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Genetic diversity and structure analysis of critically endangered *Goniurosaurus yingdeensis* (Squamata, Eublepharidae) based on SSR markers

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Abstract

The Yingde Leopard Gecko (*Goniurosaurus yingdeensis*) is a critically endangered reptile restricted to a narrow-fragmented karst region in China. Investigating the role of terrestrial islands in biodiversity generation by conducting genetic analyses of *G. yingdeensis* can offer insights into the ecological and evolutionary dynamics of inselbergs, while also providing theoretical and practical guidance for future conservation of this species. In this study, we employed 20 pairs of SSR primers to assess the genetic diversity and analyze the population structure of 86 individuals from 10 geographical populations of *G. yingdeensis*. The results revealed low genetic diversity in *G. yingdeensis*. Populations exhibited a high degree of genetic differentiation and a low level of gene flow, which may contribute to the loss of genetic diversity due to genetic drift. Clustering analyses indicated that the genetic differentiation between one population and the other nine was the most pronounced. Furthermore, the genetic distance among *G. yingdeensis* populations was found to be influenced by more than just spatial distance. This study demonstrates that for species with limited dispersal capability such as *G. yingdeensis*, karst inselbergs function similarly to aquatic islands in evolutionary processes: gene flow is restricted to a small range by geographic barriers, isolated populations evolve independently, and genetic differentiation increases over time. Among the factors contributing to population isolation in *G. yingdeensis*, rivers play the most significant role.

Key Words

conservation genetics, geographic vicariance, inselbergs, karst, microsatellite, small population

Introduction

Inselbergs, defined by old age, isolation and unique microhabitats (Vanschoenwinkel et al. 2024), serve as quintessential islands. Like oceanic islands, these terrestrial analogs provide fundamental model systems for studying ecological and evolutionary processes, including colonization, speciation, and the impacts of habitat fragmentation. The steep limestone tower karst hills of East Asia, which create dispersal barriers and act as refugia for specialized biota (Vanschoenwinkel et al. 2024; Song et al. 2025), perfectly exemplify such inselberg systems.

Among the obligate dwellers of these karst formations is the genus *Goniurosaurus* Barbour, 1908, a group of eyelid geckos currently comprise 26 extant species (Uetz et al. 2025). Their distribution ranges from the Ryukyu Archipelago in Japan southward through East Asia to northern Vietnam (Barbour 1908; Grismer et al. 2021; Ngo et al. 2021; Zhu et al. 2021; Zhu et al. 2022; Kurita and Toda 2024). Among them, *Goniurosaurus yingdeensis*

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(Fig. 1) is narrowly endemic to the karst habitats within and around the Shimentai National Nature Reserve of Guangdong Province, China (Wang et al. 2010). This species faces increasing threats from anthropogenic factors such as illegal trade and habitat loss (Ngo et al. 2019, 2022, 2023), and has been assessed as Critically Endangered (CR) by the International Union for Conservation of Nature (IUCN 2024). Furthermore, the karst landscape inhabited by *G. yingdeensis* is highly fragmented, resulting in a discontinuous distribution pattern of populations. Due to the species' poor dispersal ability, gene flow between these populations is restricted by the intervening non-karst matrix, enhancing the potential for population differentiation (Zhang et al. 2014). At the same time, small, isolated populations are highly susceptible to genetic drift, which can lead to a significant loss of genetic diversity. This scenario positions *G. yingdeensis* as an ideal model to investigate the population genetic consequences of living in terrestrial island systems.

The fragmented karst hills occupied by *Goniurosaurus yingdeensis* can be considered as inselbergs under the definition proposed by Vanschoenwinkel et al. (2024), making the principles of island biogeography directly applicable. Parallels can be drawn from studies on aquatic islands, for instance, haplotype analysis revealed that certain populations of eyelid geckos on the Ryukyu Archipelago have lost nearly all their genetic diversity (Kurita et al. 2014). However, such endangered lineages were not subjected to further assessment at the time (Honda et al. 2014). *G. yingdeensis* in terrestrial karst islands may be facing a similar crisis. To investigate this, microsatellites, or Simple Sequence Repeats (SSR), are particularly well-suited molecular markers. Due to their genomic abundance, co-dominant inheritance, multiallelic nature, high polymorphism, and reproducibility (Senan et al. 2014; Ramesh et al. 2020), SSR markers are highly informative and cost-effective for inferring infraspecific genetic relationships (Putman et al. 2014; Vieira et al. 2016). Their application has been widespread in population genetics assessment of endangered species (Abdul-Muneer 2014; Kumar et al. 2020; Sun et al. 2021). Notably, Kurita et al. (2018) successfully developed SSR markers to analyze gene flow among populations of the *Goniurosaurus kuroiwae* group in the Ryukyu islands, and detected hybridization between parapatric genetic groups. This demonstrates the utility of SSR markers in revealing fine-scale population structure un *Goniurosaurus yingdeensis* inhabiting fragmented landscapes.

In this study, we employed 20 pairs of SSR primers to estimate the genetic diversity and analyze the population structure of 86 individuals from 10 geographical populations of *Goniurosaurus yingdeensis*. By analyzing patterns of genetic differentiation and gene flow, we aim to elucidate the role of karst inselbergs in shaping the trajectory of the endemic species. Specifically, we seek to determine: (i) whether prolonged isolation in karst habitat patches has led to significant genetic structuring among populations, reflecting a pattern of colonization and limited dispersal typical of island systems; (ii) the extent to which the non-karst matrix acts as a barrier to gene flow,

Figure 1. *Goniurosaurus yingdeensis* in wild habitats.

thereby promoting population differentiation and potentially incipient speciation; and (iii) whether smaller, more isolated populations exhibit signatures of genetic drift, such as reduced genetic diversity, which would demonstrate the impacts of habitat fragmentation on population viability. Our findings will provide empirical evidence for the ecological and evolutionary dynamics of terrestrial island systems and offer a theoretical and practical framework for the future conservation of the critically endangered *G. yingdeensis*.

Methods

Field surveys

Our study encompasses the gecko's entire known range, with sampling effort focused on these 10 sites to ensure a complete representation of its distribution (Fig. 2). At each locality, we conducted systematic visual encounter surveys in areas identified as suitable microhabitat. Survey locations were strategically determined based on historical occurrence records and interviews with local forest rangers to ensure coverage of representative habitat patches characterized by evergreen forest on karst formations, high humidity, stable temperatures (20–30 °C), exposed rock substrates, and a low canopy with moderate ground vegetation. We conducted surveys during the non–hibernation season of *Goniurosaurus* in June, July and September 2020, March and July 2021. Following a preliminary reconnaissance (23 June–1 July 2020) to verify historical records and identify suitable habitat patches, we conducted systematic visual encounter surveys during three main periods (3–13 July 2020, 7–26 September 2020, and 9–20 March 2021) and one supplementary survey (6–14 July 2021). To guarantee the highest detection probability for the nocturnal species and to limit the bias of observers, excursions were carried out at night (8 p.m. to 5 a.m.). Each survey in each transect was conducted with at least two researchers. A total of 96 *G. yingdeensis* individuals were found (Suppl. material 1: fig. S1a, table S1), including 10 recaptured individuals. To test whether individuals could move between transects, we conducted individual identification using HotSpotter software (Crall et al. 2013) combined with manual verification (Suppl. material 1: fig. S1b). All recaptures occurred within the same transect where the individuals were originally captured. The maximum straight-line movement distance observed for any recaptured individual was less than 100 m (based on 10 recapture events). Given that adjacent transects were separated by at least 100 m of non-karst matrix (or geographically discontinuous karst patches), and that no movement was detected between transects, we treated each transect as a provisional geographic population for subsequent genetic analyses. These populations are designated as DYa, DYb, DR, DZ, HS, HSb, LT, PB, SQ, and YS, corresponding to the 10 sampling sites (Fig. 2). We acknowledge that this a priori delineation represents a working hypothesis based on observed movement limitations and spatial isolation; the genetic data presented below will ultimately test whether these geographically defined units indeed function as distinct genetic populations.

Tail tips were collected from each gecko during field survey for subsequent molecular experiments. A total of

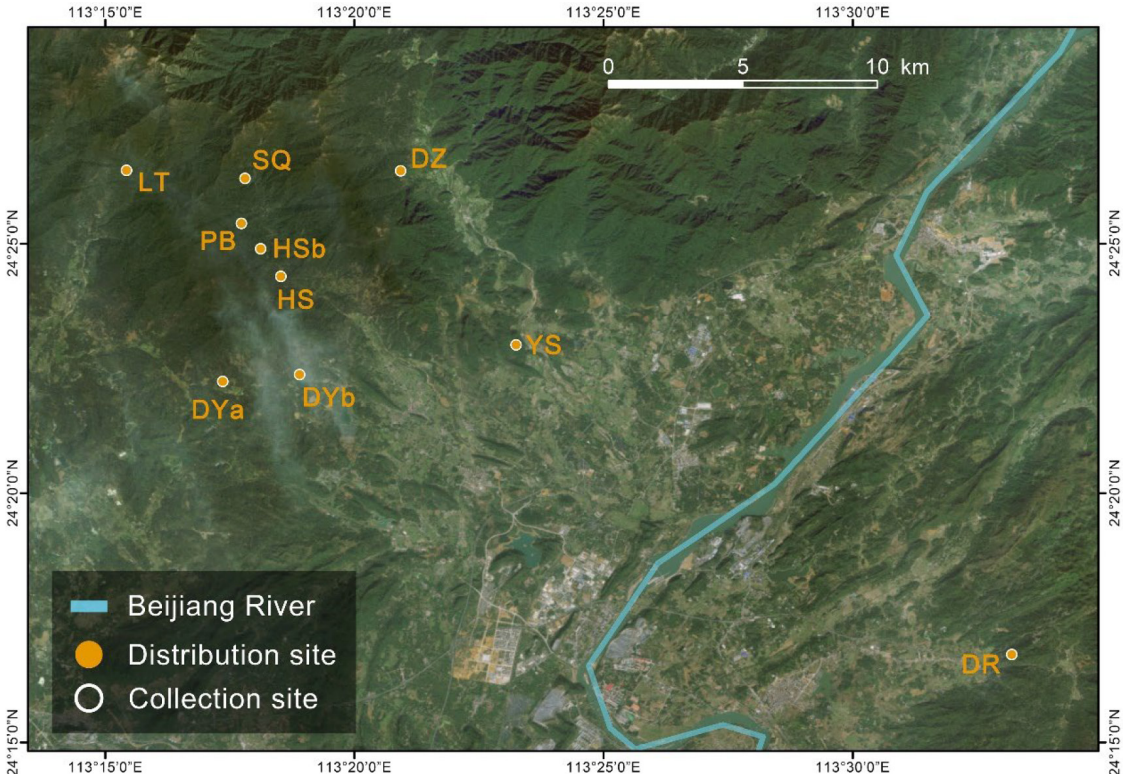

Figure 2. The collection sites of *Goniurosaurus yingdeensis* in this study, including all of the 10 known distribution sites.

96 samples from 10 populations (DYa, DYb, DR, DZ, HS, HSb, LT, PB, SQ, YS) of *Goniurosaurus yingdeensis* were collected. Samples were stored in 95% ethanol at -40 °C. All individuals were sexed, measured, and photographed before being released at their original capture sites.

DNA extraction and SSR genotyping

Total DNA was extracted from ethanol preserved tissues using the standard phenol/chloroform DNA extraction method (Sambrook et al. 1989) and stored at 4 °C.

To screen for polymorphic microsatellite loci, a preliminary test was conducted using one individual from each of the ten populations (10 individuals in total). Polymerase chain reactions (PCRs) were performed with forward primers tailed at the 5' end with the M13 universal linker sequence (TGTAAAACGACGGCCAGT). The PCR thermal profile consisted of initial denaturation at 96 °C for 3 min, followed by 30 cycles of 96 °C for 30 s, annealing at temperatures ranging from 52 °C to 62 °C (depending on the locus) for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Based on the amplification success and polymorphism level, 20 loci that showed stable amplification and high polymorphism were selected for subsequent genotyping of all samples (Suppl. material 1: table S2).

For the main genotyping procedure, all 96 individuals were amplified at the 20 selected SSR loci using the same PCR conditions described above. PCR products were genotyped by capillary electrophoresis on an ABI3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA) following the manufacturer's instructions. Allele sizes were determined against the GeneScan™ 500 ROX™ size standard and scored automatically using GENEMARKER version 2.2.0 (Soft Genetics, State College, PA, USA). All PCR amplifications and genotyping services were provided by Wuhan Tianyihuiyuan Biotechnology Co., Ltd. The SSR sequencing data of all the geckos used for subsequent analyses are provided in Suppl. material 2.

Genetic statistical analyses

A total of 96 samples from 10 populations of *Goniurosaurus yingdeensis* were collected in the field survey. Photo identification revealed 10 recaptured individuals, which were removed from the dataset. This process yielded a final dataset of 86 unique individuals across the 10 populations for subsequent study.

Genetic diversity within each population was assessed using GENALEX 6.502 (Peakall and Smouse 2006). The following categories of parameters were estimated: (i) allelic diversity indices (N_a, N_e, I); (ii) heterozygosity estimates (H_o, H_e); and (iii) fixation indices (F_{IS}, F_{IT}, F_{ST}), along with gene flow (N_m). Detailed definitions of all parameters are provided in Suppl. material 1: table S3.

The principal coordinate analysis (PCoA) was carried out in GENALEX 6.502 to divide the samples into different clusters. We conducted individual-based clustering analysis using STRUCTURE 2.3.4 (Evanno et al. 2005) to infer the population structure. The data were simulated 10 times for each number of clusters K. We used STRUCTURE HARVESTER (Earl and VonHoldt 2012) to estimate an appropriate number of K based on the second-order rate of change (delta K). We summarized the results from 10 simulations using CLUMPP (Jakobsson and Rosenberg 2007) and visualized the genetic composition of each individual using Excel.

To test for spatial autocorrelation and isolation-by-distance (IBD) patterns, we performed a Mantel test examining the relationship between genetic distance and geographic distance among all population pairs. Genetic distances were calculated as $F_{ST}/(1-F_{ST})$ following Rousset (1997). Geographic distances (in km) were log-transformed to linearize the relationship. The Mantel test was conducted using the mantel function in the R package vegan (Oksanen et al. 2022) with 9,999 permutations to assess statistical significance.

Results

Polymorphisms of the SSR markers

A total of 67 alleles were detected over these SSR loci for the *Goniurosaurus yingdeensis* populations (Table 1). The number of observed alleles (N_a) ranged from 2 to 5 with an average allele number of 3.38. The number of effective alleles (N_e) ranged from 1.38 to 3.81 with an average allele number of 2.42. The Shannon information index (I) ranged from 0.30 to 1.36 with an average of 0.88. The observed heterozygosity (H_o) ranged from 0.14 to 0.74 with an average of 0.480. The expected heterozygosity (H_e) ranged from 0.17 to 0.68 with an average of 0.482. Among 20 SSR primers, Gss007 yielded the highest number of alleles (5 alleles).

The F-statistical parameters showed that F_{IS}, F_{IT} and F_{ST} varied from -0.126 to 0.684, from 0.132 to 0.818 and from 0.193 to 0.707, respectively, with mean values of 0.009, 0.359 and 0.357 (Table 1). The N_m varied from 0.104 to 1.043, with an average of 0.516.

Genetic diversity

The genetic diversity of the *Goniurosaurus yingdeensis* has been investigated using the selected 20 SSR molecular markers. Among the ten populations, we found N_a varied from 2.000 to 5.400 with an average of 3.385 per population, and N_e ranged from 1.697 to 3.624 with an average of 2.424 per population. The I, H_o, H_e and F varied among the populations from 0.483 to 1.344, from 0.300 to 0.650 and from 0.295 to 0.676, from -0.208 to 0.065, respectively (Table 2). The average number of observed alleles was 3.38, which is

Table 1. Indices of gene diversity, *F*-statistics values and gene flow deduced by 20 SSR loci.

Locus	*Na*	*Ne*	*I*	*Ho*	*He*	F_{IS}	F_{IT}	F_{ST}	*Nm*
Gss007	5.400	3.813	1.356	0.744	0.665	-0.118	0.137	0.228	0.847
Gss008	4.000	2.766	1.031	0.551	0.533	-0.035	0.256	0.281	0.640
Gss013	3.300	2.527	0.890	0.527	0.483	-0.092	0.328	0.385	0.400
Gss016	4.900	3.627	1.330	0.679	0.682	0.004	0.196	0.193	1.043
Gss026	2.500	1.815	0.647	0.399	0.395	-0.008	0.401	0.406	0.366
Gss030	3.100	2.212	0.810	0.144	0.456	0.684	0.818	0.423	0.341
Gss033	4.000	2.187	0.901	0.444	0.481	0.077	0.401	0.352	0.461
Gss039	2.600	2.003	0.704	0.401	0.427	0.061	0.392	0.353	0.459
Gss045	2.900	1.793	0.673	0.427	0.379	-0.126	0.311	0.389	0.393
Gss063	1.800	1.381	0.296	0.171	0.173	0.014	0.711	0.707	0.104
Gss078	4.300	3.042	1.104	0.559	0.566	0.013	0.299	0.290	0.613
Gss079	3.100	2.314	0.788	0.402	0.434	0.074	0.453	0.410	0.360
Gss087	3.200	2.579	0.904	0.497	0.509	0.024	0.370	0.354	0.456
Gss090	2.900	2.068	0.779	0.527	0.468	-0.124	0.204	0.292	0.605
Gss102	3.900	2.726	1.042	0.603	0.563	-0.072	0.189	0.244	0.776
Gss108	2.700	2.132	0.746	0.380	0.441	0.138	0.453	0.366	0.433
Gss115	2.200	1.686	0.580	0.393	0.366	-0.074	0.447	0.485	0.265
Gss130	4.200	3.068	1.143	0.659	0.613	-0.075	0.231	0.285	0.629
Gss170	2.800	1.835	0.657	0.406	0.381	-0.065	0.451	0.484	0.266
Gss185	3.900	2.908	1.116	0.696	0.622	-0.119	0.132	0.224	0.868
Mean	3.385	2.424	0.875	0.480	0.482	0.009	0.359	0.357	0.516

Na: Allele number; *Ne*: Effective allele number; *I*: Shannon index; *Ho*: Observed heterozygosity; *He*: Expected heterozygosity; F_{IS}: Inbreeding coefficient within population; F_{IT}: Total inbreeding coefficient; F_{ST}: Differentiation coefficient among populations; *Nm*: Gene flow among populations.

Table 2. Genetic diversity of the gecko populations.

Pop	*Na*	*Ne*	*I*	*Ho*	*He*	*F*
DYa	5.400	3.624	1.344	0.650	0.669	0.037
DYb	5.050	3.340	1.326	0.635	0.676	0.053
HS	3.350	2.230	0.872	0.446	0.488	0.065
DR	4.650	2.849	0.968	0.481	0.457	-0.059
HSb	3.300	2.451	0.958	0.546	0.541	-0.023
DZ	2.250	1.924	0.659	0.408	0.419	0.028
LT	2.050	1.880	0.602	0.475	0.394	-0.208
PB	3.050	2.218	0.834	0.494	0.477	-0.029
SQ	2.750	2.029	0.703	0.369	0.403	0.046
YS	2.000	1.697	0.483	0.300	0.295	-0.019
Mean	3.385	2.424	0.875	0.480	0.482	-0.005

Na: Allele number; *Ne*: Effective allele number; *I*: Shannon index; *Ho*: Observed heterozygosity; *He*: Expected heterozygosity; *F*: Fixation index.

higher than that reported for *G. kuroiwae* (2.88), but lower than that for *G. orientalis* (4.41) (Kurita et al. 2014).

Population differentiation

Genetic variability of each population and genetic differentiation between populations calculated using the 20 microsatellite loci are presented in Tables 3, 4, 5. The Nei's genetic distance showed that DR and PB have the largest genetic distance (2.806), the lowest genetic identity (0.060), supporting their furthest genetic relationship. DYa and DYb have the shortest genetic distance (0.199), the largest genetic identity (0.820) and the lowest F_{ST} (0.041), supporting their closest genetic relationship.

To test whether genetic differentiation followed an isolation-by-distance (IBD) pattern, we performed a Mantel test examining the relationship between genetic distance and geographic distance (log-transformed) among the 10 populations. A significant positive correlation was detected using both Pearson's product-moment correlation ($r = 0.693$, $p < 0.001$) and Spearman's rank correlation ($r = 0.762$, $p = 0.001$) (Suppl. material 1: fig. S2).

Genetic structure

PCoA and STRUCTURE assignment were employed to categorize the gecko populations (Fig. 3). Principal coordinate analysis (PCoA) revealed two major clusters among the gecko population. Population structure analysis (PSA) revealed that when K = 2, ΔK was the largest, and 86 individuals were divided into two clusters. One cluster comprised only the DR samples. The boundary between the two clusters was obvious. The STRUCTURE assignment and the PCoA both indicated that there was a clear division between DR population and other nine populations.

Considering the large genetic differentiation between DR and other 9 populations, we also removed the DR population and employed PCoA and STRUCTURE assignment of the remaining 9 populations of 70 individuals in total (Fig. 4). Principal coordinate analysis (PCoA) revealed that there were three clusters among the gecko population. One cluster including PB, SQ and LT, one concluding YS, and the rest clustered in one. The STRUCTURE showed that all clone individuals can be clustered into two groups. PB and SQ clustered in a group, with

Table 3. Genetic distance (*D*) between different geo-populations of *Goniurosaurus yingdeensis*.

	DYa	DYb	HS	DR	HSb	DZ	LT	PB	SQ	YS
DYa	0.000									
DYb	0.199	0.000								
HS	0.323	0.425	0.000							
DR	1.917	1.873	1.842	0.000						
HSb	0.416	0.425	0.234	1.440	0.000					
DZ	0.563	0.679	0.595	1.644	0.543	0.000				
LT	0.536	0.590	0.579	2.372	0.600	0.693	0.000			
PB	0.539	0.494	0.596	2.806	0.601	0.754	0.404	0.000		
SQ	0.518	0.642	0.588	2.160	0.551	0.734	0.340	0.328	0.000	
YS	0.863	1.010	0.961	2.169	1.045	0.646	1.642	1.612	1.269	0.000

Table 4. genetic identity (*I*) between different geo-populations of *Goniurosaurus yingdeensis*.

	DYa	DYb	HS	DR	HSb	DZ	LT	PB	SQ	YS
DYa	1.000									
DYb	0.820	1.000								
HS	0.724	0.654	1.000							
DR	0.147	0.154	0.158	1.000						
HSb	0.660	0.654	0.792	0.237	1.000					
DZ	0.570	0.507	0.551	0.193	0.581	1.000				
LT	0.585	0.554	0.561	0.093	0.549	0.500	1.000			
PB	0.583	0.610	0.551	0.060	0.548	0.470	0.668	1.000		
SQ	0.596	0.526	0.556	0.115	0.576	0.480	0.711	0.721	1.000	
YS	0.422	0.364	0.382	0.114	0.352	0.524	0.194	0.199	0.281	1.000

Table 5. Differentiation coefficient among populations (F_{ST}).

	DYa	DYb	HS	DR	HSb	DZ	LT	PB	SQ	YS
DYa										
DYb	0.041									
HS	0.099	0.117								
DR	0.271	0.263	0.346							
HSb	0.103	0.101	0.083	0.296						
DZ	0.160	0.172	0.207	0.355	0.184					
LT	0.166	0.174	0.214	0.416	0.198	0.265				
PB	0.145	0.135	0.192	0.379	0.174	0.240	0.166			
SQ	0.162	0.184	0.205	0.417	0.188	0.271	0.177	0.150		
YS	0.249	0.263	0.310	0.460	0.324	0.285	0.446	0.413	0.406	

almost no gene flow between DYa, DYb, HS, HSb, DZ and YS. LT had a complex genetic structure with gene flow from both of the two genetic groups.

Discussion

Our study reveals that populations of the critically endangered *Goniurosaurus yingdeensis* harbor moderate levels of genetic diversity despite their restriction to fragmented karst habitats, yet exhibit pronounced genetic differentiation and limited gene flow. These findings provide a genetic framework for the conservation of this narrow-range endemic species and offer empirical data relevant to understanding population dynamics in fragmented karst landscapes.

The level of genetic diversity observed in *G. yingdeensis* (N_a = 3.38) intermediate compared to its island-dwelling congeners in the Ryukyu Archipelago. It is higher than the genetically depauperate *G. kuroiwae* (N_a = 2.88) but lower than *G. orientalis* (N_a = 4.41) (Kurita et al. 2014). This comparison situates a mainland karst-endemic species within the spectrum of genetic diversity documented for insular congeners facing similar evolutionary constraints. The karst hills inhabited by *G. yingdeensis*, embedded within a non-karst matrix, may impose constraints analogous to those of oceanic islands, though direct testing of this analogy requires explicit comparative frameworks beyond the scope of the present study.

The population genetic parameters we estimated strongly support this interpretation. The combination of high genetic differentiation (F_{ST} = 0.357) and low gene flow (N_m = 0.516) across all populations indicates that gene flow is insufficient to counterbalance the effects of genetic drift (Slatkin and Barton 1989; Nagylaki 1998). This pattern is consistent with expectations for species

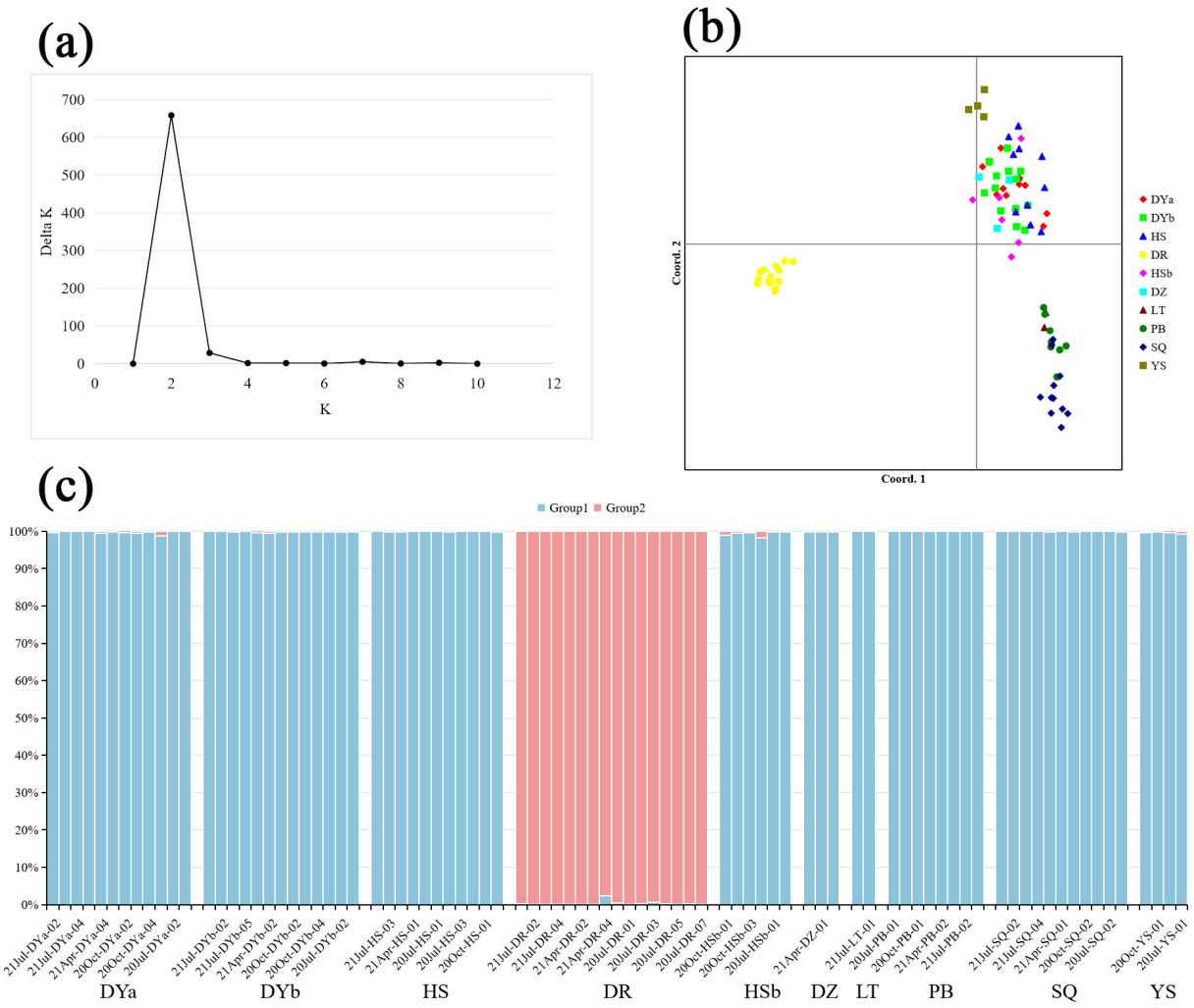

Figure 3. a. The broken line graph of ΔK and K values; **b.** Principal coordinates analysis of the 10 populations; **c.** Structure analysis for 86 gecko individuals from 10 populations (K = 2).

inhabiting inselberg systems: the intervening non-karst matrix acts as a dispersal barrier, promoting independent evolutionary trajectories and the accumulation of genetic divergence over time (Zhang et al. 2014; Vanschoenwinkel et al. 2024).

The significant isolation-by-distance (IBD) pattern detected (Mantel test, $r = 0.693$, $p < 0.001$) confirms that geographic distance is a primary factor shaping genetic structure in *G. yingdeensis*, consistent with the species' limited dispersal capacity (maximum movement < 100 m). However, the observed IBD pattern also implies spatial autocorrelation among neighboring populations—a necessary consequence of distance-limited gene flow rather than a violation of sampling independence. Critically, deviations from this IBD expectation reveal the influence of additional landscape barriers. For example, despite being geographically proximate, the LT population exhibits stronger genetic affinity to the more distant PB and SQ populations than to its closer neighbors HS and HSb (Fig. 2). This discordance between geographic and genetic distance suggests that the IBD baseline is locally overridden by specific barriers that disrupt gene flow beyond the predictions of distance alone.

Among potential barriers, the Beijiang River emerges as a likely candidate structuring the DR population. Both STRUCTURE (K = 2) and PCoA analyses separated DR from all other populations, with negligible gene flow detected. Geographically, DR is isolated by the Beijiang River (Fig. 2), which likely constitutes a formidable barrier for a terrestrial gecko with poor swimming ability. This observation is consistent with earlier hypotheses that rivers may serve as vicariant agents promoting population differentiation in continental *Goniurosaurus* (Zhang et al. 2014). However, because this pattern is based on a single population, it remains suggestive rather than conclusive; explicit landscape genetic analyses incorporating multiple river barriers and spatially explicit resistance modeling would be required to rigorously test the hypothesis that rivers are the predominant barrier to gene flow in this system.

Conclusions

This study provides a genetic assessment of diversity and population structure in the critically endangered *Goniurosaurus yingdeensis* using microsatellite markers.

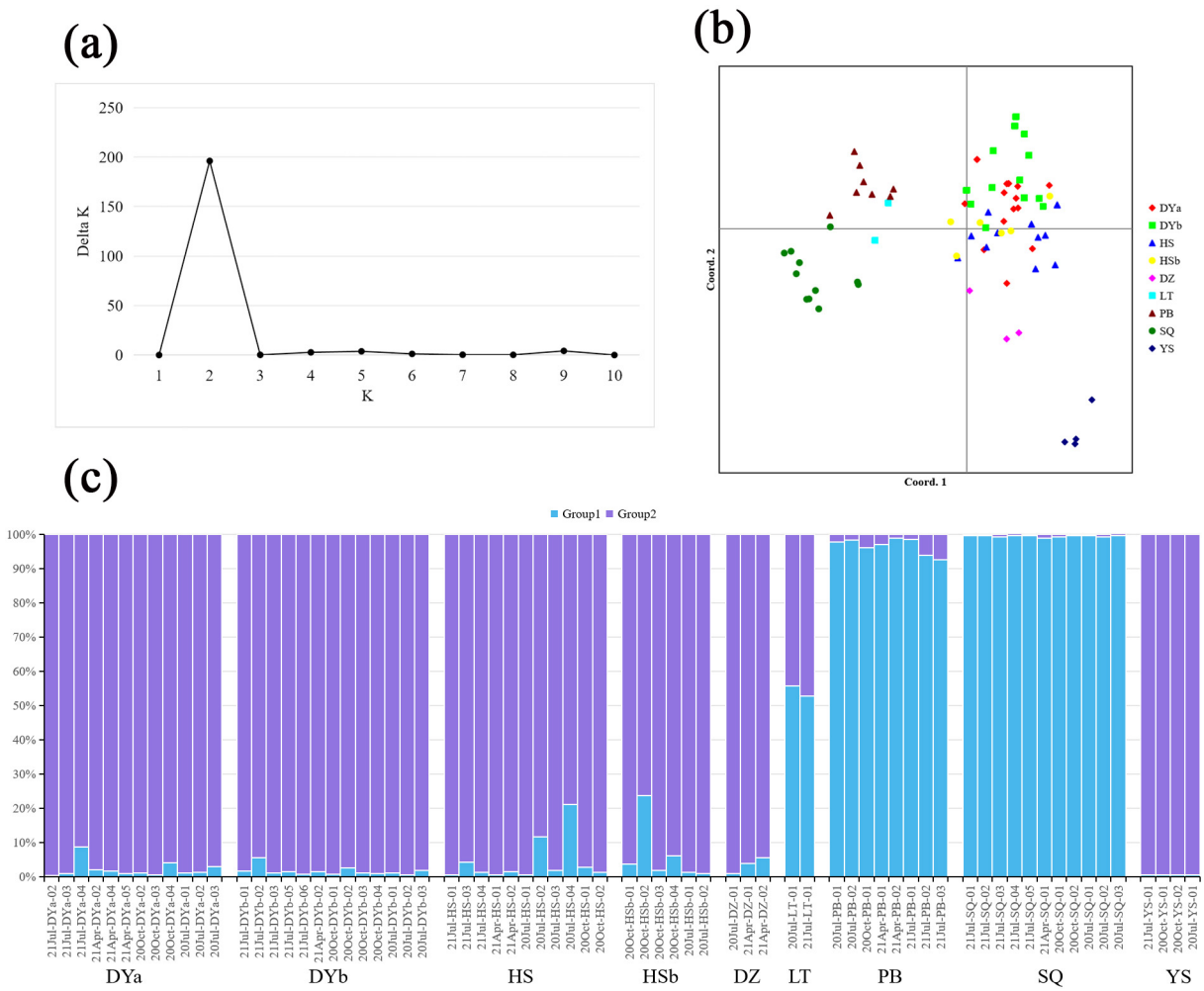

Figure 4. a. The broken line graph of ΔK and K values (DR excluded); **b.** Principal coordinates analysis of the 9 populations (DR excluded); **c.** Structure analysis for 70 gecko individuals from 9 populations excluded DR (K = 2).

Our analyses reveal that populations of *G. yingdeensis* exhibit moderate genetic diversity but are characterized by high genetic differentiation and limited gene flow. This combination of population genetic parameters suggests that genetic drift may be acting within isolated populations, potentially leading to the erosion of genetic diversity over time. The pronounced population specialization further increases the species' vulnerability to stochastic demographic events and environmental change.

The observed genetic structure, particularly the deep divergence between the DR population and all others, as well as the complex affinities among populations in the Hengshitang area indicates that different geographical populations should be recognized as distinct management units for conservation purposes. Preserving the existing genetic structure requires maintaining the integrity of remaining karst habitats and preventing further fragmentation that would exacerbate isolation.

Based on these findings, we recommend establishing a long-term monitoring program that integrates field surveys, image-based individual identification, and molecular data. A comprehensive individual database would provide the scientific foundation necessary for tracking genetic diversity dynamics, detecting population declines, and informing future conservation assessments. Such monitoring is essential for evaluating whether current levels of genetic diversity and population connectivity can be sustained under ongoing anthropogenic pressures and environmental change.

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Supplementary material 1

Supplementary information

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Data type: docx

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Supplementary material 2

Dataset

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