup variation

Analysis of variance between OTUs revealed significant differences (*P*-Value ≤ 0.05) in the characters LWB, ETD and TD among populations, and HL, ED, ETD, LWB and IOD among phylogenetic clades (Table 2). Descriptive statistics for OTUs are provided separately for populations and phylogenetic clades in supplementary Tables S2 and S3, respectively.

TABLE 1. List of morphometric and meristic characters examined in the present study along with their abbreviations.

	Character	Definition
Metric	SVL	Snout-vent length (from tip of snout to anterior edge of cloaca)
	TL	Tail length (from posterior edge of cloaca to tip of tail)
	LHF	Trunk length (gleno-acetabular distance from axilla to groin, measured from the posterior edge of forelimb to the anterior edge of hind limb insertion)
	HL	Head length (from tip of snout to the posterior edge of tympanum)
	HH	Head height (maximum distance between upper head and lower jaw)
	HW	Head width (distance between posterior eye corners)
	LFL	Length of forelimb (from top of shoulder joint to tip of 4th finger)
	LHL	Length of hindlimb (from hip joint to tip of 4th toe)
	LFO	Length of femur (from hip joint to top of knee)
	LT	Length of tibia (from top of knee to beneath wrist)
	ED	Eye diameter (distance from anterior corner to posterior corner to its posterior)
	RED	Rostral length (from tip of nostril to anterior corner of eye)
	ETD	Distance between posterior edge of eye and tympanum
	NL	Length of neck (distance between posterior edge of tympanum and shoulder joint)
	TD	Tympanum diameter (largest size)
	IOD	Interorbital distance (largest size)
	LV	Length of cloaca crevice (largest size)
	LBT	Length of widest part of tail base
	LWB	Length of widest part of belly
Meristic	MNSLS	Mean Number of supralabial scales (Right/Left)
	MNILS	Mean Number of infralabial scales (Right/Left)
	NCS	Number of gular scales along mid-line of the throat
	NGS	Number of collar scales
	NLVS	Number of longitudinal rows of ventral scales, (Maximum count in the middle of the body)
	NTVS	Number of transverse series of ventral scales (counted in straight median series between collar and the row of scales separating the series of femoral pores)
	NDS	Number of dorsal scales across midbody
	MNSDLT	Mean Number of subdigital lamellae along underside of 4th toe (defined by their width, the one touching the claw included), counted bilaterally
	MNFP	Mean Number of femoral pores (Right/Left)
Ratio	TL/SVL	Tail length / snout-to-ventral length
	HL/HW	Head length / head width
	HW/HH	Head width / head height
	LHL/LFL	Hind limb length / forelimb length
	RED/HL	Rostral length / head length
	LHF/LWB	Trunk length / abdominal width

TABLE 2. Probability values of statistical tests based on three groups of sex, populations, and phylogenetic clades among measured characters for *Eremias persica* (sensu lato) on the Iranian plateau.

		Probability Value						
		Sexual dimorphism			Population		Phylogenetic clades	
	Character	Levene's Test	T test	U test	ANOVA	Kruskal-Wallis	ANOVA	Kruskal-Wallis
Metric characters	SVL	0.61	0.92		0.06	-	0.31	-
	TL	0.27	0.08		0.50	-	0.26	-
	LHF	-	-	0.55	0.39	-	-	0.87
	HL	0.93	0.87		0.38	-	**0.01**	-
	HH	0.96	0.65		0.28	-	0.14	-
	HW	0.75	0.74		-	0.86	-	0.61
	LFL	0.54	0.69		0.08	-	-	-
	LHL	0.24	0.18		0.80	-	-	-
	LFO	0.58	0.94		0.91	-	0.15	-
	LT	0.09	0.52		0.11	-	0.34	-
	ED	0.82	0.50		0.65	-	**0.03**	-
	RED	0.88	0.95		-	0.48	-	1.00
	ETD	0.93	0.45		**0.01**	-	**0.01**	-
	NL	0.43	0.75		0.45	-	0.30	-
	TD	0.25	0.31		**0.04**	-	0.58	-
	IOD	0.27	0.82		0.26	-	**0.01**	-
	LV	0.45	0.54		-	0.68	0.63	-
	LBT	0.55	0.22		0.09	-	0.21	-
	LWB	0.26	0.17		**0.05**	-	**0.03**	-
Ratio characters	TL/SVL*	0.34	**0.03**		*	-	*	-
	HL/HW	0.79	0.59		-	-	-	0.40
	HW/HH	0.51	0.59		0.58	-	0.96	-
	LHL/LFL	0.12	0.13		0.98	-	0.85	-
	RED/HL	-	-	0.63	-	-	-	0.61
	LHF/LWB	0.26	0.52		0.05	-	0.36	-
Meristic characters	MNSLS	-	-	0.22	-	0.06	-	-
	MNILS	-	-	0.99	-	0.17	-	0.51
	NCS*	-	-	**0.04**	-	*	-	*
	NGS	-	-	0.21	-	0.91	-	-
	NLVS	-	-	0.97	-	0.70	-	0.59
	NTVS	-	-	0.47	-	0.46	-	0.44
	NDS	-	-	0.27	-	0.17	-	0.22
	MNSDLT	-	-	0.92	-	0.15	-	0.43
	MNFP	-	-	0.25	-	0.29	-	0.14
	NFPR	-	-	0.27	-	0.54	-	0.26

* Sex- dependent character

TABLE 3. Pairwise differentiation matrix of *Eremias persica* complex phylogenetic clades, *E. fahimii*, and *E. rafiqi* based on significant morphological characters (*P*-value≤0.05).

	Clade 1	Clade 2 (Type)	Clade 3	Clade 4	Clade 5	Clade 6	*E. fahimii*	*E. rafiqi*
Clade 1		HH-HW-RED-ETD-NL	HL	HL-HH-LFL-LHL-LFO-ED-ETD-MNSLS	HL-HH-HW-LFL-ED-IOD-LBT-MNSDLT	LT-ED-MNSDLT	NTVS-NDS	MNSLS
Clade 2 (Type)	HH-HW-RED-ETD-NL		ETD	LFL-LHL-ED		IOD-LWB-MNILS	LWB-MNILS-NTVS-NDS-MNFP-NFPR	
Clade 3	HL	ETD		TL-LFL-ETD-MNSLS	MNSDLT	IOD-LWB-MNSDLT	LWB-NTVS-NDS	SVL-MNFP
Clade 4	HL-HH-LFL-LHL-LFO-ED-ETD-MNSLS	LFL-LHL-ED	TL-LFL-ETD-MNSLS		LHF-ETD	LHF-HL-LFL-LHL-ETD-IOD-LWB-MNSLS	TL-LHF-HL-LHL-LFO-ETD-LWB-MNSLS	LFO-ETD
Clade 5	HL-HH-HW-LFL-ED-IOD-LBT-MNSDLT		MNSDLT	LHF-ETD		NTVS	LWB-NTVS-NDS	
Clade 6	LT-ED-MNSDLT	IOD-LWB-MNILS	IOD-LWB-MNSDLT	LHF-HL-LFL-LHL-ETD-IOD-LWB-MNSLS	NTVS		IOD-NDS	IOD
E. fahimii	NTVS-NDS	LWB-MNILS-NTVS-NDS-MNFP-NFPR	LWB-NTVS-NDS	TL-LHF-HL-LHL-LFO-ETD-LWB-MNSLS	LWB-NTVS-NDS	IOD-NDS		MNSLS-NTVS-MNFP
E. rafiqi	MNSLS		SVL-MNFP	LFO-ETD		IOD	MNSLS-NTVS-MNFP	

Considering locations as OTUs, multivariate principal component analysis identified HL, LHF/LWB, and LHL as the most weighted and the main effective characters for PC 1, and HL/HW, HW, and TL for PC 2, in separating populations. Meanwhile, discriminant function analysis identified LWB, LFL, and HH most informative characters for the first function, and IOD, LT, and ETD for the second function, for the separation of populations. The informative characters between phylogenetic clades, in the principal component analysis, were HL, HW, TD, and ETD for PC1, and MNFP, LHF/LWB, NFPR, and MNSDLT for PC2. Furthermore, the discriminant function analysis scores identified NL, HL, NDS,

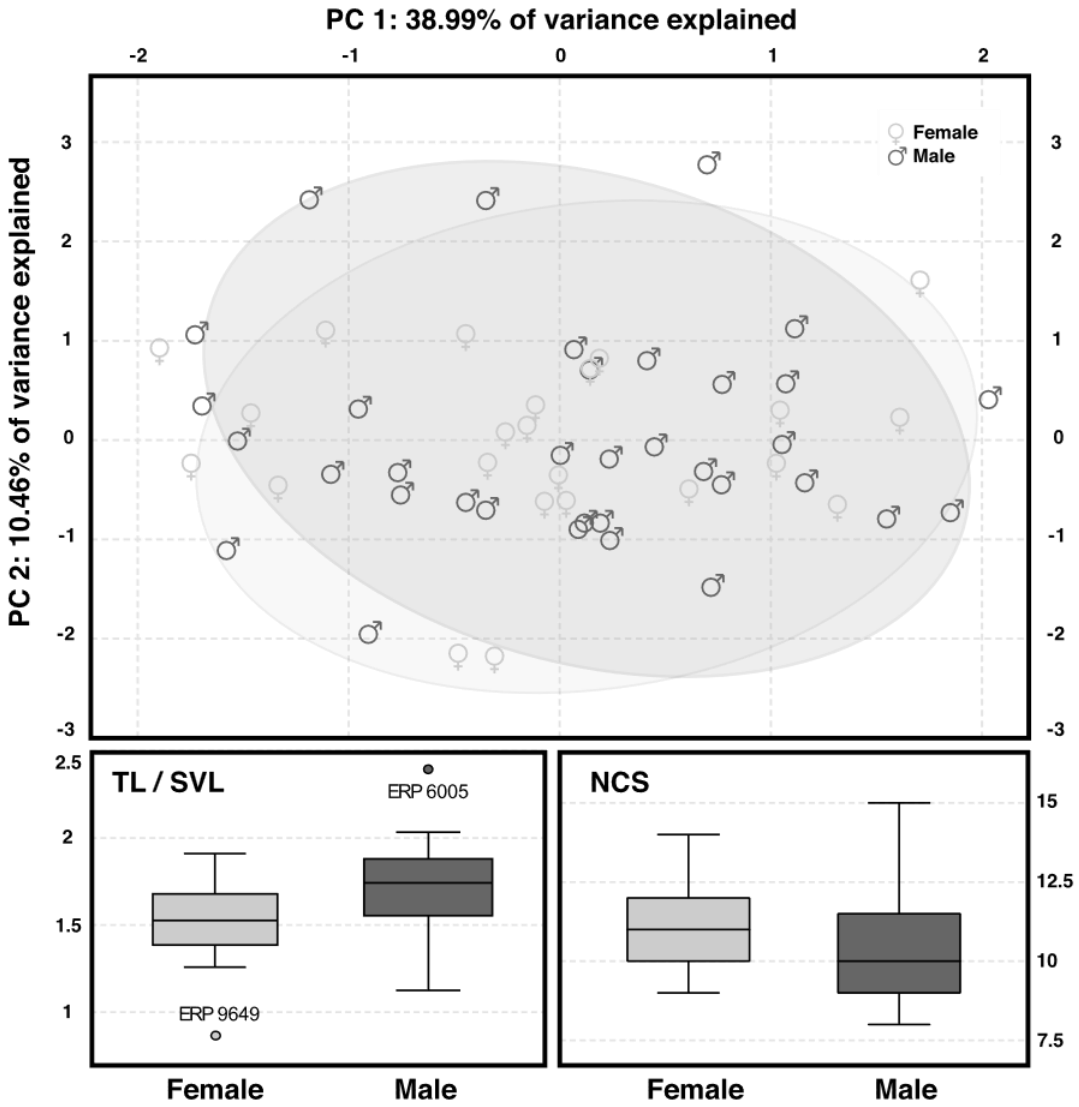

FIGURE 2. Scatter plot of the first and second principal component scores based on sex among the studied specimens, Upper; Box plot of NCS and TL/SVL characters based on sex, Bottom.

and RED for the first function, and ETD, NL, MNILS, and MNSLS for the second function, as the most weighted characters, in separating the clades. The scores of principal component analysis and discriminant function analysis for the first three components or functions are presented in supplementary Tables 4 and 5, respectively, based on locations and phylogenetic clades.

The scatter plots of DFA and PCA clearly show the differentiation of traits between populations and phylogenetic clades (Figures 3, 4). However, the distribution pattern of the distinct functions reveals that the differentiation of phylogenetic clades is more pronounced and evident than among geographic localities, indicating a clearer distinction between the clades. Based on DFA and PCA results and Table 3, the phylogenetic clades, exhibit significant levels of morphological divergence and *E. fahimii* and *E. rafiqi* are distinguished from the rest. Fars population as type locality of *E. nigrolateralis*, exhibits a lesser degree of separation compared to the other two species, *E. fahimii* and *E. rafiqi* (Figure 3). Finally, pairwise comparisons based on significant morphological characters between populations and between phylogenetic clades, as presented in Tables S6 and 3, respectively, provide a clearer delineation of divergence boundaries among these groups.

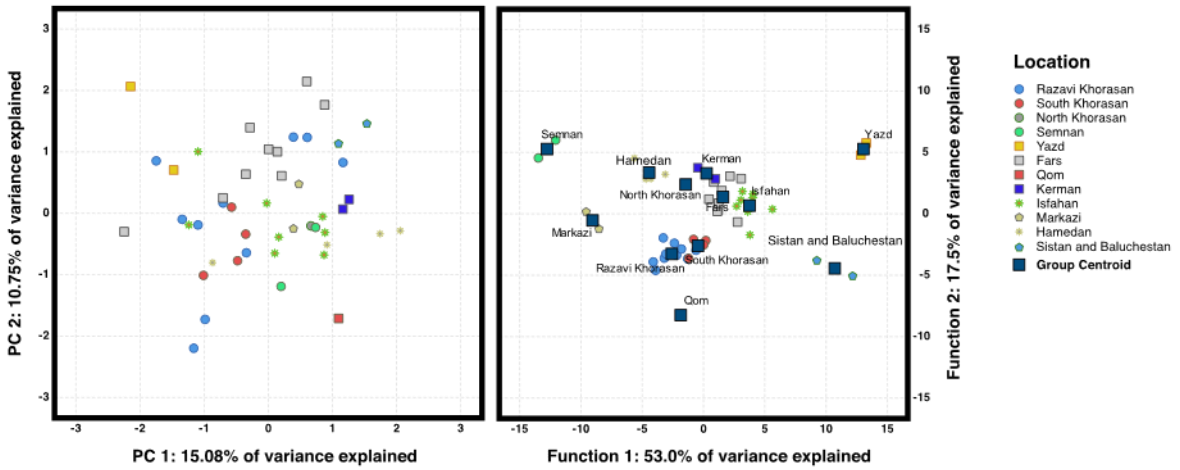

FIGURE 3. Scatter plots of the first and second principal components (PCA), left; First and second discriminant functions, right; Based on ten sampling sites of *Eremias persica* complex and *E. fahimii* from Hamedan Province, and *E. rafiqi* from Sistan and Baluchestan Province on the Iranian plateau.

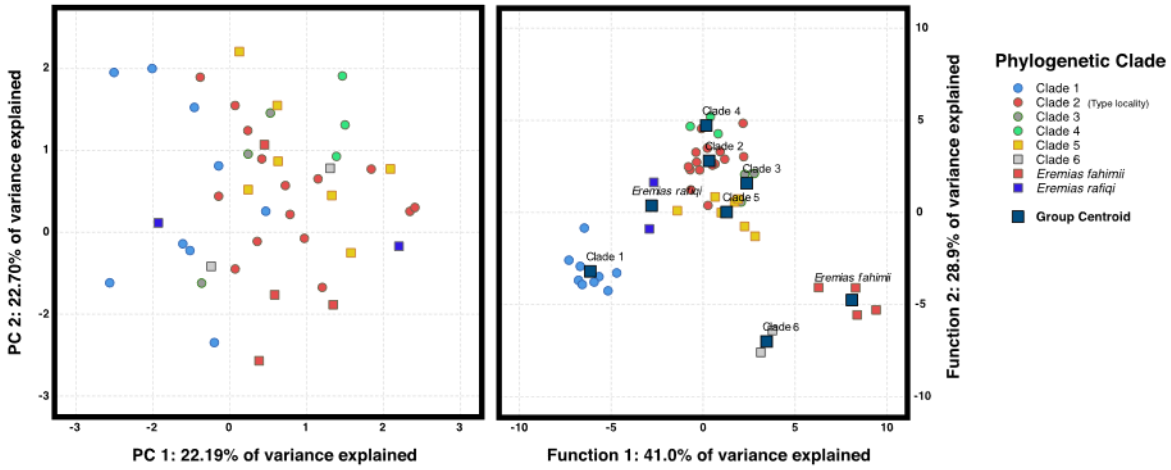

FIGURE 4. Scatter plots of the first and second principal component analysis, left; first and second discriminant functions, right; based on six phylogentic clades of the *Eremias persica* complex and *E. fahimii*, and *E. rafiqi* on the Iranian plateau.

Color and pattern variation

On the Central Iranian Plateau, thirteen dominant dorsal pattern morphs were recognised in in the adult form of *Eremias persica* (s. l.) (Figure 5). In some areas, such as the southern part of Kavir National Park, the western area of the Kalmand protected area, southern Yazd, and Ahmadabad village in Semnan Province, pattern of 14 has been observed, which is related to the sub-adult form. In the populations of Razavi Khorasan, patterns 8, 7, and 10 can be observed, while in the northeast and North Khorasan, dorsal pattern 4 is dominant, and in Semnan, pattern 3 is prevalent. Patterns 1 and 9 are seen in South Khorasan and Fars, but pattern 13 exclusively belongs to *E. nigrolateralis* from Fars and western Yazd Provinces, and in Qom, two distinct patterns, 6 and 2, can be observed. Additionally, from north of Qom to south of Tehran, the pattern 5 is seen in the populations. The patterns 11 and 12 are unique to *E. fahimii* and *E. rafiqi*, respectively, making them readily distinguishable from other species.

Adaptation to various environments has given rise to a diverse range of patterns on *E. persica* s. l., which is also evident in their coloration. In the north-east and south, the dorsal region is beige to ochraceous, while the front limbs are cream-colored, and the hind limbs and tail are cream to earthy in the

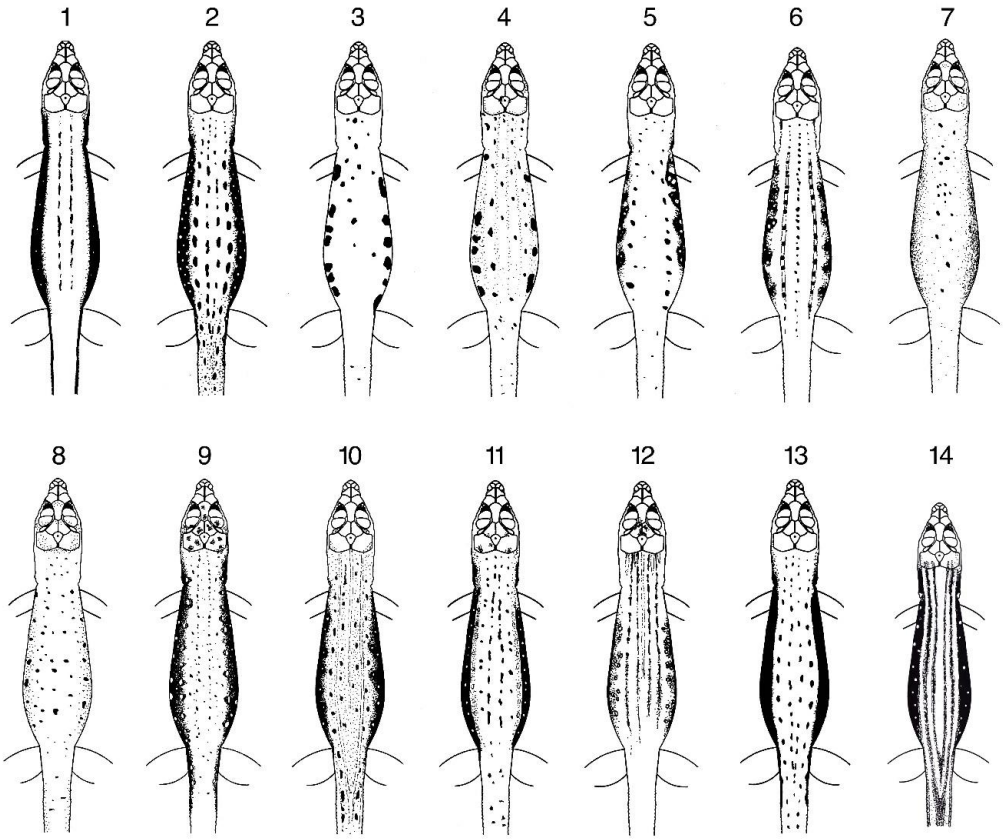

FIGURE 1. Variations in dorsal color patterns among populations of *Eremias persica* (1-10), *E. fahimii* (11), *E. rafiqi* (12), and *E. nigrolateralis* (13); Sub-adult form of *E. persica* (14); on the Iranian plateau.

southern population and grullo in the north-eastern population. In the central plateau region, the coloration is darker, with silver to fuscous colors. *Eremias rafiqi* has a lighter coloration, with a pastel gray head and viridian dorsal region, accompanied by olivaceous stripes. *Eremias fahimii* has a dominant viridescence to emerald green coloration on its head and dorsal region, with thick fuscous stripes.

DISCUSSION

Distinct lineages within *Eremias persicus* (s. l.) are suggested by the observed morphological diversity, mirroring the genetic variation previously reported in this complex species by Rastegar Pouyani et al. (2010). The morphological diversifications in *E. persicus* (s. l.), supported by molecular data, have been shaped through a divergence event dated to approximately 7–10 Mya (Rastegar Pouyani, 2007) , which has been coincident with a geological event responsible for the current configuration of the Zagros and Alborz Mountain ranges (Allen et al., 2004, Khadivi, 2010).

Sexual dimorphism in this group, similar to other *Eremias* species, is hardly recognizable (Orlova et al., 2017, Chirikova et al., 2019). Although males have a higher TL/SVL and females have more NCS, but sexual dimorphism is absent in other characters. The populations of *E. persica* (s. l.) overlap in meristic characters, making them less distinguishable from one another. In contrast, mensural traits exhibit the highest degree of differentiation among populations and phylogenetic clades. Morphological clustering is relatively apparent in *E. persica* (sensu stricto) populations on the Iranian plateau, particularly in characters related to escape strategy, especially those associated with the head region. Specifically, characters related to the head region (such as head length and snout length) and abdominal muscles reflect a unique evolutionary adaptation in the Eremiadini tribe of lizards, which is noteworthy in itself (Edwards et al., 2016, Hosseinian Yousefkhani et al., 2022). Habitat diversity on the Iranian plateau

(Dehshiri, 2018), habitat preferences, and resulting ecological limitations are the second factor that, in turn, create functional and morphological differences (Vanhooydonck & Van Damme, 1999). The adaptation of characters related to limbs and body shape (e.g., LHL, LFL, TL, LWB, and LHF/LWB in our study) is shaped by specific conditions and environmental pressures (Herrel et al., 2002). This adaptation has been observed in numerous groups of reptiles under environmental conditions (Hosseinian Yousefkhani et al., 2014, Kamali et al., 2024). In desert lacertid lizards, such as *E. persica*, which occupy relatively simple and open environments compared to their montane counterpart *E. montana*, longer hind limbs are observed (Losos, 1990, Herrel et al., 2002). This adaptation leads to increased speed and sprinting ability, rather than maneuverability. Changes in head shape are another outcome of habitat preferences (Vicent-Castelló et al., 2025). Our findings showed that HL, ED, ETD, IOD, and LWB were significantly different among phylogenetic clades, while LWB, ETD and TD were also distinct at the population level.

The presence of morphological variations in this lineage of Lacertidae, as first interpreted by Arnold (1989) for other genera of Eremiadini, can be considered as synapomorphies that arose under the influence of an adaptive radiation process (Mayer & Pavlicev, 2007). This cladogenesis may have been influenced by the formation of fragmented arid habitats resulting from the gradual cooling and desiccation of the Iranian plateau (Kargaranbafghi & Neubauer, 2018, Sun et al., 2021). Considering that lacertid lizards typically exhibit a highly conserved morphology (Arnold, 1987, Arnold, 1989), Consistent with the findings of Hosseinian Yousefkhani et al. (2013) and Boroumand et al. (2024), the morphological variations present in the *E. persica* complex may also indicate the existence of distinct lineages or species.

Considering the pattern of distribution of morphological clusters in the present study (Figure 1)and phylogenetic clades in Rastegar Pouyani et al. (2010), it can be inferred that, it is likely that geographical separations (e.g., physical barriers, isolation) contributed to the formation of the lineage. In contrast to the sister lineage, *E. montana*, where orogenesis has primarily acted as a corridor (Rastegar Pouyani & Rastegar Pouyani, 2005, Rastegar Pouyani, 2007), the speciation of the *E. persica* lineage was likely driven by the orogenic events of the Iranian plateau, which served as a barrier to population separation, and habitat loss and desertification may have intensified this process (Rodwell & Hoskins, 1996).

Concurrent with the Zagros Mountains reaching their mid-elevation in the Oligocene, the Alborz orogeny in the north began in the early-middle Miocene and continued until the late Miocene (Hatzfeld & Molnar, 2010, Pirouz et al., 2017). The initial populations of the *E. persica* lineage, after gaining access to a new and vast niche on the Iranian plateau, dispersed throughout the region, entering the area while the Kopet Dagh and Khorasan mountains were forming in the northeast and east of the country (Robert et al., 2014).

Accordingly, the similarity between all measured mensural traits between the populations of Fars and Kerman provinces in southern Iran can be attributed to the integrity and similarity of their habitats (Table S6), something that is not observed in the isolated population of western Yazd province, despite genetic similarity (Rastegar Pouyani et al., 2010). As previously suggested by Rastegar Pouyani et al. (2010), *E. nigrolateralis* can be considered conspecific with the type population in southern Isfahan in most morphological characters. It is emphasized that the most significant morphological characteristic of *E. nigrolateralis* is its color pattern, which has also been observed in certain subadult forms of eastern populations and adult forms from western population of Yazd Province. In conclusion, despite *E. nigrolateralis* being rejected as one of the Phenotypes within *E. persica*, it is proposed that the morphological clusters previously separated by molecular data (Rastegar Pouyani et al., 2010), should be classified as subspecies or distinct species. Future comprehensive studies on the phylogeny of this lineage, addressing the gaps in previous research, can help clarify the remaining ambiguities regarding the taxonomy of the clades within this species complex. Additionally, examining the changes in head shape as a strong taxonomic signal in this group of lacertid lizards can be helpful. (see Edwards et al. 2016). Also, identifying the suitable niches and the degree of habitat overlap between the morphological and genetic clusters of *E. persica* can provide valuable insights.

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SUPPLEMENTARY MATERIAL
Supplementary material is available at Iranian Journal of Animal Biosystematics online.

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Distribution and habitats of Near Threatened Iranian stream salamander *Paradactylodon persicus* (Eiselt & Steiner, 1970) (Caudata: Hynobiidae)

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Abstract

The mountain salamanders of the genus *Paradactylodon* Risch, 1984 of the family Hynobiidae with three known species are distributed in Iran and Afghanistan. Based on morphological characteristics, the genus *Paradactylodon* consists of three species *P. mustersi* (Afghanistan), *P. persicus* and *P. gorganensis* (Iran); the Iranian stream salamander *P. persicus* was first reported from the Talesh Mountains and then the Gorgan cave salamander *P. gorganensis* was described from the eastern Alborz Mountains; the two Iranian *Paradactylodon* species are distinct based on morphological characteristics. Due to its conservation significance and numerous threats, this taxon is classified as Near Threatened on the IUCN Red List. The distribution range of *P. persicus* is Ardabil, Guilan, and Mazandaran Provinces, and *P. gorganensis* is Golestan Province. Initially, the study involved reviewing previous research, consulting local residents and experts, and conducting field visits to collect data and photographs of both known and newly discovered habitats. Two specimens of the Persian salamander (*Paradactylodon persicus*) were reported on 8 May and 4 August 2024 from Masal Mountain, Chesli Village (37°16'49" N and 49°2'17" E; 375 m above sea level) from Guilan Province (western part of Alborz Mountain Range, northern Iran). The first report of this species in 1970, was based on five salamander larvae found in a stream at an altitude of 800 meters in the Asalem region of the Talesh Mountain Range in the western Alborz Mountains. this research aims to find new habitats and assess the conservation status of the Iranian salamander *Paradactylodon persicus*, and suggests that habitat changes should be prevented population decline and further protect this species.

Keywords: Iranian salamander, habitat, distribution, *Paradactylodon persicus*, Guilan Province.

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INTRODUCTION

Amphibians are a group of vertebrates that are evolutionarily after fish and before reptiles (Kent & Miller, 1997). The habitat of all Iranian tailed amphibians is in the mountainous areas or the edge of the Alborz and Zagros forests (Safaei-Mehro, 2014). Among these areas (habitats) are the western Alborz mountain range (Ardabil, Guilan and Mazandaran Provinces) and the eastern Alborz (Golestan Province). Schmidtler and Schmidtler (1971) collected a number of larvae from the type locality; they gave a brief description of the young salamanders after their metamorphosis in captivity. In 1978, a new population of Hynobiid salamanders was discovered by Clergue-Gazeau and Thorn in the eastern Alborz mountains in Golestan Province; the species *Paradactylodon gorganensis* was described based on morphological features of a single adult specimen (Clergue-Gazeau & Thorn, 1978). The second Iranian Hynobiid taxon

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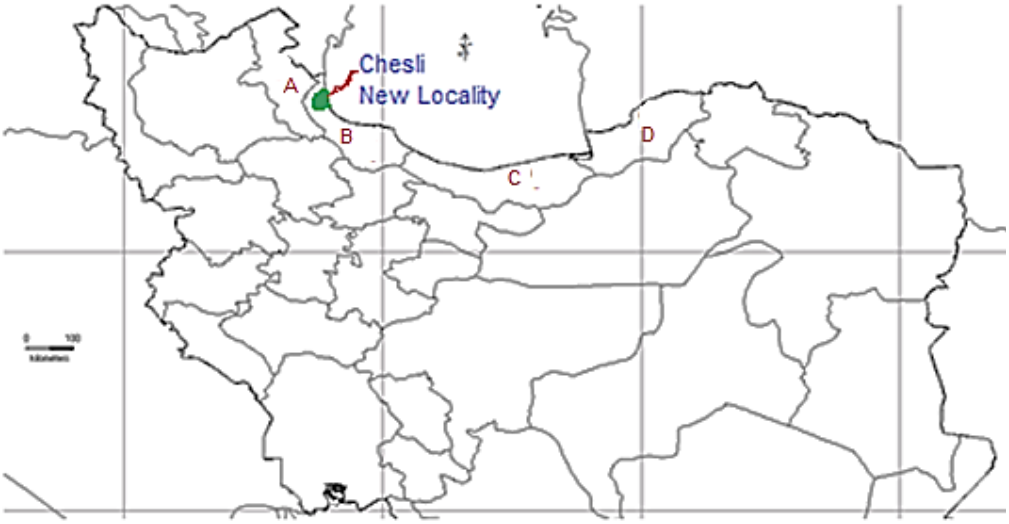

FIGURE 1. Distribution of *Paradactylodon persicus* in Ardabil (A), Guilan (B) and Mazandaran (C) Provinces and distribution of *Paradactylodon gorganensis* in Golestan Province (D) **.**(Green dot on the map of Guilan Province is the new location of the description of two salamander specimens) (Chesli, Masal, Guilan).

is based on 23 cm long adult male specimen kept in the National museum of Natural History in Paris (MNHN); this cave salamander was discovered on the eastern edge of the Hyrcanian Corridor (Shirabad cave, Golestan Province) and was only later described as *Batrachuperus gorganensis* (Clergue-Gazeau & Farcy, 1978). Based on a study conducted by Risch (1984), the salamanders taken between Gorgan and Aliabad were introduced with the name *Paradactylodon gorganensis* and he considered the name *Paradactylodon* as a valid name. However, the morphological evidence for such a decision was not convincing enough and the name was quickly synonymized with *Batrachuperus* by Reilly (1987). The first species of this genus, *Paradactylodon mustersi*, was described by Smith (1940) from the Paghman Mountains near Kabul, Afghanistan. Stöck (1999) compared the morphological features of larval and subadult topotype specimens of *P. persicus* and *P. gorganensis* and introduced *P. gorganensis* as a subspecies. The Iranian stream salamander was reported by Kami (2004) and Ebrahimi et al. (2004) from two locations: the village of Vissar in southeastern Chalus, Mazandaran Province, and the village of Deilmadeh in southeastern Khalkhal, Ardabil Province. The Iranian stream salamander *Batrachuperus persicus* has been transferred to the genus *Paradactylodon* based on genetic studies by Zhang et al. (2006). The name *Iranodon* has also been used for the genus *Paradactylodon* by a group of authors (Zhang et al. 2006; Raffaëlli, 2007; Poyarkov, 2010; Dubois & Raffaëlli.2012). Ahmadzadeh et al. (2011) reported *Paradactylodon persicus* in the Dasht-e Doman region of Rezvanshahr, Guilan Province. This species is a protected species under national laws.The Gorgani cave salamander is one of the endangered species, the only habitat of this species is a cave in Shirabad, Golestan Province (Yousefi siyahkalroudi et al., 2015). Using the skeletochronology method, the age structure of *Paradactylodon gorganensis* has been investigated, and the lifespan of females is 13 years and that of males is 11 years (Zivari & Kami, 2017). Stöck et al. (2019) have investigated the genetic structure of the western (*P. persicus*) and eastern (*P. gorganensis*) taxa and proposed that they belong to a single species *P. persicus s.l.* The distribution range of *P. persicus* is Ardabil, Guilan and Mazandaran Provinces and *P. gorganensis* Golestan Province. The aim of this study is to report a new record of the distribution of the western taxon (*P. persicus*) in the Guilan and Ardabil regions and to compare the morphological and biometric characteristics of the specimens studied in these regions.

FIGURE 2. Adult Persian Salamander *Paradactylodon persicus*, 8 May 2024 (Chesli, Masal, Guilan Province).

MATERIAL AND METHODS

This research was conducted in the form of a field visit to the areas and stations of Guilan Province in the months of May, June, July, August and September, using previous sources (research and articles) and obtaining information from experts and local people. By describing and showing people photos of the salamander, awareness of its presence or absence in a specific area was obtained. Then, with great effort, the desired location was reached and the samples collected in their habitat were recorded, photographed, biometrics and tissue samples from the fingers and tail were obtained. In order to protect them, the species were released alive in the same place where they lived. A new record of the Iranian salamander in the Masal mountains and Chesli village of Guilan Province (western part of the Alborz mountain range, northern Iran), of which two specimens of the Iranian salamander (*Paradactylodon persicus*), the first specimen was reported on 8 May and the second specimen on 4 August 2024 .After collecting the samples by hand, they were identified based on morphological identification key Yousefi SiahKalroodi et al. (2013). These Iranian salamander specimen was reported in a salmon pond in the village of Chesli, which is located about 15 Km from Masal and at an altitude of about 375 m above sea level. This pond is located in the heart of the forest and its water supply is also from springs in the upper regions; Since the only way water enters this pool is through the canal and water pipe, it seems that these salamanders entered this pool through the canal and water pipe, which was then identified and diagnostic traits were reported (Fig. 1).

Habitat: This species lives in cold streams and small pools among dense Hyrcanian forests and highlands where the water temperature is 13-15°C.

RESULTS

From two specimens of the Persian salamander (*P .persicus*) that were caught on and in the village of Chesli, Masal County, Guilan Province, at an altitude of about 375 m above sea level, the time interval between the two specimens was about 88 days, and both specimens had passed the larval stage and breathed by the lungs and were in the juvenile stage, and both specimens were almost similar in terms of age, size, and appearance (Fig .2). Both specimens entered the pond space from a spring located in higher areas through canal water, meaning that the spawning and larval stages and entry into the juvenile stage took place in the same upstream spring and then entered the pond through canal water (Fig.3). In macroscopic examination, the specimens have a large and broad head that gradually compressed towards the neck, palatal blade teeth arranged in two arcuate rows, the locomotor organs have four fingers without nails, a wide tail compressed from the sides and a dorsal ridge in the upper area that extends along theentire length of the tail. This apparent structure of the tail helps the animal in swimming; it has 10

FIGURE 3. Habitat and new locality report of the Iranian salamander *Paradactylodon persicu*s (Chesli, Masal, Guilan).

FIGURE 4. Young Persian salamander *Paradactylodon persicus* (Gilvan, Khalkhal, Ardabil Province).

lateral grooves and a distinct vertebral groove .It has smooth skin and numerous pores that play a role in the animal's skin respiration. The dorsal area is dark or light brown and sometimes yellowish, but the lower body and sides are lighter and often pinkish or yellow .Also, irregular light yellowish, pinkish or orange lines or spots are seen in the specimens. This coloring is uniform throughout the body .The eyes are black, large and prominent .The background color of the body in living specimens is yellowish gray or deep purple with a metallic sheen with pale yellow spots. In part of the body and in the tail area, it has blackish brown spots and the abdominal area is somewhat brighter and more transparent .In these young specimens, the skin is completely smooth. The length of the tail in adult specimens is smaller or larger than the total length of the head and body .Four fingers are seen on the forelimbs and also four fingers on the hind limbs, all of which are pale, thick and circular (Table 1).

FIGURE 5. Habitat of the Persian salamander *Paradactylodon persicus* (Gilvan, Khalkhal, Ardabil Province).

The salamanders described from Gilvan village, Khalkhal county, Ardabil Province in September 2024 (37° 18' 6" N and 48° 48' 41" E); altitude about 1735 mabove sea level, compared to the sample of the newts reported from Chesli village, the dorsal area is darker (difference) and also irregular lines or spots of yellowish, pinkish or orange color are seen in the samples from both Ardabil and Guilan Provinces (Fig. 4). This coloring is the same throughout the body of the salamanders from both Guilan and Ardabil Provinces (similarity). It seems that this difference is due to the habitat of the salamander, the geographical conditions of the village of Gilvan khalkhal and the altitude above sea level. Due to its high latitude, Ardabil Province is considered as one of the cold Provinces of the country and it should be noted that the annual air temperature of Ardabil Province is cooler in summer and colder in winter than that of Guilan (Fig.5) .The approximate distance from the place of description of the Gilvan Khalkhal salamanders from Ardabil Province to the place of description and report of the salamanders of the village of Chesli Masal, Guilan Province, is about 60 km (Table 1).

TABLE 1. Metric characters of specimens of the Persian salamander *Paradactylodon persicus* (Guilan and Ardabil province).

	Collection date	Fishing location	Hind legs mm	Front legs mm	Tail size mm	Head size mm	Body size (head and Trunk) mm
1	8 May 2024	Chesli, Masal, Guilan	11.23	9.75	39.41	11.75	44.03
2	4 August 2024	Chesli, Masal, Guilan	9.79	9.04	31.71	10.20	40.18
3	6 September 2024	Gilvan, Khalkhal, Ardabil	12.75	12.03	47.75	10.22	50.57
4	6 September 2024	Gilvan, Khalkhal, Ardabil	14.59	12.22	46.47	13.10	57.90

DISCUSSION

Batrachuperus Boulenger, 1878 are mainly distributed in the Hengduan Mountains, southwest China with the most complex topography on Earth; *Batrachuperus* species Boulenger, 1878 occupy mountain streams at altitudes of 1500 to 4200 m above sea level and are aquatic all year round (Zhao & Yang 1997). The genus *Paradactylodon* is widespread in the mountainous regions of the Middle East. This genus consists of three species: *Paradactylodon mustersi* (Afghanistan), *Paradactylodon persicus*, and

Paradactylodon gorganensis (Iran). Initially, *P. persicus* was reported from the Talesh Mountains, and then *P. gorganensis* was described from the eastern Alborz Mountains; The two Iranian *Paradactylodon* species are distinct based on morphological features; According to species distribution models (SDMs) and molecular (two mitochondrial DNA markers, 16S and COI) analyses the Iranian *Paradactylodon* populations were affected by the Quaternary glaciation and, based on haplotype networks, haplotype diversity was higher in the western part of the species range and, given the low genetic distance among all specimens; It has been suggested that *Paradactylodon gorganensis* is synonymous with *Paradactylodon persicus*; but Populations from the eastern Alborz Mountains of Iran have long been known as *Paradactylodon gorganensis*; Ahmadzadeh et al. (2020) used mitochondrial DNA markers to study geographic diversity and placed *Paradactylodon gorganensis* within *Paradactylodon persicus*; Dubois and Raffaelli (2012) considered the name *Paradactylodon* invalid and replaced it with the name *Iranodon*; but the amphibian website (Amphibia Web.org) still uses the name *Paradactylodon*. The Hyrcanian forests are a unique Tertiary relict ecosystem covering the northern slopes of the Alborz and Talesh (Iran, Azerbaijan) and are a unique biodiversity hotspot with many cryptic species that have not been studied (Stöck et al. 2019). According to the International Union for Conservation of Nature (IUCN) Red List, these two Iranian hynobiid species are considered "critically endangered" (*P. gorganensis*) or "near threatened" (*P. persicus*); These salamanders can be considered as biological indicators for "intact and healthy" riverine ecosystems of Hyrcanian forests; however, in some cases, *Paradactylodon persicus* may survive for decades, even after human deforestation has long destroyed native vegetation (Ahmadzadeh & Kami, 2009). As long as intact streams or springs allow remnant populations to survive, these Hynobiids have survived in the Hyrcanian forests since the Tertiar; Therefore, humans bear a great responsibility for the long-term conservation of these species in their ecosystems, along with the unique biodiversity of their habitats (Akhani et al., 2010).This record represents the first record of the *P. persicus* (Eiselt & Steiner, 1970), from Northern Iran. The range extension is approximately more 80 km Northern from the closest previously known locality in Asalam area in Talash mountain range of guilan Province of Iran (Ahmadzadeh et al., 2011); According to SVL measurements, our specimens were a few small than previously measured specimens from Guilan (Baloutch & Kami, 1995) and Ardabil Provinces (Ahmadzadeh & Kami, 2009); but color pattern they are similar with previously specimens.

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New insights into the distribution and ecological modeling of the Paghman Mountain Salamander *Paradactylodon mustersi* (Caudata: Amphibia) in Afghanistan

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Abstract
This study provides new insights into the distribution and ecological modeling of the Paghman Mountain Salamander (*Paradactylodon mustersi*), an endemic species in Afghanistan, based on field surveys across 53 strategically selected localities in high-altitude streams from March to September 2024. We confirmed the species' presence in all surveyed sites, reflecting targeted sampling of suitable habitats rather than widespread abundance, consistent with its Endangered status (IUCN Red List). Using MaxEnt modeling with cross-validation to minimize overfitting, we identified key environmental variable influencing habitat suitability, as precipitation patterns (BIO13 at 46.1%), with excellent predictive accuracy (AUC = 0.945). The species showed presence in disturbed secondary growth habitats, suggesting some resilience to habitat alteration, though this requires further investigation. Ongoing monitoring and targeted conservation strategies are critical to ensure the long-term survival of this threatened species amidst pollution and climate change pressures. However, ongoing monitoring and targeted conservation strategies are essential to ensure the long-term survival of this threatened species amidst changing environmental conditions.

Keywords: Amphibian, habitat requirements, Conservation biology, water quality, pollution.

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INTRODUCTION

The Paghman Mountain Salamander, *Paradactylodon mustersi*, is an endemic amphibian of the family Hynobiidae, found exclusively in the highland streams of Afghanistan's Hindu Kush mountain range. Initially described as *Batrachuperus mustersi* by Smith (1940), its taxonomic status has been debated, with Dubois and Raffaëlli (2012) questioning the validity of *Paradactylodon* due to the lack of a clear diagnosis and proposing the monotypic genus *Afghanodon* for this species (Böhme & Jablonski, 2022). We adopt *Paradactylodon mustersi* based on its broader use in recent literature. The species occurs in the Paghman mountains on the southern slopes of the Hindu Kush, including three tributaries of the Paghman Stream, as well as Gardan Diwal in the Koh-i-Baba Massif, Salang Pass, Sanglakh in Maidan Province, and Dasht-i-Nawar, representing five or fewer threat-defined locations with an extent of occurrence of 2,847 km² (Wagner et al., 2016)

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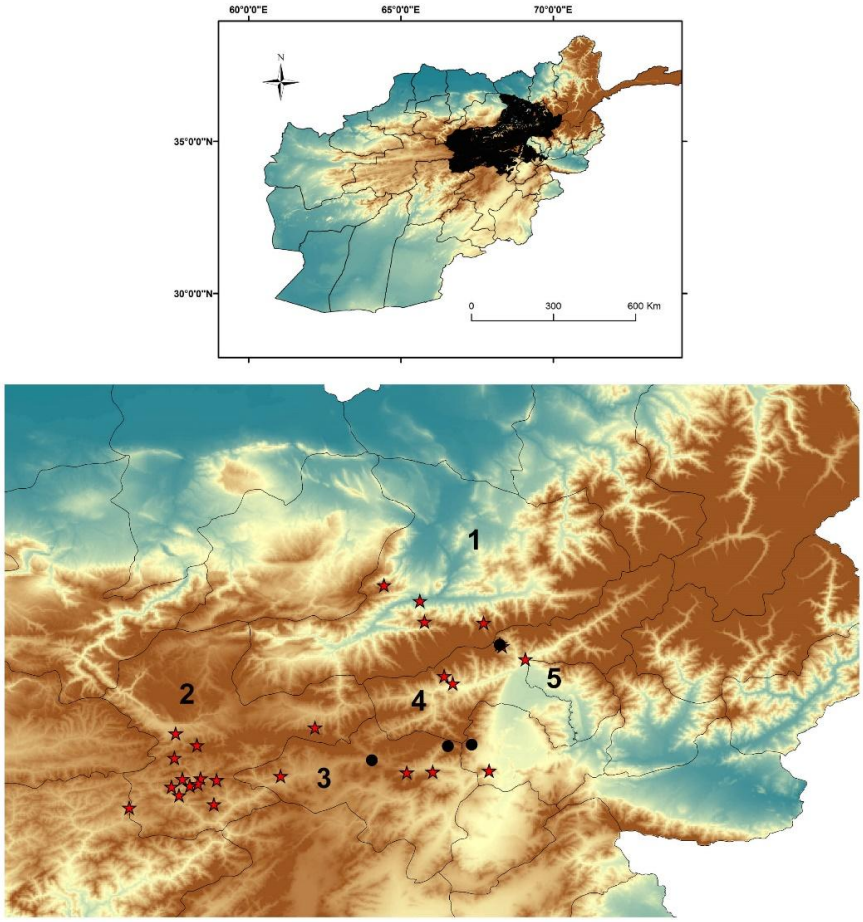

FIGURE 1. Study area and current distribution of *Paradactylodon mustersi* across Afghanistan, based on fieldwork (Stars refer to the newly identified localities) and literature (Black circles). 1) Baghlan; 2) Bamyan; 3) Wardak; 4) Parwan; 5) Panjshir.

Habitat and morphology

Paradactylodon mustersi inhabits glacier-formed valleys, primarily in the Paghman streams area of Kabul Province, at elevations of 2,440–3,750 m above sea level (Wagner et al., 2016). It thrives in cold, fast-flowing streams with water temperatures between 0 and 14 °C (Ayobi et al., 2022). The salamander's morphological characteristics include a dark olive-brown to yellowish-olive body speckled with tiny pigmented dots and well-developed limbs, enabling effective navigation in its aquatic environment (Reilly, 1983). Recent surveys (2017–2021) confirmed its presence across tributaries of the Paghman stream and extended its known range to Panjsheer Province, highlighting its adaptation to high-altitude aquatic ecosystems (Ayobi et al., 2022).

Threats and research gaps

Classified as Endangered (EN) by the IUCN Red List, *P. mustersi* has an estimated population of 1,000–2,000 adults, threatened by habitat disturbance from water pollution, agricultural expansion, and water diversion (Wagner et al., 2016; AmphibiaWeb, 2020[1]; Ayobi et al., 2022). Prolonged political instability in Afghanistan has limited zoological research, with comprehensive assessments of its distribution and

[1] AmphibiaWeb. 2020. *Paradactylodon mustersi*. Retrieved from https://amphibiaweb.org/species/3872 (accessed 30 Nov. 2025)

ecology last conducted nearly 40 years ago (Reilly, 1983; Stuart et al., 2008). Understanding its ecological requirements and distribution patterns is critical for developing effective conservation strategies to ensure the long-term survival of this unique species.

The taxonomic classification of the Paghman Mountain Salamander as *Afghanodon mustersi* or *Paradactylodon mustersi* remains debates in scientific community. The species, described as *Batrachuperus mustersi* (Smith, 1940) and was reassigned to the monotypic genus *Afghanodon* by Dubois and Raffaëlli (2012) due to the lack of a clear diagnosis for *Paradactylodon*. However, Böhme and Jablonski (2022) advocate for the genus *Paradactylodon*, based on morphological traits (e.g., limb structure) and genetic evidence indicating closer phylogenetic ties to other *Paradactylodon* species within the Hynobiidae family. We adopt *Paradactylodon mustersi* following its broader use in recent literature (e.g., Wagner et al., 2016; Ayobi et al., 2022), though ongoing phylogenetic studies are needed to resolve this classification.

This study aims to provide new insights into the distribution and ecological modeling of *P. mustersi*, emphasizing the importance of ongoing research and conservation efforts in Afghanistan's unique ecosystems. We assessed *P. mustersi*'s abiotic water parameters of the endangered species habitat.

MATERIAL AND METHODS

Field Surveys and Data Collection

Field surveys were conducted from March to September 2024 across 53 newly identified localities to assess the population of the Paghman Mountain Salamander (*P. mustersi*). The specific field activities conducted in each province include Baghlan Province (Six localities), Bamyan Province (31 localities), Panjshir Province (Two localities), Parwan Province (Six localities) and Wardak Province (Nine localities) (Figure 1 and Appendix 1). Presence records for the Paghman Mountain Salamander (*P. mustersi*) were obtained through direct fieldwork and literature (Ayobi et al., 2022). In total, 53 presence records were gathered and georeferenced.

Species Distribution Modelling (SDM): To predict current distributions, bioclimatic variables were derived from observational data available in WorldClim version 2.1 (Fick & Hijmans, 2017), with a spatial resolution of 30 arc seconds (~1 km²). Nineteen bioclimatic variables (Table 1) for the present and altitude were downloaded and clipped for Afghanistan at the same spatial resolution. The data were converted to ASCII format using DIVA-GIS version 7.5 (Zhang et al., 2021). A jackknife analysis was performed to assess the contribution of bioclimatic variables, and model fit was evaluated using the area under the receiver operating characteristic curve (AUC). The layers were cropped using ArcGIS (ESRI) to focus on the study area within Afghanistan.

All nineteen bioclimatic layers and altitude were analyzed by ENMTools 1.3 (Warren et al., 2010) to evaluate the pairwise correlation and then select the layers with lower correlation than 0.75. The following variable were selected: BIO3; BIO4; BIO11; BIO13; BIO14. Species distribution modeling was conducted using MaxEnt version 3.4.1 software (available at http://www.cs.princeton.edu/schapire/Maxent/). This software was utilized to simulate and predict the potential geographical distribution probability of *Paradactylodon mustersi* under current conditions (Coban et al., 2020; Ye et al., 2020). During the modeling process, 70% of the occurrence data samples were randomly selected as training data, while the remaining 30% were reserved for testing. A total of 10,000 randomly generated background points were used (Zhang et al., 2021). The regularization multiplier was set to 0.1 to prevent overfitting (Phillips & Dudik 2008), and linear, quadratic, and hinge features were included in the model. A total of 100 runs were conducted for model building (Flory et al., 2012), employing the Jackknife method in the environmental parameter settings while keeping other parameters at their default values. Model evaluation was performed using threshold-independent receiver operating characteristic (ROC) analyses, with AUC values used to estimate model accuracy (Elith et al., 2006). Model performance was classified as failing (0.5–0.6), poor (0.6–0.7), fair (0.7–0.8), good (0.8–0.9), or excellent (0.9–1), with higher AUC values indicating better model performance. The lowest training presence threshold define as 0.2, namely the species can tolerate a wider range of habitat conditions, even those with lower suitability scores.

TABLE 1. Description of predictive bioclimatic variables used in this study.

BIO1	Annual Mean Temperature	BIO10	Mean Temperature of the Warmest Quarter
BIO2	Mean Diurnal Range [Mean of Monthly (Max Temp – Min Temp)]	BIO11	Mean Temperature of the Coldest Quarter
BIO3	Isothermality	BIO12	Annual Precipitation
BIO4	Temperature Seasonality	BIO13	Precipitation in the Wettest Month
BIO5	Maximum Temperature of the Warmest Month	BIO14	Precipitation in the Driest Month
BIO6	Minimum Temperature of the Coldest Month	BIO15	Precipitation Seasonality
BIO7	Temperature Annual Range	BIO16	Precipitation in the Wettest Quarter
BIO8	Mean Temperature of the Wettest Quarter	BIO18	Precipitation in the Warmest Quarter
BIO9	Mean Temperature of the Driest Quarter	BIO19	Precipitation in the Coldest Quarter

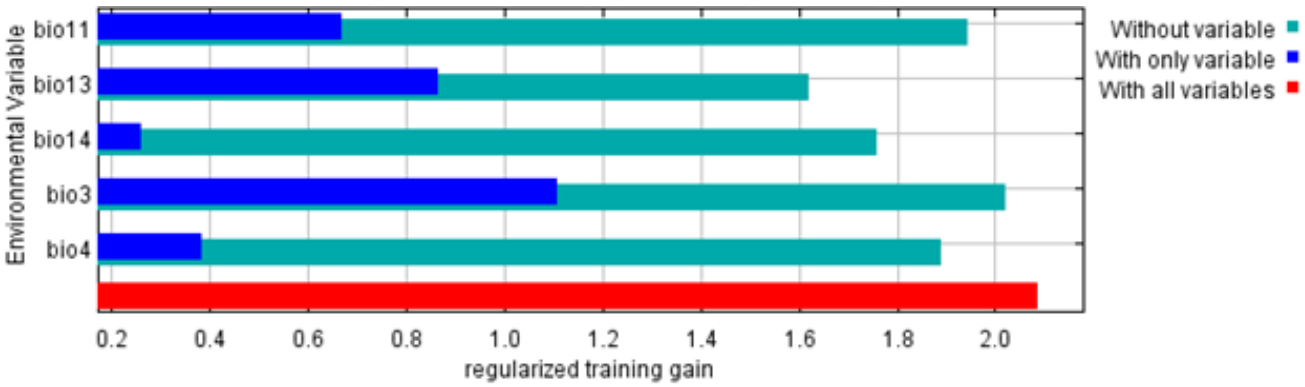

FIGURE 2. Results of Jckknife of regularized training gain for *Paradactylodon mustersi*.

RESULTS

Field surveys conducted across 53 newly identified localities from March to September 2024 yielded critical data on the distribution and ecological requirements of the Paghman Mountain Salamander (*P. mustersi*). The surveys confirmed the presence of the species in all surveyed locations, contributing valuable presence records for future conservation efforts.

Environmental Variable Contributions

To understand the ecological factors influencing the distribution of *P. mustersi*, a species distribution model was developed using MaxEnt. The analysis revealed the percent contribution and permutation importance of various environmental variables, which are summarized in Table 2 and Figure 2. BIO13 (Precipitation in the Wettest Month) was identified as the most effective variable on habitat suitability, contributing 46.1% to the model's predictive power and demonstrating a permutation importance of 43.6%. Mean Temperature of the Coldest Quarter (BIO11) followed closely, with a percent contribution of 14.2% and a permutation importance of 13.7%, indicating its relevance in understanding the species' habitat preferences. Other precipitation variable also played crucial roles, BIO14 (Precipitation in the Driest Month), which had a percent contribution of 14.0% and a relatively high permutation importance of 26.0% and BIO3 (Isothermality) contributed 13.2% with a permutation importance of 13.3%. Other factor as Temperature Seasonality (BIO4) shows minimal contributions to habitat suitability, with contribution of 12.5%.

The performance of the habitat suitability model was evaluated using the Area Under the Curve (AUC) metric. The mean AUC value was found to be 0.945, indicating excellent predictive accuracy for

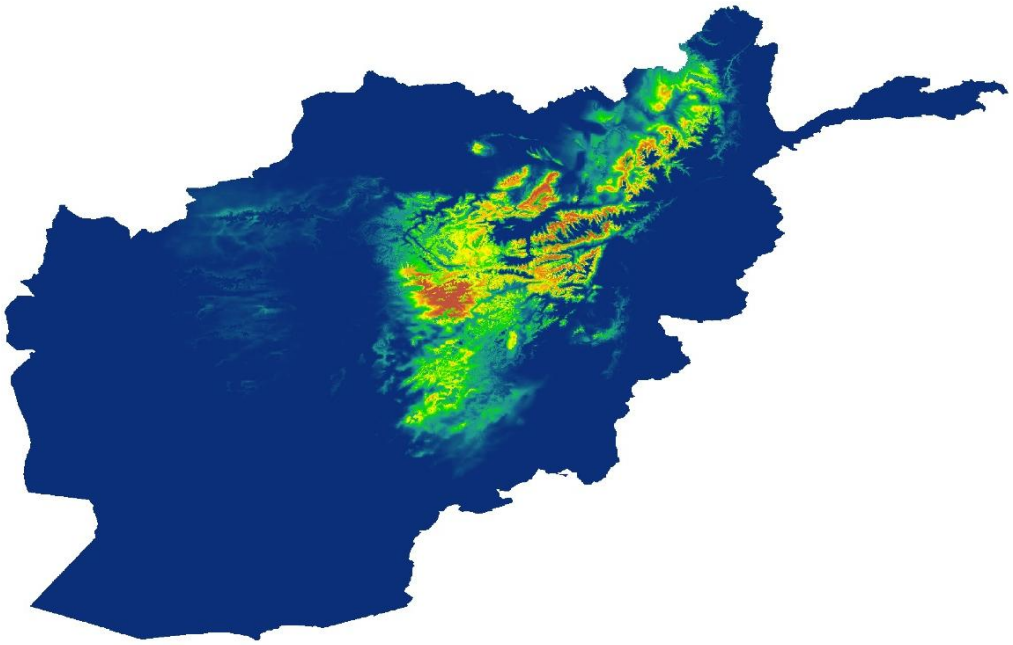

FIGURE 3. Habitat suitability prediction and the favorable area for *Paradactylodon mustersi* in Afghanistan under current climate conditions (Worldclim 2.1) based on the Maxent model. Red color refers to the most suitable regions (1) and the blue color shows unsuitable regions (0).

identifying suitable habitats for *P. mustersi*. This high AUC value suggests that the model effectively distinguishes between suitable and unsuitable habitats based on the environmental variables analyzed. The high suitable regions in the modeled map, clearly shows that valleys and streams is the best regions for species presence and needs more consideration (Figure 3).

DISCUSSION

The results of this study offer critical insights into the distribution and ecological requirements of the Paghman Mountain Salamander (*Paradactylodon mustersi*) in Afghanistan, a region where amphibian ecology remains poorly documented. The confirmation of *P. mustersi* presence across all 53 surveyed localities from March to September 2024 underscores the value of systematic field surveys in generating robust baseline data for conservation in Central Asia. The MaxEnt species distribution model, with a high predictive accuracy (AUC = 0.945), effectively delineates suitable habitats, particularly valleys and streams, which are pivotal for the species' survival. These findings contribute to the broader understanding of amphibian ecology in montane environments and align with regional studies on salamander distribution patterns.

Precipitation-related variables, notably BIO13 (Precipitation in the Wettest Month, 46.1% contribution) and BIO14 (Precipitation in the Driest Month, 14.0% contribution), emerged as the primary drivers of habitat suitability for *P. mustersi*. This strong dependence on water availability mirrors patterns observed in other Middle Eastern salamanders, such as *Salamandra infraimmaculata* in Turkey and Iran, where precipitation is a key determinant of habitat suitability (Kurnaz, 2022; Vaissi, 2021a). For instance, Kurnaz (2022) reported that precipitation during the wettest periods significantly influenced the distribution of *S. infraimmaculata* in Turkey, a finding echoed in our study. Similarly, ecological niche modeling of *Neurergus derjugini* in Iraq highlighted the critical role of precipitation in sustaining stream-dependent amphibians (Khwarahm et al., 2021). The high permutation importance of BIO14 (26.0%) in our model suggests that *P. mustersi* is particularly sensitive to dry season conditions, a trait shared with

Table 2. Variable Contributions to Habitat Suitability Model. Bold value refers to the high contributed layer in modeling.

Variable	Percent Contribution (%)	Permutation Importance (%)
BIO3 (Isothermality)	13.2	13.3
BIO14 (Precipitation in the Driest Month)	14.0	26.0
BIO13 (Precipitation in the Wettest Month)	**46.1**	**43.6**
BIO4 (Temperature Seasonality)	12.5	3.5
BIO11 (Mean Temperature of the Coldest Quarter)	14.2	13.7

Paradactylodon populations in Iran, where niche evolution is shaped by precipitation gradients (Vaissi et al., 2023). These consistent patterns across taxa underscore the vulnerability of stream-associated salamanders to changes in hydrological regimes.

Temperature-related variables, including BIO11 (Mean Temperature of the Coldest Quarter, 14.2% contribution) and BIO3 (Isothermality, 13.2% contribution), also significantly influence *P. mustersi* distribution. The importance of cold season temperatures aligns with findings for *Salamandra infraimmaculata*, where cooler, high-elevation habitats were preferred (Vaissi, 2021a). In contrast, the limited contribution of BIO4 (Temperature Seasonality, 12.5%) suggests that *P. mustersi* is less affected by seasonal temperature fluctuations compared to hynobiid salamanders in western Japan, which show greater sensitivity to temperature variability (Shin et al., 2021). This may reflect *P. mustersi*'s adaptation to the stable microclimates of valleys and streams, which provide thermal and moisture refugia. Comparable studies on *Neurergus barani* and *N. strauchii* in Anatolia further support the importance of stable microhabitats for montane salamanders (Kurnaz & Şahin, 2021). The prominence of valleys and streams in our habitat suitability maps (Figure 3) reinforces their role as critical habitats, consistent with findings for other stream-breeding amphibians in the Middle East (Vaissi, 2021b).

The biogeographic context of the Paghman Mountains, characterized by arid conditions and fragmented habitats, amplifies the conservation significance of our findings. The high AUC value of our model indicates that conservation efforts should prioritize protecting valley and stream ecosystems, which serve as refugia for *P. mustersi*. This is particularly urgent given the vulnerability of montane amphibians to habitat loss and climate change, as observed in *Neurergus* species in the Zagros Mountains (Hosseinain Yousefkhani, 2021; Kurnaz & Şahin, 2021; Vaissi et al., 2023). Projections for *Salamandra infraimmaculata* suggest potential range contractions under future climate scenarios due to reduced precipitation and rising temperatures (Vaissi, 2021a; Khwarahm et al., 2021), a threat likely applicable to *P. mustersi* given its reliance on moisture-rich habitats. Additionally, studies on three rare salamanders species in East Asia indicate that climate-driven shifts in precipitation could disrupt breeding cycles of stream-dependent amphibians (Ma et al., 2020), further highlighting the need for climate-informed conservation strategies for *P. mustersi*.

Our study also underscores the utility of ecological niche modeling in data-scarce regions like Afghanistan. By integrating our findings with those from neighboring regions, such as Iran, Turkey, and Iraq, we demonstrate that *P. mustersi* shares ecological traits with other Middle Eastern salamanders, including dependence on moist microhabitats and sensitivity to climatic extremes (Vaissi et al., 2023; Kurnaz, 2022; Khwarahm et al., 2021). However, the unique environmental conditions of the Paghman Mountains, including their high-altitude streams and isolation, suggest potential niche specialization. For example, studies on the ecological niche and population genetic of two groups of *Hynobius* on Tsushima Island indicate that isolated populations often exhibit distinct ecological adaptations (Niwa et al., 2022), a hypothesis worth exploring for *P. mustersi* through genetic and population studies.

In conclusion, this study provides a robust framework for understanding the ecological drivers of *P. mustersi* distribution and emphasizes the critical role of valleys and streams as conservation priorities. By drawing parallels with regional studies on *Salamandra*, *Neurergus*, and *Hynobius* species, we highlight

shared ecological vulnerabilities and the pressing need for targeted conservation in Central Asia. Future research should incorporate climate change projections and genetic analyses to assess long-term risks and enhance conservation strategies for this enigmatic species.

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RESEARCH ARTICLE Open access

Assessment of population dynamics, agricultural impact, and conservation challenges facing the Indian Peafowl (*Pavo cristatus*) in Tirupur District, Tamil Nadu, India

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Abstract
Indian peafowl, the national bird of India, faces significant threats from various anthropogenic activities. A study was conducted to assess the population status, habitat choice, and conservation challenges faced by the species in Tirupur, Tamil Nadu, India. The study utilized road transects to estimate population, opportunistic surveys to determine tree roosting preferences, and structured questionnaires to assess people's perceptions and conservation threats. The study recorded a total of 807 individuals (1.92 individuals/km) observed during 546 sightings along 420 km of road transects, with adult females having the highest number of sightings. Tree preferences for roosting identified seven species, with Cocos nucifera most preferred. Major conservation risks included habitat modification, food scarcity, hunting, and encroachment onto forest areas. Alarmingly, 93 Indian peafowls were documented as deceased due to poisoning incidents from 2016 to 2022. This study provides valuable data on the ecology and conservation needs of the Indian peafowl, emphasizing the importance of addressing these threats for their long-term survival in the Tirupur district.

Keywords: Population dynamics, Roosting ecology, Conservation strategies, Habitat alteration, Wildlife-human conflict, Population monitoring.

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INTRODUCTION

Indian peafowl (*Pavo cristatus*), commonly called the Blue Peafowl, occupies a significant position in India. In addition to being designated the country's national bird in 1963 (Ali *et al.*, 1987), it is also a prominent character in aesthetics and cultural mythology. The Indian peafowl is a member of the Phasianidae family in the Galliformes order, as classified by Delacour in 1977. This family encompasses many species, including pheasants, partridges, and quails. The family consists of 155 species found in the Old World. They are native to the Indian subcontinent and abundantly distributed inside protected

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reserves and rural landscapes, and found in certain regions in Nepal, Sri Lanka, and parts of Pakistan (Ali *et al*., 1987). These species are essential markers of the health of forests and ecosystems (Johnsgard, 1986). They are renowned for their ecological significance and cultural importance throughout the Indian subcontinent, increasingly leading to conflicts with human populations. The Indian peafowl's diverse diet, consisting of seeds, fruits, insects, small animals, and reptiles (Grimmett *et al*., 2016), highlights its capacity to adapt to many food sources and environments, ranging from deciduous forests to cultivated fields. Despite their abundance, Indian peafowl populations have declined in specific areas, necessitating an assessment of their conservation status and management approaches (Roberts, 1991). However, the major threats they face include habitat degradation, anthropogenic disturbances, illicit trafficking, and the adverse impacts of intensive agricultural practices. *Human activities degrade the peafowl's natural habitat, leading to conflicts from crop damage.* As a result, these issues have pushed the limits of their survival, ultimately resulting in the extinction of species in specific areas, such as Pakistan (Ramesh & McGowan, 2009; Jain & Rana, 2013). Therefore, the aforementioned human activities elucidate the increasing concerns related to conflicts between humans and peafowls. (Grewal *et al*., 2002; Rasmussen, & Anderton, 2005). Thus, comprehensive management strategies are required to ensure its survival to address potential threats.

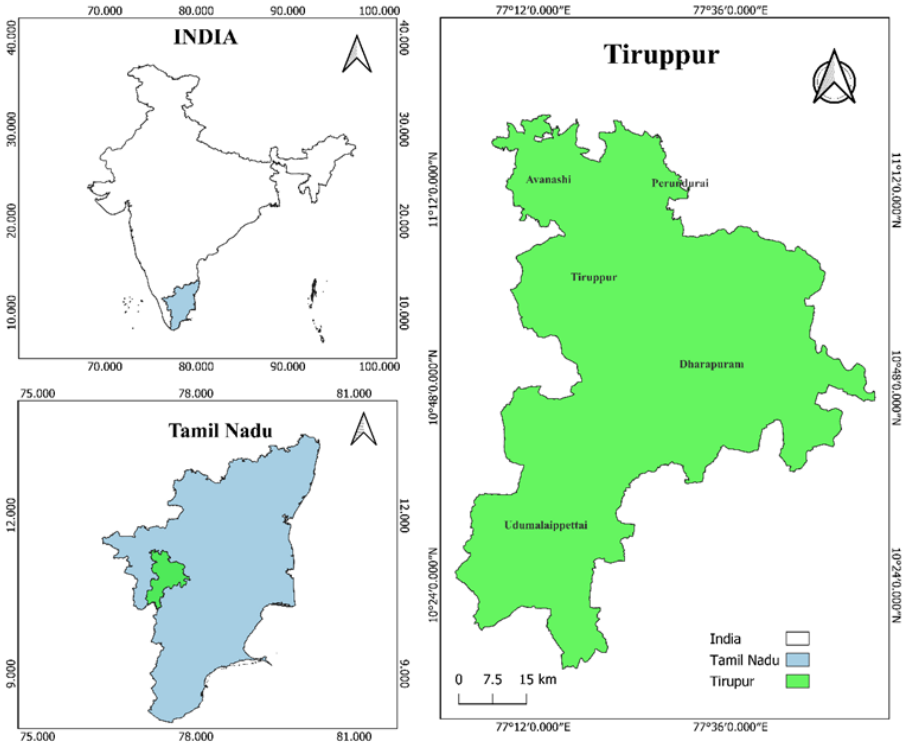

FIGURE 1. Study area map of Tirupur District, Tamil Nadu.

Tirupur district in Tamil Nadu has a pleasant climate with noticeable seasonal changes. The winter season spans from September to January and delivers lower temperatures and agreeable weather conditions. In contrast, the monsoon months of June, July, and August bring about a time of increased precipitation and reduced temperatures, providing respite from the hot summer weather. The temperatures from March to June fluctuate between 20°C and 38°C, making it the warmest time of the year. Tirupur receives moderate rainfall during this period, adding to the region's yearly precipitation. The city predominantly experiences precipitation from the Southwest monsoon, crucial for maintaining the local environment and supporting agricultural endeavours. Tirupur has an average annual rainfall of 700 mm, essential for replenishing water supplies and ensuring the sustainability of irrigation and ecological

balance (Thenmozhi & Kottiswaran, 2016). Tirupur's climate changes significantly impact agricultural methods, wildlife behaviour, and human activities (Loganathan & Elangovan, 2017). River Noyyal, originating from the Velliangiri Hills and flowing through the city centre in the Easterly direction and the Western Ghats on the near west, are the major geographical features of this region (Udayakumar *et al*., 2011). Tirupur has 231.28 sq. km of moderately dense forest and 46.65 sq. km of very dense forest (FSI, 2021[1]).

The present study therefore aims to assess the population status of Indian peafowl in Tirupur District, quantify the extent of crop damage attributed to peafowls, investigate the perceptions and attitudes of local communities regarding peafowl presence, and evaluate key threats and conservation risks to this species in the region.

MATERIAL AND METHODS

Study Area and Survey Design

The population assessment of Indian peafowl in Tirupur District (Figure 1) was carried out via the road transects method (Buckland *et al*, 2001). Strategically positioned road transects were placed throughout the Tirupur district, covering various habitat types. A four-wheeler was utilized for surveys during the early morning (06:00 to 10:00) and late evening (15:00 to 19:00). A total of 420 km of transects were built, each being examined once. During the surveys, researchers documented the presence of Indian peafowl. They collected data on the number of individuals classified by age and sex (Adult Male [AM], Adult Female [AF], Sub Adult Male [SAM], Sub Adult Female [SAF], and Juveniles [JV]), group size, vegetation type, and topographical characteristics. The sex of the individuals was determined using the method described by Samson and Ramakrishnan (2018).

Roosting Tree Preferences

Indian peafowl's roosting tree preferences were identified via opportunistic searches and observations. The identification of roosting trees was conducted through visual estimation throughout the early morning and late evening hours, with additional support from secondary markers, such as droppings and feathers beneath the trees. The presence of Indian peafowl on the roosting tree was verified using high-powered binoculars (10X52 Nikon). The study recorded many attributes of roosting trees, such as the number of trees (n), the height of the trees (m), the height of the roost (m), the girth of the trees at breast height (GBH) in centimetres, the characteristics of the habitat, and the date and time of the observation. Furthermore, it was deduced that the accumulation of droppings indicated the extended use of roosting trees. The tree species were identified using known literature as a reference (Matthew, 1982).

Newspaper Reports

Incidents of peafowl poisoning were compiled from regional newspaper archives dating from 2016 to 2022. No specific inclusion/exclusion criteria were established for report selection. To improve reliability, this information was supplemented with records and informal communications from the local forest department, offering additional verification for recorded mortality events.

Community Perceptions and Questionnaire Surveys

A questionnaire-based technique was used to evaluate the opinions of local communities, including residents and agricultural workers, regarding protecting Indian peafowl. Two sets of questionnaires, specifically labelled "Precise and Closed" and "Broad and Open-ended", were created for this objective. The focused communities, primarily composed of individuals with high literacy levels, actively used the local language to ensure clear and efficient communication. Before conducting the survey, the researchers described the study's goal to the participants: to reduce any confusion or uncertainty. Face-to-face interviews were conducted to enhance comprehension of the questions and reduce potential misinterpretations. Data was gathered using open-ended inquiries, enabling participants to articulate their perspectives and concerns about the conservation of Indian peafowl. The research methods employed

[1] https://fsi.nic.in/forest-report-2021-details (Date accessed: 22-01-2025)

were the recommendations put forward by Balakrishnan and Ndhlova, (1992) and Samson and Ramakrishnan, (2020).

Statistical analysis

The study used PAST 3.1, a widely used statistical software, to analyse the distribution, abundance, and habitat preferences of Indian peafowl in a specific area. The mean and standard error were computed to determine the average and variability of peafowl sightings. At the same time, the encounter rate was calculated by dividing the total number of individuals observed by the length of the study area transects by km. Correlation and regression analyses were employed to investigate the connections between variables associated with roosting trees and the presence of Indian peafowl.

RESULTS

The population and demographic status of Indian peafowl in Tirupur district were assessed, revealing 807 individuals observed in 546 sightings across 420 km (1.47±0.87), with an encounter rate of 1.92 individuals/km. The majority of the peafowl (Table 1) were observed in individuals, indicating solitary behavior. The sex ratio of Males was 0.64 and females was 1.54. The number of sightings varied among categories, with the lowest recorded figures being 4 for AM, 26 for AF, 6 for SAM, 21 for SAF, and 6 for JV. The mean sightings and Standard Errors (SE) were used to determine the average value and analyse the variability. The average number of sightings per category was 1.47±0.87, with an overall average of 1.47±0.87 individuals per sighting. The Encounter Rate per Km (ER/Km) was calculated to measure the density of Indian peafowl populations in the research area, with values of 0.40 for AM, 0.68 for AF, 0.30 for SAM, 0.40 for SAF, and 0.13 for JV, resulting in a total ER/Km of 1.92.

Table 2 presented the preferred roosting trees and significant characteristics of the Indian peafowl in Tirupur District. The data includes the count of observed individuals, diameter at breast height (DBH), tree height, and roost height. *Cocos nucifera* was the most frequent roosting place, followed by *Borassus flabellifer* and *Prosopis juliflora*. The average DBH was 323.82 cm, tree height was 17.14 m, and roost height was 10.33 m.

Statistical analysis showed a weak, negative correlation between the diameter at breast height (DBH) of roosting trees and the height at which Indian peafowls roosted ($r=-0.277$, $r^2=0.08$, $p=0.054$; Figure 2). However, this relationship was only marginally non-significant at the conventional threshold ($p<0.05p<0.05$) and explained little of the observed variation. In contrast, a positive correlation was found between the height of the roosting trees and the height of the peafowl roost ($r = 0.708$, $p < 0.074$; $r^2 = 0.5025$), as depicted in (Figure 3). These findings indicate that Indian peafowl prefer taller roosting trees with higher perches. Additionally, the diameter of the trees decreases as the height increases, suggesting specific habitat preferences and utilisation patterns of the species.

A total of 360 participants were questioned for the study. Of these, 195 included individuals of both genders, with females (n=108) outnumbering males (n=87). The average age for males was 45.44±1.65, while for females, it was 47.11±2.34. In terms of educational attainment, the survey revealed that the most significant proportion of participants were unable to read or write (40%; n=145), followed by those who had completed primary education (1-5 STD) (26%; n=92), secondary education (5th STD to 10th STD) (23%; n=84), and a smaller percentage who had attained higher education (12th STD to Degree) (11%; n=40).

The crops that observed the most substantial harm were onions (*Allium cepa*), with a damage rate of 14%. Chillies (*Capsicum annuum*) followed with a damage rate of 11%, while groundnuts (*Arachis hypogaea*) and cluster beans (*Lablab purpureus*) both had a damage rate of 9%.

The region's agricultural landscape faces crop loss year-round, where 180 farmers cultivate between four to 18 crop types, primarily due to wild boar (*Sus scrofa*) and Indian peafowl (Table 3). Survey data reveals that a significant majority of farmers, ranging from 80% to 90%, recognize the influence of Indian peafowl on crops. This impact is most significant during the post-growth period, followed by the pre-growth stage, while the growth stage is least susceptible to their effects. The extent of damage caused by

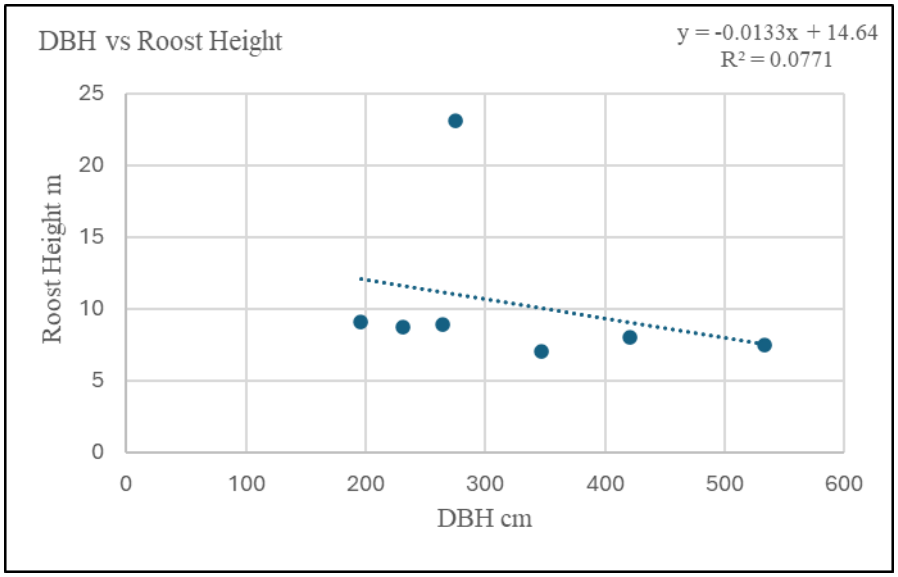

FIGURE 2. Relationship between tree diameter (DBH, cm) and roost height (m) for Indian peafowl in Tirupur District.

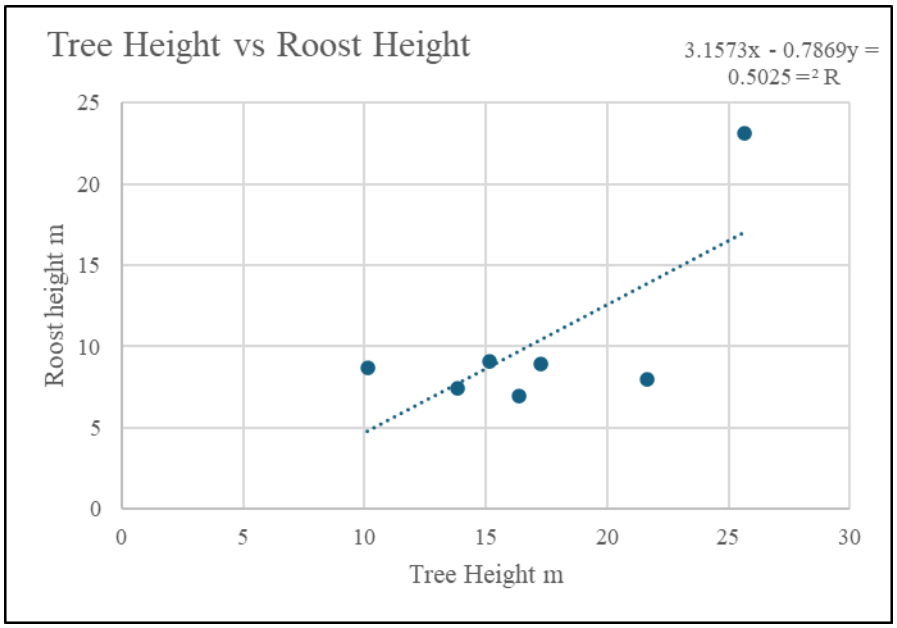

FIGURE 3. Relationship between tree height (m) and roost height (m) for Indian peafowl in Tirupur District.

peafowl intake varies greatly, affecting essential crops such as onion, chilli, peanuts, beans, ragi, maize, and tomato.

Although crop damage causes distress, many farmers (n=40) consider the hazards associated with agriculture in forested environments acceptable. Farmers universally endorse Indian peafowl conservation activities, emphasizing the importance of addressing populations in decline caused by habitat modification, scarcity of food, and expansion into forested regions. Local perceptions indicate that Indian peafowl do not harm settlements, highlighting the intricate relationship between human-wildlife interactions and conservation efforts in the Tirupur area.

TABLE 1. Population and demographical status of Indian Peafowl in Tirupur district.

	Variables	AM	AF	SAM	SAF	JV	Total
1	**Sightings**	147	172	97	106	24	546
2	**Individuals**	169	288	126	168	56	807
3	**Maximum**	1	1	1	1	1	1
4	**Minimum**	4	26	6	21	6	46
5	**Mean SE**	1.14±0.15	1.67±0.34	1.29±0.27	1.58±0.17	2.33±0.68	1.47±0.87
6	**ER/Km**	0.40	0.68	0.3	0.4	0.13	1.92

TABLE 2. Roosting tree preference and its characteristics of Indian Peafowl in Tirupur district.

	Tree Species	No	DBH	Height	Roost Height
1	*Cocos nucifera*	56	275.45	25.65	23.12
2	*Borassus fabellifer*	47	195.76	15.14	9.12
3	*Prosopis juliflora*	39	231.10	10.12	8.7
4	*Azadiracta indica*	35	264.3	17.25	8.9
5	*Tamarindus indica*	32	346.36	16.34	7.0
6	*Ficus benghalensis*	4	533.57	13.82	7.47
7	*Ficus religiosa*	3	420.23	21.66	8.0
	Total/ Mean	**216**	**323.82**	**17.14**	**10.33**

The evaluation of the risk posed by Indian peafowl in Tirupur district, as determined from public newspaper reports, indicates a concerning sequence of poisoning deaths. Between 2016 and 2022, 93 Indian peafowl individuals died due to poisoning, as shown in Table 4. These incidents emphasize the danger that poisoning poses to the Indian peafowl population in the Tirupur district.

DISCUSSION

The current study carried out in the Tirupur district in Tamil Nadu revealed a significant presence of robust peafowl populations in their natural habitat, as determined by population assessment. There were 807 individuals observed in a surveyed area of 420 km, with a total of 546 sightings. This provides us with an encounter rate of 1.92 individuals per km. Furthermore, the adult peafowls had a sex ratio that was rather equitable, with one AM observed for every fourFM. This suggests a consistent and stable reproductive pattern within the community. Similar patterns were found in investigations undertaken by Deepan *et al*., (2021) in the Karur district and Veeramani *et al*., (2019) in the rural parts of Annur and Avinashi in the district. A total of 1356 peafowls were observed in the Karur district, leading to an encounter rate of 2.26 individuals per km. The AM-to-FM sex ratio was about equal, suggesting a well-balanced demographic makeup. In addition, the research conducted by Veeramani *et al.* (2019) focused on the observation patterns and frequency of encounters in the rural regions of Annur and Avinashi Kongu districts. The study also emphasised the high occurrence of AM among the population. In contrast, research carried out by Samson and Ramakrishnan (2018), Veeramani (1990), Sharma (1979), Johnsingh and Murali, 1981, and Rajadurai (1988) in various areas of Tamil Nadu and India revealed differences in population dynamics and gender ratios. The observed differences might be attributed to a range of reasons, including the suitability of the habitat, anthropogenic pressure , and the individual ecological conditions in each research region.

The correlation between the abundance of peafowls and water presence supports studies highlighting water sources' role in population density, especially during reproduction periods in arid seasons (Brickle, 2002). Riparian environments, which are located near water bodies, are ideal habitats for bird populations

because they have plentiful food sources, such as a variety of insects and plants that produce fruit. In riparian ecosystems, the existence of large trees is crucial as they offer necessary shade, which helps Indian peafowls to roost and also acts as a strategy to avoid predators (Gadgil & Ali, 1975; Gadgil, 1972). Agricultural fields also substantially impact the successful survival of Indian peafowl populations (Jain &Rana, 2013). The presence of diverse food sources, such as insects and cultivated crops, as well as water sources and sufficient shade in these locations, contributes to the high population of peafowls observed in fields like those in the Tirupur district. In contrast, the Tirupur district has the fewest peafowls in areas where humans live. This is in line with research that suggests that Indian peafowls are less likely to thrive in areas with human presence since they are naturally cautious around humans (Brickle, 2002).

TABLE 3. Damage caused by Indian peafowl in agricultural fields in Tirupur district.

	Scientific Name	Common Name	Local Name	Damage %
1	*Allium cepa*	Onion	Vengayam	14%
2	*Capsicum annuum*	Chilli	Milagai	11%
3	*Arachis hypogaea*	Groundnut	Verkadalai	9%
4	*Lablab purpureus*	Cluster beans	Avarai	9%
5	*Eleusine coracana*	Ragi	Kelvaragu	8%
6	*Zea mays*	Maize	Makka Kollam	8%
7	*Solanum lycopersicum*	Tomato	Thakkali	7%
8	*Abelmoschus esculentus*	Ladies finger	Vendai kai	6%
9	*Solanum melongena*	Brinjal	Kattari	6%
10	*Manihot esculenta*	Tapioca	Maravalli	5%
11	*Curcuma longa*	Turmeric	Manjal	4%
12	*Beta vulgaris*	Beet Root	Beet Root	3%
13	*Brassica oleracea*	Colliflower	Pookosu	3%
14	*Cocos nucifera*	Coconut	Thengai	2%
15	*Jasminum sambac*	Jasmine	Malli	2%
16	*Musa paradisiaca*	Banana	Vazhai	1%
17	*Saccharum officinarum*	Sugar cane	Karumbu	1%
18	*Phyllanthus emblica*	Gooseberry	Nelli kai	1%

The conservation needs of Indian peafowls are greatly affected by their roosting behaviour. Research indicates that peafowls primarily roost at dawn and dusk on robust branches, exhibiting distinct preferences for particular tree species that differ depending on the geographical area. Parasharya and Mukherjee (1999) observed that the main tree species that roost in the Tirupur district are *Cocos nucifera* and *Borassus fabellifer*. Deepan *et al*., (2021) determined that *Tamarindus indica, Tectona grandis, Thespesia populnea,* and *Syzygium cumini* are the most prevalent species in Karur. In contrast, Veeramani *et al*. (2019) observed that *Cocos nucifera*, *Borassus fabellifer*, and *Azadirachta indica* are the preferred species in the Annur and Avinashi areas. Samson and Ramakrishnan (2018) conducted a study in the Sigur Plateau where they observed various species, including *Tectona grandis, Erythroxylum monogynum,* and *Cassine glauca*, which were particularly noticeable. Veeramani (1990) emphasised the presence of *Acacia sundra* in Mudumalai, while Rajadurai (1988) recorded *Tamarindus indica* as the prevailing species in semi-wild areas. Trivedi and Johnsingh (1995) observed that the peafowl chose *Pongamia pinnata and Holoptelia integrifolia* in Gujarat's Gir forest for roosting. Gaining knowledge of the specific roosting preferences of animals is crucial for conservation efforts as it helps to guide the development of methods to preserve their habitats.

TABLE 4. Indian peafowl Threats assessment in Tirupur district.

	Date	Reason for death	No of Peafowl dead	News Paper Source
1	26/10/2016	Poisoning	14	https://timesofindia.indiatimes.com/city/coimbatore/another-farmer-poisons-14-peafowls-to-death-arrested/articleshow/55059439.cms
2	12/11/2016	Poisoning	16	https://www.newindianexpress.com/states/tamil-nadu/2016/nov/12/farmer-arrested-for-poisoning-peacocks-to-death-1537826.html
3	18/07/2021	Poisoning	54	https://www.thehindu.com/news/national/tamil-nadu/54-peafowl-poisoned-near-tiruppur-in-nine-days/article35391007.ece
4	15/03/2022	Poisoning	9	https://www.instanews.city/tamil-nadu/tiruppur/avanashi/peacocks-dead-near-avinasi-1118238
	Total		**93**	

During this study, it was observed that the Indian peafowl primarily foraged at a height ranging from 7 to 23 m, with an average height of 10 m. Trivedi and Johnsing (1995) observed that the optimal rooting height for Indian peafowl in the Gir forest is 15 m above ground level. The study revealed that Indian peafowls exhibit activity patterns at specific times of the day: 0640 and 1000 hours, as well as 1600 and 1830 hours. There is a significant amount of research that corroborates our current observation. Hillgarth (1984) observed that Indian peafowls exhibited their highest levels of activity throughout the time intervals of 0900 to 1100 hours and 1700 to 1800 hours. Navaneethakannan (1984) discovered that peafowls had the highest level of activity during the early morning and afternoon. Rathinasabapathi (1987) noted that peafowls exhibit peak activity between 0600 and 1100 hours and 1600 and 1800 hours. They repose between 1100 and 1500 hours in the shade of *Prosopis juliflora* scrub. The present study supports the study conducted by Rathinasabapathi (1987) where he recorded Indian peafowls roosting in *Prosopis juliflora* in the Tirupur district.

The Indian peafowl, renowned for its omnivorous feeding habits, ingests a diverse range of food sources such as seeds, insects, fruits, small animals, and reptiles (Johnsingh, 1976). Observations indicate that there is a tendency to favour plants, whereas snakes and animal-based products make up a lower proportion of their diet (Navaneethakannan, 1981). Peafowls have been seen to consume a wide variety of crops in agricultural fields. The survey identified 18 cultivated crop species that were included in the diet, with Onion, Chilli, Groundnut, Beans, Ragi, Maze, and Tomato being the most prevalent (Samson & Ramakrishnan, 2018). Veeramani (1990) observed similar behaviour in the Mudumalai Wildlife Sanctuary, while Johnsingh and Murali (1981) reported the same in other areas. Peafowls were reported to feed on various crops, including paddy, bajra, tomato, chilly, and bananas. Indian peafowls are considered agricultural pests due to their feeding habits, which cause substantial crop damage despite their natural diet. McGowan and Garson (1995) emphasised the danger presented by the utilisation of pesticides and insecticides, specifically to young peafowl. In addition, peafowls are confronted with risks posed by human activity, including shooting for their plumage, and flesh, and the extraction of 'peacock oil'. The encroachment of human settlements and the increase of agriculture intensify clashes between peafowls and farmers. Human-wildlife conflicts and habitat degradation have grown in the Tirupur district as a result of the conversion of fallow lands into agricultural fields. Preserving the connection between habitats and ensuring uninterrupted access to water sources are crucial strategies for the conservation of Indian peafowl populations (Johnsingh, 1976; McGowan & Garson, 1995).

CONCLUSION

In conclusion, this study in Tirupur district, Tamil Nadu, reveals significant insights into the population status and conservation challenges of the Indian peafowl (*Pavo cristatus*). The results of our study, which included a total of 807 individuals, indicate that the population structure is imbalanced, with a higher proportion ofAM. Additionally, we observed a distinct predilection for roosting in *Cocos nucifera*. The

study notably emphasises significant crop destruction caused by peafowls and severe occurrences of poisoning, resulting in 93 deaths documented over a span of six years (Table 4). The presence of these problems highlights the immediate requirement for comprehensive conservation plans that tackle both the safeguarding of wildlife and the involvement of local communities in order to reduce conflicts between humans and wildlife. This research offers crucial data for conservation plans that seek to guarantee the survival of the Indian peafowl in landscapes that have been altered by human activity.

Therefore, it is recommended to a) conduct more longitudinal surveys along the same transects in order to precisely track the population trend of Indian peafowls in the Tirupur district of Tamil Nadu; b) Implementing compensation schemes for crop damage caused by Indian peafowls could be a useful technique to reduce conflicts and prevent more deaths resulting from poisoning occurrences; c) Specific awareness programs designed for agricultural communities are essential to promote comprehension and collaboration. This will ultimately decrease the number of deaths caused by poisoning and ensure the preservation of this iconic species.

Limitations

The documentation of peafowl poisoning incidents was based on newspaper reports supplemented with forest department records; while this approach provided valuable information into a difficult-to-monitor threat, some cases may remain unreported. The survey period (December 2023–March 2024) provides a focused snapshot of peafowl populations and crop damage, though it may not represent seasonal differences. Road transects were used for population assessments, a method widely employed for large, ground-dwelling birds in agricultural landscapes; while effective, this may slightly favor detections closer to human-modified areas.

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RESEARCH ARTICLE Open access

Distribution patterns, acoustic-external features, and ecological niche modelling of the pale bent-wing bat, *Miniopterus pallidus* (Chiroptera: Miniopteridae) in Iran

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Abstract
In this review, we summarize the historical and recent knowledge on the distribution and ecology of the pale bent-wing bat (*Miniopterus pallidus*) in Iran. We revisit earlier records and incorporate 14 newly identified localities documented between 2014 and 2017. To explore the species' ecological preferences, a presence-only niche model was developed using MaxEnt, which showed strong predictive performance (AUC = 0.85; regularized gain = 0.67), with annual precipitation contributing 81.7% to the model. The predicted distribution aligns closely with montane regions of the Zagros and Alborz Mountains, highlighting the role of topography and climate in shaping the species' range. We further review available data on the species' acoustic characteristics and present new echolocation call parameters from 44 individuals across six localities. The recorded FM-type calls suggest adaptation to relatively open habitats. Additionally, external morphological measurements from 96 individuals at ten localities showed no significant sexual dimorphism across 13 traits. This integrative review provides updated insights into the spatial ecology, bioacoustic features, and potential habitat suitability of *M. pallidus* in Iran, emphasizing the value of combining historical data with recent field records and modelling approaches for conservation-oriented assessments.

Keywords: distribution, niche modelling, morphological, acoustic, echolocation.

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INTRODUCTION

The bat fauna of Iran has been increasingly studied over the years, with the number of recorded species rising steadily. Earlier studies reported about 38 species (DeBlase, 1980), which later increased to 45 species (Karami et al., 2008) and subsequently to 49 species (Benda *et al*., 2012). The first record of Nathusius' Pipistrelle, *Pipistrellus nathusii* (Naderi *et al*., 2017), brought the total to 50 species. The most recent assessment of bat diversity in Iran reports a total of 51 species, with the latest addition being *Rousettus leschenaultii* (Chiroptera: Pteropodidae) reported by Mohammadi *et al*. (2022).

Since early 21st century, research on bats in Iran has gone beyond simply listing species. More focus has been put on their biology and ecology, including distribution patterns, acoustic features, external morphology, habitat suitability modeling, and phylogeography and phylogeny (e.g., Sharifi *et al*., 2000; Akmali *et al*., 2015; Sharifi & Javanbakht, 2017; Mehdizadeh *et al*., 2018; Shahabi *et al*., 2019; Yousefi & Sharifi, 2020; Mehdizadeh *et al*., 2021). These studies have helped improve our understanding of the life history and conservation needs of species like *Miniopterus pallidus*, which is the main focus of this study.

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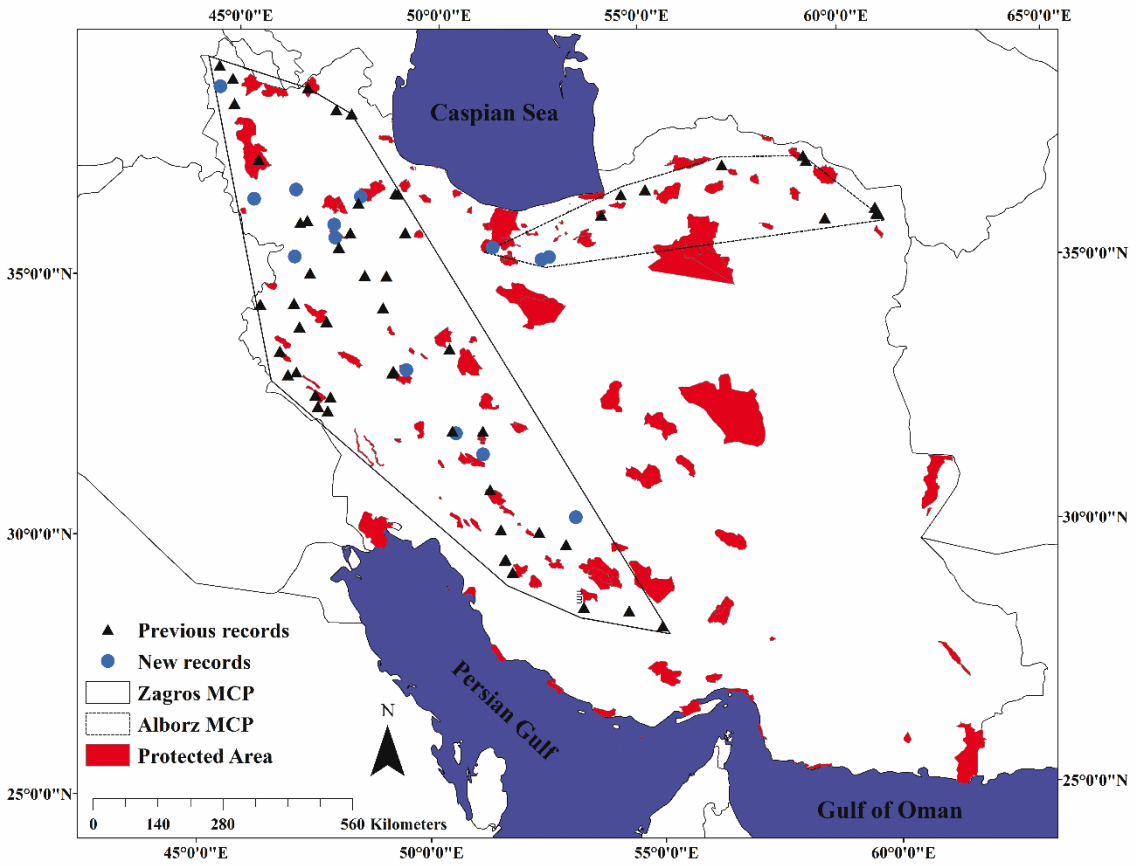

FIGURE 1. Geographical distribution of *Miniopterus pallidus* in Iran portrayed by two separate minimum convex polygons (MCP) encompassing all localities along Zagros and Alborz Mts. Blue circles illustrate new record (Appendix I) obtained in present study and black triangles present previous records (Appendix II) from published data. Areas depicted with red representing protected areas.

The Miniopteridae family comprises a singlegenus, *Miniopterus*, which includes around 30 recognized species (Monadjem *et al*., 2020). Members of this genus, commonly known asbent-winged bats, are distinguished by their unique wing morphology—specifically, the second phalanx of third finger is approximately threetimes longer than first (Nowak, 1994). These bats have short, slightly rounded ears with a moderate posterior fold and a short, blunt tragus curved slightly forwards (Srinivasulu *et al*., 2010). Their fur is typically black or dark brown, occasionally displaying reddish patches or entirelyreddish coloration (DeBlase, 1980). All *Miniopterus* species are medium-sized insectivorous bats with strong morphological similarities, which can make species-level identification difficult (Francis & Barrett, 2008). While forearm length and body weight may help distinguish individuals,skull measurements are often required for definitive identification (Francis & Barrett, 2008). Although pelage coloration may varybetween some species (Harrison, 1956), there is also considerable variationwithin species (Lewis & Harrison, 1962). The pale bent wing bat, *Miniopterus pallidus*, was first recordedby Thomas (1907), who identified it as *Miniopterus schreibersii* near Bandar-i-Gaz in northern Iran. Later, Lay (1967) described it as as *M. schreibersii pallidus* based on specimens from Shiraz (Shahpur cave) in southwestern Iran. In 1980s, DeBlase expanded its known distribution by listing additional localities inwestern and northwestern Iran. Subsequent records were provided by Benda *et al*. (2006, 2012) and Fathipour *et al*. (2014, 2016). Historically, there has been considerable uncertainty regarding thetaxonomic status of *M. pallidus* in Iran and neighboring countries. However, recent studies suggest that *M. schreibersii* species complex in southwestern Palaearctic and supraSaharan Africa comprises two main lineages: the

nominotypical *M. schreibersii*, distributed in Europe, supra-Saharan Africa, western Asia Minor, and *M. pallidus* found in southwestern Asia (including Iran, eastern Asia Minor), and eastern Afghanistan (Bilgin *et al*., 2008; Furman *et al*., 2010; Šrámek *et al*., 2013; Akmali *et al*., 2015). In Iran, *M. pallidus* has beenreported from various regions including the north, west, northeastern (DeBlase, 1980; Benda *et al*., 2006, 2012; Akmali *et al*., 2015; Malekpourfard & Akmali, 2022).

Species distribution models (SDMs) are widely used tools for predicting suitable habitats based on environmental variables and presence records, and are increasingly important in conservation biology (Tsoar *et al*., 2007; Elith & Leathwick, 2009). Among these, MaxEnt (Phillips *et al*., 2006) is one of the most effective methods, particularly suitable for species with limited occurrence data (Wisz *et al*., 2008; Engler *et al*., 2004). In recent years, these approaches have been increasingly applied in Iran to study the distribution and ecological preferences of various bat species, which play critical roles in maintaining ecosystem balance. For instance, Najafi *et al*. (2018) used SDMs to estimate the potential distribution of the Mediterranean horseshoe bat (*Rhinolophus euryale*), while Shahabi *et al*. (2019) focused on the greater horseshoe bat (*Rhinolophus ferrumequinum*), both highlighting habitat suitability patterns based on present climatic variables. In another study, Kafaei *et al*. (2021) assessed the ecological niche breadth and projected future range shifts of *Rhinopoma muscatellum* under various climate change scenarios, revealing the heightened vulnerability of arid and semi-arid mountain ecosystems in Iran. Furthermore, Mehdizadeh *et al*. (2018) combined molecular markers and environmental data to study population structure *Miniopterus pallidus*, showing that climatic variables such as temperature and precipitation influence genetic differentiation by limiting gene flow and promoting local adaptation. Collectively, these studies demonstrate the value of SDMs for understanding habitat dynamics, identifying conservation priorities, and predicting species responses to climate change, particularly in biologically diverse and climatically sensitive regions like Iran.

In present study our main objectives were (i) to delineate the species range and present additional records of *M. pallidus* from Iran, (ii) to characterize information on the external characteristics and acoustic features of the species in different parts of the country and (iii) to generate predicted habitat with only existing presence records using MaxEnt software.

MATERIAL AND METHODS

Study Area and Distribution

Iran, encompassing approximately 1,648,195 km², is geographically positioned at the intersection of Mediterranean and arid Central Asian climatic zones. The country's topography is dominated by prominent mountain ranges, including the Zagros Mountains—extending over 2,000 km from Turkey to the Strait of Hormuz—the Alborz Mountains, characterized by an elevated (>5000 m), narrow (~100 km wide) belt along the southwestern and southern coasts of the Caspian Sea, and the Kopet Dag range, a linear mountain belt spanning approximately 700 km from the southeastern Caspian Sea to the Afghanistan border (Tavakoli, 2007; Hollingsworth *et al*., 2006). These montane systems harbor extensive cave networks, which constitute critical habitats for diverse bat species, including the pale bent-wing bat, *Miniopterus pallidus*.

Between 2014 and 2017, systematic field surveys were conducted targeting caves and crevices within the mountainous regions of western, northern, and eastern Iran. In total, 24 caves were examined, 14 of which were surveyed for the first time. All records were mapped and are included in Appendix 1. Geographical position for each cave was recorded using a Garmin GPS unit (GPSMAP 60CSx; Garmin International, Inc., city, state, USA). Bats were netted with mist nets (6 × 3 m) placed on caves entrances or collected using hand nets. In addition, air temperature and humidity were measured in the field and inside caves using a digital thermo-hydrometer. Minimum convex polygons (MCPs) for species localities were created using ArcGIS 10.3 software. The extent of MCPs and their associated protected areas encompassing the Zagros and Alborz Mts. were calculated separately. Published data for *Miniopterus pallidus* were compiled from the scientific literature, including DeBlase (1980), Karami *et al*. (2008), Benda *et al*. (2012), and Fathipour *et al*. (2014, 2016).

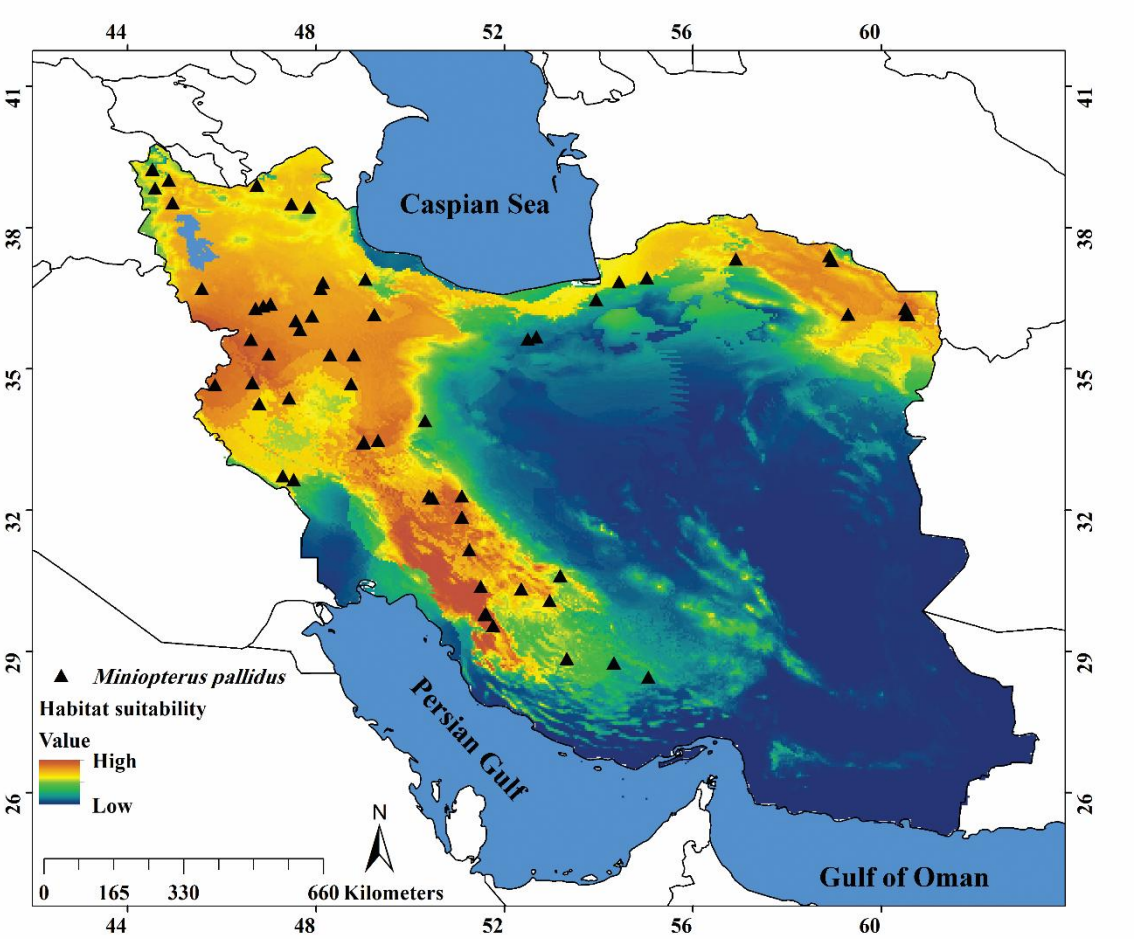

FIGURE 2. Presence records (triangles) in Iran considered for the development of a maximum entropy model (MaxEnt) predicting habitat suitability for *Miniopterus pallidus*.

Ecological niche modelling

We used SDM with the program MaxEnt (Phillips *et al*. 2006) in order to predict the distribution of suitable habitat for *M. pallidus*. The distribution records of *M. pallidus* in Iran were based on 64 localities shown in Figure 1. Climatic data representing contemporary climate conditions (~ 1960–1990) were obtained from WorldClim, version 1.4 (http://www.worldclim.org/) at a spatial resolution of 2.5 arc-minutes (~ 4.5 km). We used all 19 standard bioclimatic variables (BIO1–BIO19), which describe various aspects of temperature and precipitation relevant to ecological and evolutionary processes. These variables were subsequently processed usingArcGIS 10.3 to derive the bioclimatic variables. A full description of the variables is available on the WorldClim website. We used experiment designer ENMTools software version 1.3 to detect correlation values higher than 0.8. The following environmental variables were included in the models: BIO2 (Mean Diurnal Range, Mean of monthly), BIO3 (Isothermality (BIO2/BIO7) (*100)), BIO4 (Temperature Seasonality), BIO5 (Max Temperature of Warmest Month), BIO8 (Mean Temperature of Wettest Quarter), BIO12 (Annual Precipitation), BIO15 (Precipitation Seasonality) and BIO18 (Precipitation of Warmest Quarter).We ran 10 model replications and selected replicate with the highest test and train AUC percentages as the appropriate model. Model performance was evaluated based on the area under the curve (AUC) of the receiver operator characteristics (ROC) value. AUC values between 0.7 and 0.8 indicate acceptable model performance, values between 0.8 and 0.9 indicate good performance, and values above 0.9 indicate excellent performance (Elith *et al*. 2006; Phillips *et al*, 2006).

TABLE 1. New records of the pale bent wing bat, *Miniopterus pallidus*, with other bat species in 14 caves in Iran. Presence of bats is depicted by present records (+) and ■ previous records.

No	Cave	*M. pallidus*	*M. blythii*	*M. capaccinii*	*P. macrobullaris*	*R. ferrumequinum*	*R. hipposideros*	*R. mehely*	No Species
1	Alisheikh	+	+		+	+	+		5
2	Dehbokr	+	+	+		+		+	5
3	Jabiglu	+				+			2
4	Kamtaran	+				+		+	3
5	Katale khor	+	+			+	■		4
6	Ghar Darwish Olia	+				+			2
7	Salavatabad	+				+			2
8	Nesar	+	+			+			3
9	Rudafshan	+	■						2
10	Bornik	+	+						2
11	Imamzadeh Davood	+							1
12	Ghar-e-Agha seyyed isa	+				+			2
13	Eshkaft-e Zolaikha	+	■				■		3
14	Shoparak	+	+			+		+	4

TABLE 2. External body measurements (mm) of male and female *Miniopterus pallidus* sampled in present study. n, number of bats which were measured.

External features	n	Male	Number	Female	p-value
HB	27	53.48 ± 2.47	26	53.58 ± 2.38	0.38
T	22	59.09 ± 1.67	26	58.56 ± 1.52	0.74
HF	31	21.09 ± 0.50	18	21.12 ± 0.94	0.12
E	17	10.46 ± 0.46	13	10.29 ± 0.66	0.57
Tr	13	5.12 ± 0.31	9	5.27 ± 0.40	0.52
D4P1	27	10.20 ± 0.58	12	9.80 ± 0.62	0.18
D4P2	25	17.46 ± 0.94	12	18.10 ± 0.53	0.65
D3P1	27	11.10 ± 0.34	12	11.20 ± 0.72	0.12
D3P2	27	37.80 ± 0.92	12	37.76 ± 0.67	0.23
D4M	24	41.37 ± 0.74	9	42.20 ± 0.61	0.46
D3M	30	42.93 ± 0.77	27	42.80 ± 0.19	0.59
FA	52	46.40 ± 0.95	34	46.37 ± 0.98	0.07
WE	11	7.45 ± 0.29	5	7.64 ± 0.36	0.06

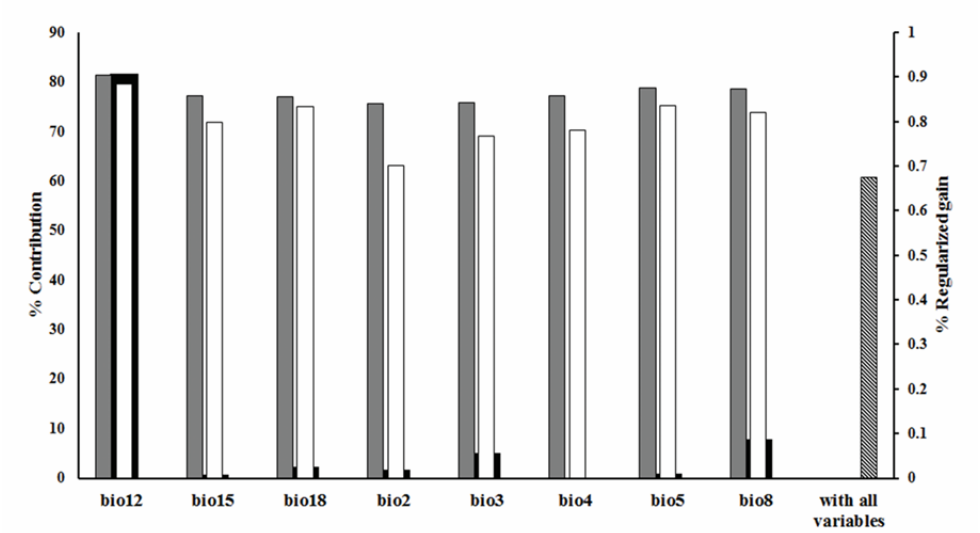

FIGURE 3. Contribution provided by the eight environmental variables considered to develop the MaxEnt model for *Miniopterus pallidus*. Black bars show the percentage contribution of each variable to the model and corresponding values are given on the left axis. Jackknife results for the model (values on the right axis) are also shown for single variables (white), for all variables except the one selected (grey) and for all variables (diagonal shade).

The MaxEnt model produced continuous habitat suitability maps for *Miniopterus pallidus*. These maps were reclassified into binary presence-absence maps, where areas with suitability values above a selected threshold were classified as suitable (presence = 1), and those below as unsuitable (absence = 0). This classification enabled identification of regions with favorable climatic conditions for *M. pallidus* populations.

External features

Live bats were sexed, aged and their external characteristics measured at ten sites including, Alisheikh, Dehbokr, Karaftu, Kilasefid, Mahidasht, Cara Tarik, Ghanjekuh, Mozduran, Sarab, and Tadovan caves (Table1, Appendix 1). Bat species identification was based on the criteria described by DeBlase (1980). Following body measurement and echolocation recording, all bats were released to their roost. Thirteen external body measurements were taken from 96 live bats using calipers. These measurements included: length of forearm (FA); length of ear (EAR); width of ear (WE); length of head to body (HB), length of tail (T); length of hind foot (HF); the metacarpal of the third and fourth digit (D3M, D4M); first phalange of the third and fourth digit (D3P1, D4P1); two phalange of the third and fourth digit (D3P2, D4P2); Length of tragus from base to tip (Tr).

Acoustic recordings

Acoustic recordings were carried out using an ultrasonic bat detector (Petterson Ultrasound Detector D240x) connected to an ultrasound recorder (EDIROL R-09), employing a time expansion of 10× to 1.7 seconds. We recorded calls of adult bats at Alisheikh, Mahidasht, Ghanjekuh, Mozduran, Sarab and Tadovan caves (Table 3). The recording microphone was placed 20 cm in front of bats inside a white bag. When necessary, bats were gently stimulated by a finger touch during recording to observe vocalizations changes in response to the stimuli. Recorded calls were analyzed using BatSound software (Pettersson Elektronik AB, Uppsala) with spectrogram setting of 512 and a Hanning window. For acoustic analysis, at least 10 pulses were randomly selected from each individual. Four parameters were measured for each pulse tocharacterize echolocation calls: call duration (CD), defined as the time between the start and end of a pulse in milliseconds (ms) from the oscillogram, peak frequency (PF), representing the frequency withthe greatest amplitude in kilohertz (kHz) from the power spectrum, start (SF); and end (EF) frequency of the pulse from the spectrogram, both in kHz.

Table 3. Frequency modulate (FM) echolocation calls of adult *Miniopterus pallidus* from 6 localities in Iran. Values are given as Mean ± SD for start frequency (SF), end frequency (EF), peak frequency (PF), call duration (CD), and inter-pulse interval (IPI). n, number of bats which were recorded.

Location	n	PF (kHz)	SF (kHz)	EF (kHz)	CD (ms)	IPI (ms)
Tadovan	6	57.27 ± 1.49	102.67 ± 4.49	51.26 ± 1.04	3.67 ± 0.47	101.95 ± 25.65
Sarab	10	59.48 ± 1.50	100.77 ± 4.22	51.12 ± 0.65	3.30 ± 0.44	93.63 ± 14.23
Alisheikh	9	57.04 ± 1.38	104.30 ± 3.57	49.41 ± 0.74	3.24 ± 0.27	69.32 ± 32.53
Mahidasht	3	58.19 ± 1.79	104.66 ± 2.35	48.44 ± 1.02	3.02 ± 0.28	99.50 ± 4.61
GanjeKuh	4	55.34 ± 3.55	94.43 ± 4.48	49.57 ± 1.71	3.53 ± 0.29	105.07 ± 57.93
Mozduran	12	57.12 ± 1.08	98.30 ± 5.99	48.82 ± 0.97	2.95 ± 0.38	80.89 ± 28.73

TABLE 4. Typical and continuous distress calls parameters from *Miniopterus pallidus*: start frequency (SF), end frequency (EF), peak frequency (PF), call duration (CD), and inter-pulse interval (IPI). Values are given as Mean ± SD.

Distress calls	PF (kHz)	SF (kHz)	EF (kHz)	CD (ms)	IPI (ms)
Typical (90)	22.40 ±3 .57	31.62 ± 4.20	13.15 ± 4.36	2.68 ± 0.58	4.16 ± 1.22
Continuous (14)	24.20 ± 2.98	29.43 ± 4.13	19.29 ± 3.07	45.74 ± 12.34	-

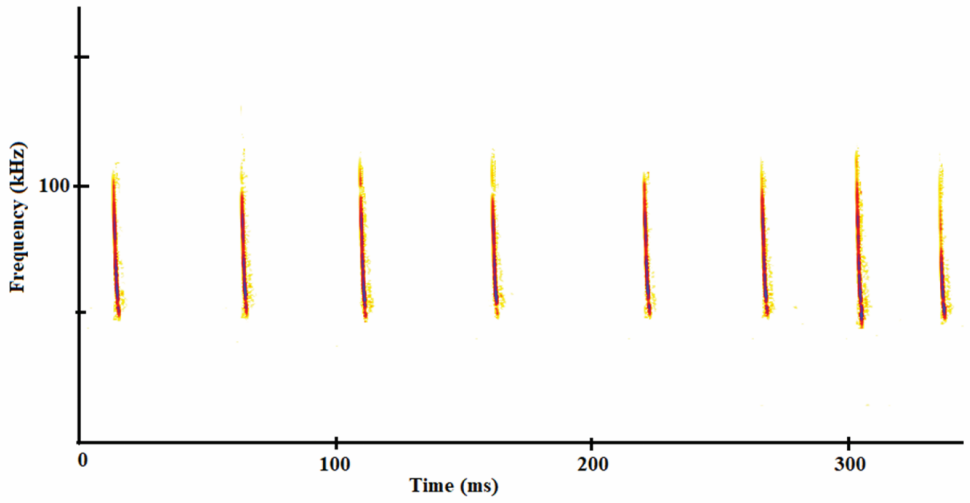

FIGURE 4. A typical echolocation calls of male *Miniopterus pallidus*.

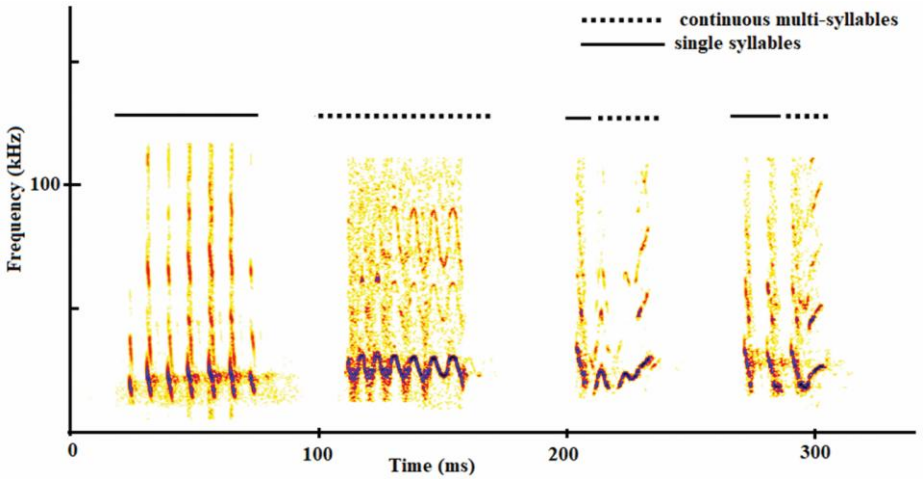

FIGURE 5. Sonogram of distress calls (single syllables) and continuous distress calls (continuous multi-syllables) from adult individuals of *Miniopterus pallidus*.

Descriptive statistics were calculated separately for each geographic group and sex, includig the mean (M), minimum (min), maximum (max), and standard deviation (SD). Sexual size variation was analyzed using an independent samples t-test. All analyses were conducted using SPSS 20.0 and Excel 2013.

RESULTS

Distribution

The occurrence points of *M. pallidus* reported in the present study were shown in Figure 1 and described in Appendix 1. on the 24 *M. pallidus* localities documented in this study, 14 were reported for the first time. Of these new localities, only three were located in the Alborz Mountains and while the remainingwere in the Zagros Mountains. A reviewot the scientific literature revealed 54 previously published records of the species across various regions of Iran (Figure 1, Appendix 2). Over 75% of localities reported for *M. pallidus* belong to the Zagros Mountains. No specimens have been reported from major biogeographical regions such as the central deserts of the Iranian Plateau, the Persian Gulf littorals, the eastern and southeastern territoriesand the southern Caspian Sea coastal areas. *Miniopterus pallidus* was observed in mountainous area ranging in elevation from 745 m a.s.l (Bazangan cave) to 2581 m a.s.l (Sarab cave). Minimum convex polygons (MCP) for all localities in Zagros and Alborz Mountainswere conveniently separated (Figure 1). The extend of the MCPs for the Zagros and Alborz Mountainswas approximately 452,370 km^2 and 107,578 km^2, respectively.

Among the 14 new localities reported for *M. pallidus* in this study, three were identified asnursery colonies: Dehbokr Cave (West Azerbaijan Province), Katalekhor Cave (Zanjan Province) and Nesar Cave (Lorestan Province). The largest bat aggregationwas recorded in Dehbokr Cave, where with *M. pallidus* the most abundant species, with anestimated population of approximately of one thousand individuals. Bat assemblages that included*M. pallidus* alongside other cave-dwelling bat species are summarizedin Table 1. Large summer aggregations in caves often consisted of two or more bat species, most commonly *M. pallidus*, *Rhinolophus ferrumequinum*, and *M. blythii*. In addition, *Miniopterus pallidus* was found to co-occur with *Plecotus macrobullaris*, *Myotis emarginatus*, *M. capaccinii*, *Pipistrellus kuhlii*, *P. pipistrellus*, *R. hipposideros*, *R. euryale*, *R. mehely*, *R. blasii*, *Rhinopoma microphyllum* and *R. muscatellum* (Table 1).

Ecological niche modelling

The ecological niche modelling using MaxEnt closely matched the known distribution of *Miniopterus pallidus* across Iran (Figure 2), confirming the robustness of the model. Notably, the model did not predict suitable habitats in central and southeastern Iran—regions where the species has not been recorded—highlighting its ecological fidelity. The model yielded a high Area Under the Curve (AUC) value of 0.85, indicating strong predictive performance, and a regularized gain of 0.67, suggesting a good fit to the presence data. Among the environmental variables tested, Annual Precipitation (Bio12) was the most influential, contributing 81.7% to the model, followed by Mean Temperature of the Wettest Quarter (Bio8, 7.8%), Isothermality (Bio3, 5.0%), and other temperature and precipitation-related variables (Figure 3). These results emphasize the key role of precipitation in shaping the distribution of *M. pallidus*, and suggest that climate-driven environmental heterogeneity may act as a limiting factor for the species' range in Iran.

External feature

Biometric data for *M. pallidus* specimens are presented in Table 2. The external body measurements obtained from live bats include the length of the forearm, length and width of ear, head to body length, tail length, hind foot length, length of the third- and fourth-digit metacarpal, first and second phalange of the third and fourth digit, and length of tragus (from base to tip). No statistically significant differences were found between males and females in any of the thirteen external morphological traits (Table 2).

Echolocation features

The echolocation calls of *M. pallidus* recorded in this study were classified as FM/QCF (frequency modulated/quasi-constant frequency) type. These calls typically swept from a maximum frequency of

approximately 104 kHz to a minimum of around 48 kHz (Figure 4). The species emitted broadband calls characterized by a quasi-constant frequency tail, which carried the highest energy. This dominant tail frequency ranged between 47.13 and 52.32 kHz across the sampled individuals in Iran (n = 44). Average pulses of *M. pallidus*, which were recorded in different localities in Iran are shown in Table 3. A total of 906 echolocation calls were analyzed from 44 adult individuals across six localities in Iran, with each bat contributing between 3 to 37 calls (Table 3).

In addition to echolocation, adult *M. pallidus* individuals also produced rapid sequences of, referred to as distress calls (Figure 5). These calls typically consisted of three to eight pulses per sequence, although the number could range from 3 to 30. Compared to echolocation pulses, distress calls were shorter in duration and exhibited lower frequencies (Table 4). Furthermore, *M. pallidus* was observed to emit distinctive distress pulses, either as continuous multi-syllabic sequences or discrete single pulses, differing in form from the typical distress calls (Figure 5). Continuous distress calls, recorded from four males and two females, displayed similar frequency ranges to discontinuous calls but were of shorter duration. These vocalizations may reflect prolonged anxiety or agitation in this species (Table 4).

DISCUSSION

Until the present study, *Miniopterus pallidus* had been recorded across much of Iran, with the exception of the southeastern and central regions (DeBlase, 1980; Benda *et al*., 2012). As expected, most of the newly documented localities align well with the predicted distribution from our ecological niche model (Figure 2). The majority of *M. pallidus* records are concentrated in the elevated Zagros Mountains, with only three from the Alborz range (Rudafshan and Bornik caves, and Imamzadeh Davood; Appendix 1, Figure 1). Except for Imamzadeh Davood—where bats were collected from a fissure in a building wall—all specimens were found roosting in natural caves.

Our species distribution model identified key environmental variables shaping the range of *M. pallidus* across a highly heterogeneous landscape in western and eastern Iran. The model demonstrated high predictive accuracy (AUC > 0.85; Elith, 2002), improving our understanding of the species' ecological requirements and informing future conservation planning. Nonetheless, it is important to note that the model does not account for biotic interactions, climate change, or anthropogenic pressures, which may influence habitat suitability. Despite these limitations, the model remains a valuable tool for identifying priority areas in poorly surveyed regions and guiding protected area design. Both this ecological niche modeling study and the previous mitochondrial DNA analysis (Mehdizadeh *et al*., 2018) independently highlight the key role of climatic variables in shaping the distribution and genetic structure of *Miniopterus pallidus* in Iran. These findings demonstrate that climate plays a central role in both the current and historical patterns of this species, reinforcing the importance of considering environmental heterogeneity in conservation planning.

Echolocation data collected in this study revealed considerable variability and structural complexity. Distinct differences were observed between single distress calls and multi-syllabic continuous sequences, particularly in call duration (Table 3). These variations likely reflect different types of social signaling in response to capture or disturbance. Similar complexity in distress vocalizations has been reported in other bat species, where such calls convey individual identity and serve as warnings to conspecifics (e.g., *Pipistrellus nathusii*, *P. pipistrellus*, *P. pygmaeus*, Russ *et al*., 2004; *Cynopterus sphinx*: Mariappan *et al*., 2013; *Saccopteryx bilineata*, Eckenweber & Knörnschild, 2016).

Our findings confirm that *M. pallidus* is predominantly associated with high-elevation mountainous habitats in Iran, particularly the Zagros and Alborz Mountains. This distribution pattern aligns with the species' morphological traits. The long and narrow wings of *M. pallidus* are characteristic of fast, energy-efficient flight in open or semi-open environments at higher altitudes (Norberg & Rayner, 1987), likely enhancing maneuverability between cliffs and roosting sites.

Acoustic data support this ecological interpretation. The species' FM/QCF echolocation calls, with dominant constant-frequency tails around 47–52 kHz, are well-suited to open and moderately cluttered habitats. Broadband FM components enable precise spatial resolution and prey detection, which are essential for navigation and foraging in complex mountainous terrains (Schnitzler & Kalko, 2001). The

absence of pure constant-frequency calls—typical of species foraging in dense vegetation—further supports the adaptation of *M. pallidus* to relatively open environments.

The ecological niche model also highlights the influence of climate, particularly annual precipitation, on the species' distribution. The mountainous regions inhabited by *M. pallidus* experience unique precipitation regimes, which likely affect prey abundance and roosting microclimates. The species' absence from central deserts and low-precipitation zones suggests a preference for cooler, wetter environments, consistent with its physiological and behavioral adaptations. Together, the morphological, acoustic, and environmental data present a coherent picture of *M. pallidus* as a mountain-adapted species, employing specialized flight and echolocation strategies optimized for life in high-elevation ecosystems. Conservation efforts should therefore prioritize these habitats, which support the largest populations and most critical nursery colonies.

The first attempt to assess the conservation status of Iranian bats by Sharifi et al. (2000) categorized *M. pallidus* as common based on relative abundance data, including geographic range, number of localities, and specimen counts. However, the species has yet to be assessed globally by the IUCN. Quantitative population estimates for Iranian bats remain scarce due to limited data availability. Historical records from the 1960s document large aggregations of bats, including *M. pallidus*, such as the >1,500 individuals reported from Gara Tarik Cave (DeBlase, 1980). Recent surveys in the same and nearby sites have shown steep population declines. For example, no bats were observed in Azad Khan and Moghan caves despite past reports (Etemad, 1964–1984; DeBlase, 1980; Benda et al., 2012; Akmali et al., 2015). These declines raise serious concerns about the long-term viability of *M. pallidus* populations and highlight the urgent need for systematic monitoring and conservation action.

CONCLUSION

Our findings show that *Miniopterus pallidus* is closely associated with mountainous habitats in Iran, especially in the Zagros and Alborz ranges. Its morphological and acoustic traits reflect adaptations to open, high-elevation environments. Ecological niche modeling confirmed the key role of precipitation in shaping its distribution. Notably, declines in historical roost sites raise concerns about population trends. This study provides essential data for future research and highlights the need for conservation efforts focused on protecting critical habitats of this species.

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Rediscovery of an 'extinct' endemic mammal Sonnerat's Shrew (*Diplomesodon sonnerati* Cheke, 2012) in Nilgiris, Tamil Nadu, India

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Abstract
The family Soricidae comprises 385 species in 26 genera, including the monotypic genus Diplomesodon, previously thought to contain only the Central Asian Piebald Shrew *D. pulchellum*. The enigmatic Sonnerat's Shrew *D. sonnerati*, described over 200 years ago from Pondicherry, India, was long considered possibly extinct due to the absence of confirmed specimens. On 1 October 2022, we photographed a dead shrew in Udhagamandalam (Nilgiris, Tamil Nadu) exhibiting distinctive grey pelage with a transverse white band and a stout tail, consistent with historical descriptions of *D. sonnerati*. The specimen measured 143 mm (head–body) and 26 mm (tail). Despite extensive live-trapping (15 traps, October–December, winter season), no additional individuals were detected. The Nilgiri record lies ~350 km from the type locality, indicating a notable biogeographic disjunction that may represent either a relict population or part of a wider, under-documented range. Targeted surveys in intervening habitats, coupled with genetic analyses, are urgently needed to assess distribution, population connectivity, and taxonomic validity, and to inform conservation strategies for this potentially rare and evolutionarily distinct shrew.

Keywords: Biogeographic disjunction, *Diplomesodon sonnerati*, India, Nilgiris, Rediscovery, Small mammal survey

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The family Soricidae comprises 385 shrew species in 26 genera (Wilson & Reeder, 2011), classified into three extant subfamilies: Crocidurinae (white-toothed shrews), Myosoricinae (African shrews), and Soricinae (red-toothed shrews). Members of Crocidurinae are further divided into 11 genera (*Crocidura, Diplomesodon, Feroculus, Myosorex, Paracrocidura, Ruwenzorisorex, Scutisorex, Solisorex, Suncus, Surdisorex, and Sylvisorex*). Current generic boundaries within this subfamily are based on limited morphological characters, and the delimitation of some genera remains the focus of ongoing genetic and morphological studies.

Diplomesodon is a monotypic genus containing the extant Piebald Shrew *Diplomesodon pulchellum* (Nowak, 1999). An extinct species, *D. fossorius*, is known from the Early Pleistocene of South Africa, far removed from the present Caspian region distribution of *D. pulchellum* (Repenning, 1965). A third

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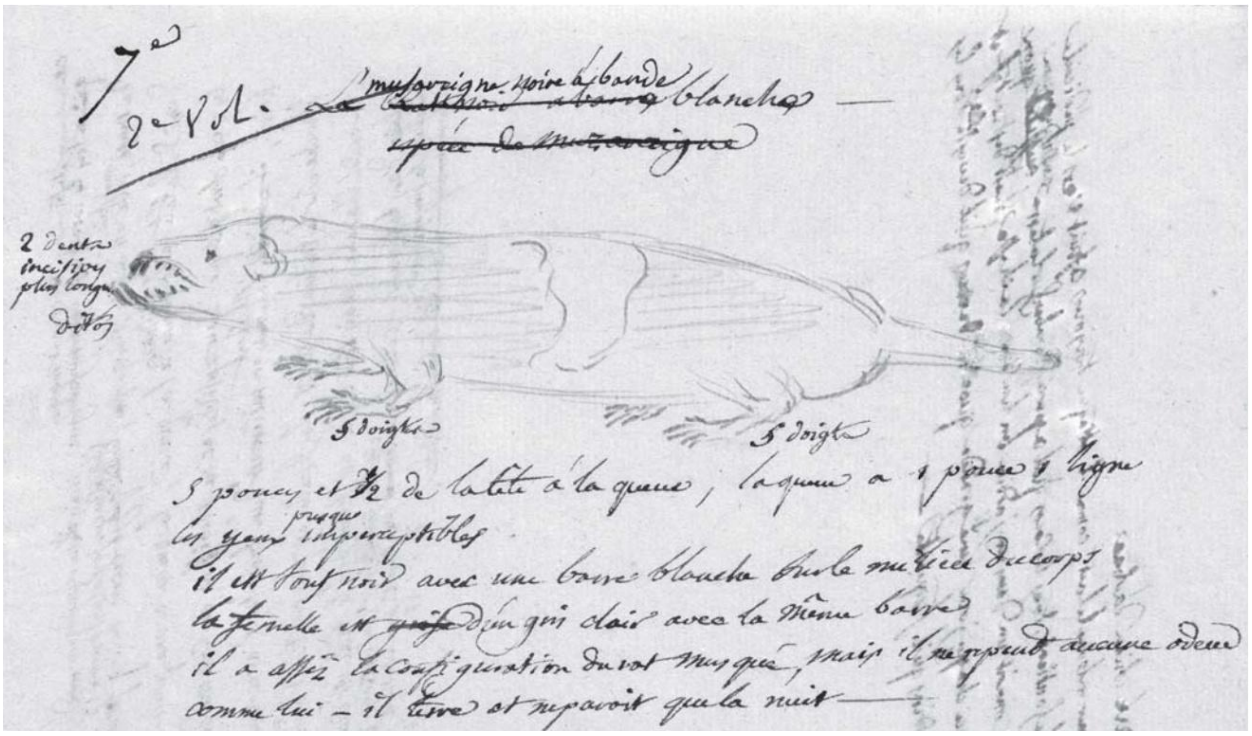

FIGURE 1. Pen-sketch representation by Pierre Sonnerat of the holotype of *Diplomesodon sonnerati* (from Cheke 2012, Published with permission of the New South Wales State Library). The original is Item No. PX*D83 p.32 in the Mitchell Library, Melbourne, Australia; The vertical writing is show-through from the other side of the paper).

putative member of the genus is the enigmatic Sonnerat's Shrew *Diplomesodon sonnerati* (Cheke), an Indian species previously considered possibly extinct. It was originally described only from an old written account and a crude pen-sketch of the holotype (Figure 1) (Cheke, 2011; Cheke & Hume, 2018). Its generic assignment is tentative, based primarily on pelage similarity with *D. pulchellum*. Sonnerat's Shrew is reported to be approximately twice as long as the Central Asian species (head + body 54–76 mm; Nowak, 1999). If indeed regularly larger than *Suncus murinus*, as noted by Sonnerat, it would be among the largest shrews globally. *S. murinus* reaches 150 mm (Nowak, 1999) or 160 mm (Alfred et al., 2006) in head-body length—comparable to the size described for *D. sonnerati*. Molecular phylogenetic analysis by Dubey et al. (2008) nested Diplomesodon within Crocidura, but the authors retained it as a distinct genus due to its morphological distinctiveness, despite this rendering Crocidura paraphyletic. Cheke (2011) suggested that Sonnerat's Shrew might still persist in southern India and recommended that voucher specimens of each sex be collected if rediscovered, with one designated as a neotype.

On 1 October 2022, we photographed a dead shrew in Udhagamandalam (Ooty), Nilgiris District, Tamil Nadu, India, that was morphologically distinct from other shrews regularly encountered in the region (*Suncus dayi*, *S. montanus*, *S. murinus*, *S. niger*, and *Feroculus feroculus*). Although the specimen was not collected, photographs were taken for subsequent examination. The individual exhibited silky grey fur with a transverse white band across the mid-body (Figure 2) and a stout, stubby tail features consistent with the description of female *D. sonnerati* (males reportedly being black where females are grey). The measured head-body length was 143 mm and tail length 26 mm. The specimen was located beside a railway line, adjacent to a small wetland patch dominated by *Juncus inflexus*, near Ooty Lake.

Photographs were shared with shrew specialists in India and abroad, including Anthony Cheke, who originally described the taxon. He noted that the pelage patterning was almost unique among shrews and sought expert opinion from Paula Jenkins (UK Natural History Museum), who confirmed that such a pattern was highly unlikely to represent partial leucism.

FIGURE 2. Photograph of Sonnerat's shrew (*Diplomesodon sonnerati*) in Nilgiris, Tamil Nadu India, 22 October 2022 (Photo Courtesy: Sirajudeen Mohammed Shahir).

Sonnerat's Shrew was first documented by Pierre Sonnerat during his stay in southern India between 1786 and 1813 (Deloche & Ly-Tio-Fane, 2010). Sonnerat reported the species from lowland agricultural landscapes near Pondicherry, approximately 350 km (aerial distance) from the Nilgiri locality reported here. His account included both a general description of southern Indian fauna and a specific diagnosis with an accompanying sketch (Figure 1). The absence of physical type material and the reliance on historical description had cast doubt on its validity, leading to the assumption that it was extinct (Cheke, 2012; Cheke & Hume, 2018). Our photographic evidence, matching Sonnerat's original description and sketch, demonstrates that the taxon persists in the wild.

Given that the Nilgiri specimen was discovered in an atypical location adjacent to a railway, and despite extensive trapping (15 traps operated from October to December during the winter season) no additional specimens were captured, accidental transport from lowland areas cannot be ruled out. Consequently, surveys should be extended to Sonnerat's type locality and intervening habitats.

The rediscovery of *D. sonnerati* in the Nilgiris highlights a significant biogeographic disjunction relative to its type locality. This distributional gap may indicate either a broader, under-documented range reflecting the cryptic habits of shrews and the scarcity of targeted small-mammal surveys or a relictual population persisting in ecological refugia following historical range contraction. Suitable habitats occur along the Eastern Ghats and Nilgiri foothills, suggesting potential dispersal corridors. Focused trapping efforts in these regions, across multiple seasons, combined with genetic analyses, are needed to assess connectivity between populations and clarify the taxonomic and conservation status of this species.

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