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Enhanced risk assessment framework integrating distribution dynamics, genetically inferred populations, and morphological traits of *Diploderma* lizards

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ABSTRACT

Assessing the threat status of species in response to global change is critical for biodiversity monitoring and conservation efforts. However, current frameworks, even the IUCN Red List, often neglect critical factors such as genetic diversity and the impacts of climate and land-use changes, hindering effective conservation planning. To address these limitations, we developed an enhanced extinction risk assessment framework using *Diploderma* lizards as a model. This framework incorporates long-term field surveys, environmental data, and land-use information to predict distributional changes for 10 recently described *Diploderma* species on the Qinghai-Xizang Plateau, which hold ecological significance but remain underassessed in conservation assessment. By integrating the distribution data and genetically inferred effective population sizes (*N*e), we conducted scenario analyses and used a rank-sum approach to calculate Risk ranking scores (RRS) for each species. This approach revealed significant discrepancies with the IUCN Red List assessments. Notably, *D. yangi* and *D. qilin* were identified as facing the highest extinction risk. Furthermore, *D. vela*, *D. batangense*, *D. flaviceps*, *D. dymondi*, *D. yulongense*, and *D. laeviventre*, currently classified as “Least Concern”, were found to warrant reclassification as “Vulnerable” due to considerable threat from projected range contractions. Exploring the relationship between morphology and RRS revealed that traits such as snout-vent length and relative tail length could serve as potential predictors of extinction risk, offering preliminary metrics for assessing species vulnerability when comprehensive data are unavailable. This study enhances the precision of extinction risk assessment frameworks and demonstrates their capacity to refine and update risk assessments, especially for lesser-known taxa.

Keywords: Lizard; Extinction Risk Assessment; IUCN Red List; Conservation Status; Effective Population Size; Morphological Traits

INTRODUCTION

Assessing the threat status of species involves systematically categorizing taxa based on extinction risk, which is critical for biodiversity monitoring and conservation planning (Bachman et al., 2019). The International Union for Conservation of Nature (IUCN) Red List of Threatened Species is widely recognized as a leading framework for evaluating extinction risk (Brooks et al., 2019), applying five quantitative criteria regarding species distribution, population size, structure, and trends (Supplementary Table S1). These criteria include reduction in population size (Criterion A), restricted geographic range (Criterion B), and very small or declining populations (Criterion D). The Red List serves as an essential tool for guiding biodiversity conservation, informing sustainable development, and shaping policy decisions (Anderson, 2023; Betts et al., 2020; Le Breton et al., 2019; Williams et al., 2021).

However, the IUCN Red List has faced criticism for not adequately incorporating threats from climate change and land-use modifications (Dakhil et al., 2021; Peng et al., 2023). This gap can result in underestimations of extinction risk for various taxa, including plants, mammals, birds, reptiles, and amphibians (Li et al., 2024; Mi et al., 2023; Peng et al., 2023; Santini et al., 2019). In addition, severe data limitations and insufficient research pose significant challenges in maintaining accurate and up-to-date assessments. Currently, only a small

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proportion of known species have been assessed as threatened and prioritized for conservation action (Cazalis et al., 2022), and many remain classified as "Data Deficient" (DD), even within otherwise thoroughly assessed groups (Borgelt et al., 2022; Cazalis et al., 2022). Studies indicate that DD species are more likely to face vulnerability compared to data-sufficient (DS) species (Borgelt et al., 2022; Roberts et al., 2016). An improved framework is urgently needed to address these deficiencies by integrating both current and future threats, such as climate and land-use changes, into assessments of species distribution ranges and population sizes, with a particular focus on DD species.

The geographic distribution of a species constitutes a fundamental unit of biodiversity conservation (Hamilton et al., 2022; Jetz et al., 2019). For most species, understanding and characterizing geographic ranges, along with their changes over time, is fundamental to assessing extinction risk, forming the basis of the IUCN Red List of Threatened Species (Cazalis et al., 2022; IUCN, 2024). To quantify distributional ranges, the IUCN applies two key measures: extent of occurrence (EOO) and area of occupancy (AOO). EOO represents the geographical range of a species, calculated as the area within the minimum convex polygon encompassing all known, observed, or inferred locations of occurrence (Anderson, 2023; IUCN, 2024). Complementarily, AOO denotes the area actually occupied by a species, typically estimated by mapping known or projected distribution points onto a 2 km×2 km grid and counting the number of occupied cells (IUCN, 2024). These metrics are not only vital for well-documented species in the IUCN Red List but are also integral to evaluating regional endemic, rare, keystone, and flagship species, highlighting their critical role in extinction risk assessments (Braby et al., 2018; Chakona et al., 2022; Dong et al., 2023; Gaston & Fuller, 2009; Marcer et al., 2013).

Climate and land-use changes are driving a profound global redistribution of life (Pecl et al., 2017), reshaping ecosystems and threatening biodiversity. These shifts in distribution represent some of the most significant threats to species survival (Root et al., 2003; Yu et al., 2022). Species distribution models (SDMs) have become indispensable tools for predicting variations in spatial distribution under scenarios of climate and land-use change and informing conservation strategies (Hamann & Aitken, 2013; Liang et al., 2024). Outputs from SDMs, particularly those focused on climate-based scenarios, play a crucial role in informing extinction risk assessments, providing quantitative data relevant to "population reduction" under Criterion A or "continuing decline" under Criterion B or C2, which pertain to EOO, AOO, and small population sizes (IUCN, 2024). However, despite the projected acceleration of climate change, the incorporation of climate projections into IUCN Red List assessments of population trends remains a considerable challenge and is limited to a minority of species (Mancini et al., 2024).

Genetic parameters are increasingly recognized as essential components of conservation assessments, especially when ecological and demographic data alone prove insufficient for accurately evaluating extinction risk (Garner et al., 2020; Hoban et al., 2020). Among these, long-term effective population size (*N*e) represents a fundamental metric, reflecting the size of an idealized population experiencing genetic drift at a rate equivalent to the actual population (Wright, 1931). Traditionally, conservation genetics has applied *N*e thresholds of 50 and 500 to assess short- and long-term population viability. The post-2020 framework of the Convention on Biological Diversity (CBD) recently designated *N*e values exceeding 500 as a critical benchmark for gauging a population's ability to maintain genetic diversity and mitigate extinction risk (Hoban et al., 2020; Mastretta-Yanes et al., 2024).

Lizards in the genus *Diploderma* provide an ideal model for evaluating the efficacy of enhanced risk assessment frameworks. As ectothermic animals, lizards rely heavily on environmental temperatures and habitat for thermoregulation, making them particularly vulnerable to the impacts of climate and land-use changes. This vulnerability is compounded in *Diploderma*, which is the most species-rich lizard genus in China, comprising 47 recognized species, 41 of which are endemic to the region (Cai et al., 2024). The recent surge in *Diploderma* species discoveries has highlighted significant knowledge gaps in their life history, distribution, and habitat. As such, a high proportion of these species are classified as DD, complicating conservation efforts and limiting their prioritization (Wang et al., 2021, 2024). Additionally, nearly 70% of *Diploderma* species are concentrated in the Hengduan Mountains and nearby regions, where many are confined to narrow valleys. These habitats have been severely impacted by human infrastructure (e.g., hydropower projects and roads), leading to habitat degradation, frequent roadkill incidents, and the destruction of limestone environments (Shi et al., 2023; Wang et al., 2021, 2022). In recognition of their vulnerability, seven species were designated as nationally protected in 2021, underscoring the urgency of developing comprehensive and accurate risk assessments.

Given the urgent need to assess the conservation status of DD species, recent studies have combined IUCN threat assessments with additional data to predict extinction risks. Functional traits such as morphology, life history, demographics, and range size are frequently employed in extinction risk analyses (Senior et al., 2021). Extensive research has demonstrated strong correlations between these traits and threat status across diverse taxa (Chichorro et al., 2019). Among these, morphological traits offer distinct advantages for extinction risk assessment, as they are closely associated with factors influencing population dynamics and habitat specialization (Miles, 2020). Importantly, morphological traits can often be quantified from museum and historical collections, providing valuable insights even in the absence of contemporary ecological or demographic information. Therefore, exploring the relationship between morphological traits and extinction risk is essential for advancing extinction risk assessment frameworks.

This study presents an enhanced risk assessment framework using *Diploderma* species as a model system. By integrating long-term field survey data with environmental and land cover datasets, we simulated the AOO for 10 *Diploderma* species and predicted changes in their AOO under three future climate and land-use scenarios (SSP126, SSP370, and SSP585). Population genetic data obtained from field sampling were used to estimate the *N*e for each species. This study aimed to: (1) develop an enhanced framework for assessing extinction risk and conservation priorities that integrates projections of future climate and land-use changes as well as genetically inferred *N*e, addressing key limitations of the IUCN Red List methodology; (2) apply this framework to *Diploderma* species, addressing critical gaps in conservation knowledge for this ecologically significant but understudied genus; and (3) establish a preliminary method for assessing extinction risk in DD species by correlating morphological traits with distribution dynamics, habitat area, and genetically inferred *N*e. Overall, this integrative approach highlights significant shortcomings in existing IUCN assessments and provides a foundation for the development of more targeted conservation strategies for *Diploderma* species and lesser-known taxa in regions that may serve as future climate refuges.

MATERIALS AND METHODS

Ethics statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were strictly followed. All relevant procedures involving animal experiments presented in this study were compliant with the ethical regulations regarding animal research and conducted under the approval of the Chengdu Institute of Biology, Chinese Academy of Sciences (Approval No.: CIBDWLL2022004).

Studied species

Ten recently described *Diploderma* species from the Qinghai-Xizang Plateau were selected to evaluate the effectiveness of the enhanced risk assessment framework (Figure 1A). These species are distributed across the Nujiang, Lancang Jiang, Jinsha Jiang, Yalong Jiang, and Dadu river valleys (details in Supplementary Table S2).

Prediction of distribution range using ensemble of small models

The geographic ranges of each species were quantified using EOO and AOO. EOO was defined as the area within a minimum convex polygon (MCP) for each *Diploderma* species constructed in ArcGIS based on known distribution records (Shi et al., 2023). The coordinates of distribution records were primarily obtained from field surveys conducted between 2020 and 2023, supplemented with data from published literature (Flower et al., 2013; Thomas et al., 2012; Wang et al., 2016, 2019). Specific information and sources are shown in Supplementary Table S3. After removing duplicate records and those lacking precise geographic information, 249 species occurrences were retained, comprising 19 for *D. aorun*, 14 for *D. batangense*, 28 for *D. drukdaypo*, 14 for *D. dymondi*, 12 for *D. flaviceps*, 26 for *D. laeviventre*, 13 for *D. qilin*, 39 for *D. vela*, 28 for *D. yangi*, and 56 for *D. yulongense*. However, given the limited number of occurrences for these species (Guisan et al., 2017), a rare species modeling strategy, ensemble of small models (ESM) (Liao et al., 2022), was employed. As dispersal assumptions are critical for *in situ* conservation (Thuiller et al., 2019), AOO was calculated within the empirical distribution range. To achieve this, the distribution ranges predicted by the ensemble model were intersected with the EOOs in ArcGIS, and the area of the intersection was used to estimate the AOO. The total number of grid cells within the intersected area was then used to quantify the AOO for each species.

To predict species distribution, 19 bioclimatic variables, net primary productivity (NPP), and growing season length (GSL) were utilized, sourced from the CHELSA V2 (CMIP6) dataset for both the current period (1981–2010) and future projections (2071–2100) (http://chelsa-climate.org/). The ecological relevance of these variables is provided in Supplementary Table S4. Prior to modeling, 21 variables were pre-screened to avoid multicollinearity issues (Villemant et al., 2011) using a variance inflation factor (VIF) threshold of less than 5, implemented with the *vifstep* function in the R package "usdm" (Naimi, 2014).

Species distribution shifts were predicted under three climate change scenarios outlined in the Intergovernmental Panel on Climate Change (IPCC) Sixth Assessment Report: SSP126 (low-emission scenario), SSP370 (medium-to-high emission scenario), and SSP585 (high-emission scenario) (Kissling et al., 2010). To account for uncertainties in climate projections, data from five Earth system models were incorporated, including GFDL-ESM4, IPSL-CM6A-LR, MPI-ESM1-2-HR, MRI-ESM2-0, and UKESM1-0-LL. The ESM approach was applied to predict the distribution ranges of *Diploderma* species under current and future climate conditions. Four algorithms were selected for their ability to balance computational efficiency and predictive accuracy, including regression tree (CTA), artificial neural network (ANN), generalized boosted regression model (GBM), and generalized additive model (GAM). Model parameters were adjusted using the best-fitting ESM approach, based on observed distribution records and 10 000 randomly sampled pseudo-absence points within the study region (Breiner et al., 2018).

The Somers' D score was employed to evaluate model performance, with 75% of the sampling data randomly selected for model training and the remaining 25% used for testing. Bivariate models yielding a Somers' D score below zero were assigned a value of zero and excluded from the final ensemble of the ESM. Model accuracy was evaluated using two metrics: the area under the receiver operating characteristic curve (AUC) and the true skill statistic (TSS). The receiver operating characteristic (ROC) curve connects all possible threshold points, plotting the false positive rate as the abscissa and the true positive rate as the ordinate (Phillips et al., 2006). The AUC value represents the area of the ROC curve enclosed by abscissas, serving as a threshold-independent measure of model performance (Phillips et al., 2006). AUC values range from 0 to 1, with values closer to 1 indicating better predictive accuracy. Models with an AUC below 0.7 are generally considered to have poor predictive accuracy, while those with an AUC above 0.8 are considered to have good predictive accuracy (Peterson et al., 2011; Zank et al., 2014). TSS, calculated as the sum of sensitivity and specificity minus one, measures the proportion of correctly predicted validation points (Chu et al., 2017). TSS values closer to 1 indicate higher predictive accuracy of the model, with values exceeding 0.5 generally regarded as acceptable for predictive purposes.

The potential distribution area for each species was estimated based on binary predictions, and changes in distribution size were quantified under current and future periods. Outputs from the five Earth system models were arithmetically weighted. To generate binary distribution maps, the maximum training sensitivity plus specificity (MTSS) threshold was applied, converting continuous habitat suitability into suitable (1) and not suitable (0). Changes in distribution areas were analyzed by calculating the portions of habitat expected to decrease, increase, or remain the same under each future climate scenario. Crucially, the predicted distributions were refined based on extensive field sampling data and expert knowledge of the actual distribution ranges of the 10 species (Shi et al., 2023). This refinement involved

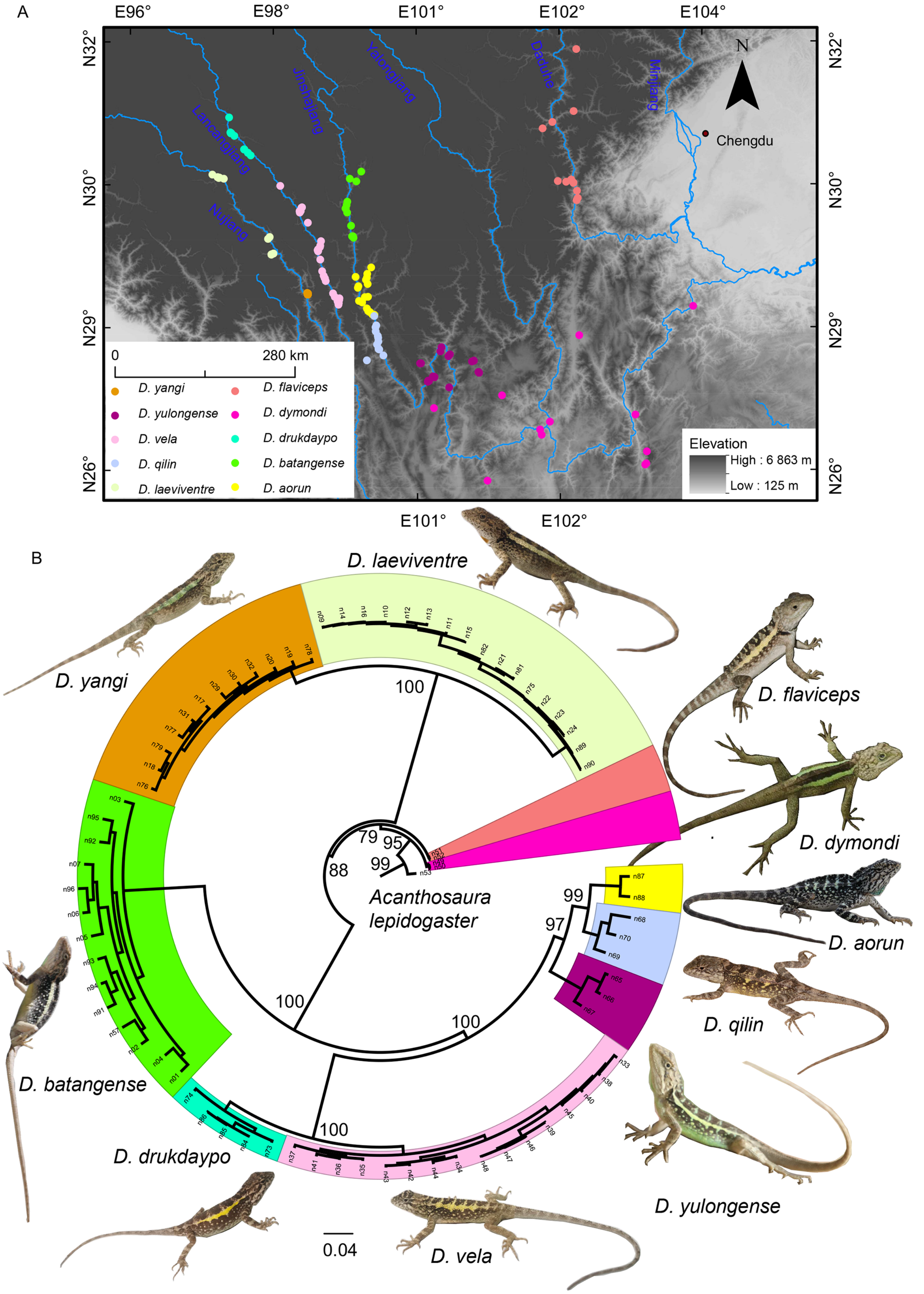

Figure 1 Distribution of sampling sites and phylogenetic relationships of *Diploderma* species

A: Distribution of sampling sites for *Diploderma* species. B: Phylogenetic tree of 10 *Diploderma* species analyzed in this study. *D. qilin*, photo by Kai Wang; *D.aorun* and *D. flaviceps*, photos by Ya-Yong Wu; *D. batangense*, *D. yulongense* and *D. dymondi*, photos by Xiu-Dong Shi; *D. drukdaypo*, *D. laeviventre*, *D. vela* and *D. yangi*, photos by Lin Shi.

constraining the modeled distributions to ecological niches currently occupied by the species, thus limiting predictions to the basins and major branch streams where these species have been observed.

Genetic analyses and effective population size estimation

To analyze genetic diversity and estimate *N*e for each species, 2–16 toe-clipping tissue samples were collected. Detailed locality information is provided in Supplementary Table S5. Genomic DNA was extracted using a MolPure Cell/Tissue DNA Kit (Yeasen, China), double-digest restriction site-associated DNA (ddRAD) sequencing was performed to generate individual genotypes. The RAD library was prepared following standard protocols (Peterson et al., 2012), with some modifications (Yang et al., 2018), and sequencing was carried out using the Illumina NovaSeq platform (Novogene, China). Given the absence of a robust reference genome, the RAD data were demultiplexed and analyzed using the "denovo_map.pl" pipeline in STACKS (v.2.64; Catchen et al., 2011; Rochette et al., 2019) to identify genetic variants under the Marukilow model (Maruki & Lynch, 2017). Genome-wide nucleotide diversity (θ_π) was calculated using vcftools (v.0.1.16) with a sliding window of 10 kbp (Danecek et al., 2011). *N*e was estimated under the simplifying assumption of mutation-drift equilibrium using

$$Ne = \theta_\pi / 4\mu \quad (1)$$

where μ represents the mutation rate. As specific mutation rates for *Diploderma* species are unavailable, the mean mutation rate for reptiles, derived from available genomic data (1.17×10^{-8} per site per generation, 95% confidence interval of the mean=5.34×10^{-9} to 1.80×10^{-8}), was used (Bergeron et al., 2023). *N*e values of 50 and 500, long recognized as the minimum thresholds for avoiding inbreeding decline in the short term and maintaining evolutionary potential over the long term, respectively, were applied in this study (Jamieson & Allendorf, 2012). These thresholds play an important role in the development and implementation of the IUCN Red List categorization system for threatened species (Frankham et al., 2014), particularly as genetic data become more accessible and affordable.

Risk assessment

Building on the dynamics of species distributions and genetically inferred population sizes, we developed an enhanced extinction risk assessment framework, encompassing five scenarios.

For scenario 1 (S1), the RRS for each species was derived by integrating EOO, AOO, and *N*e (Figure 2). The EOO, AOO, and estimated *N*e values of each species were first ranked in descending order, with ranks summed to determine the relative threat status of each species. Lower cumulative scores indicated a lower extinction risk to the species.

For scenario 2 (S2), both current climatic and habitat suitability were considered. Climatically suitable areas that do not align with the habitat preferences of the species were excluded (Figure 2). To achieve this, the global land cover projection dataset, which maintains a consistent 1-km spatial resolution and includes 20 land types projected for the 2015 to 2100 period at five-year intervals, was employed. These projections incorporate the latest IPCC scenarios that account for interactions between socioeconomic factors and climate change (Chen et al., 2022). Within the AOO, land-use classification of each grid cell was identified, designating croplands and urban areas as unsuitable habitats, and needleleaf and deciduous forests and grasslands as suitable habitats (Supplementary Table S6). Suitable habitat areas were then ranked in descending order and combined with the rankings of EOO, AOO, and *N*e to compute the updated RRS. Lower RRS values corresponded to reduced risk to the species.

For scenario 3 (S3), both current and future climatically suitable areas were considered. The AOO was calculated for each climate scenario across five Earth system models (Figure 2). The differences in AOO between the current climate and future projections were averaged across the models for each scenario (SSP126, SSP370, and SSP585) (Eq.1). The average rates of AOO loss under the three climate scenarios were ranked from smallest to largest, with these ranks added to those obtained from Scenario 1 to derive an updated RRS for each species.

$$AOO_{(\text{Loss of future climate scenario})} = \left(\sum (AOO_{(\text{Current})} - AOO_{(\text{Future})} / AOO_{(\text{Current})}) 100\right)\% / 5 \quad (2)$$

For scenario 4 (S4), habitat loss within suitable areas was considered under land-use change scenarios based on the RRS calculated in S2 (Figure 2). Specifically, habitat loss rates were averaged across the different Earth system models for each climate scenario (similar to Equation 2). For each species, the average habitat loss rates under each climate scenario were ranked from smallest to largest (values ranging from 1 to 10).

For scenario 5 (S5), the cumulative extinction risk was determined by summing all RRS values from the preceding four scenarios (S1 to S4) (Figure 2). This comprehensive score was calculated by aggregating the rank summaries of non-overlapping assessment criteria across all scenarios. Species were then ranked from smallest to largest values, with smaller scores indicating a lower risk for the species.

Testing the relationships between morphological characters and risk status

To facilitate the initial assessment of some DD species, the influence of morphological traits on extinction risk was evaluated. Eight functionally relevant characteristics were measured for each species, including competition-related snout-vent length (SVL), head length (HL), and head width (HW); movement-related tail length (TL), forelimb length (FLL), and hind limb length (HLL); and sensory-related diameter of the eye (DE) and snout-eye distance (SED). To enable meaningful multi-species comparisons, all measurements were exclusively taken from males, using either field-collected live individuals or specimens. A vernier caliper (Pr'sKit) with an accuracy of 0.01 mm was employed for all measurements, and at least five male individuals were sampled per species to ensure reliability.

To account for the influence of phylogenetic relationships on morphological differentiation, a phylogenetic tree was constructed for related lizard species based on simplified genomes, with *Acanthosaura lepidogaster* designated as the outgroup (Figure 1B). Variant data from RAD sequencing were concatenated, and the maximum-likelihood (ML) phylogeny was inferred under a GTR+ASC model with 1 000 bootstrap replicates using IQ-TREE (Nguyen et al., 2015).

To explore the correlation between morphological traits and relative extinction risk, phylogenetic generalized least-squares (PGLS) regression was used. Prior to formal analysis,

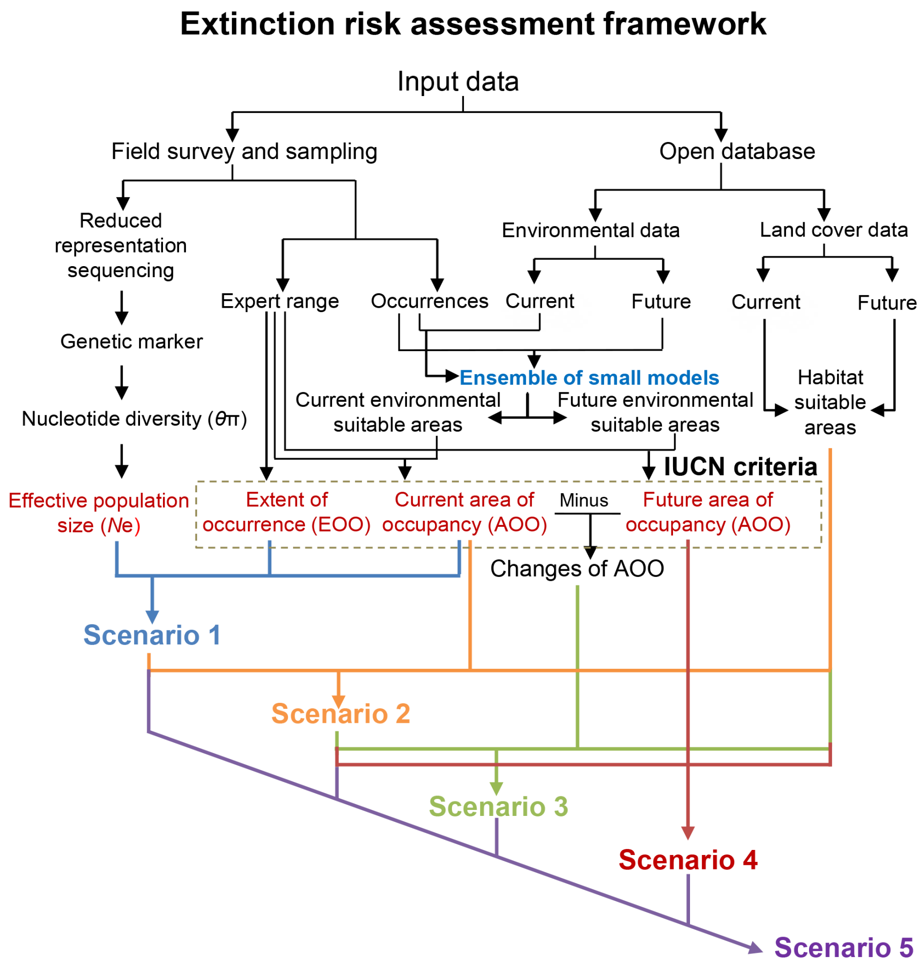

Figure 2 Risk assessment framework

The framework is divided into multiple sections to evaluate risks associated with changes in species distribution under climate and land-use change scenarios.

morphological measurements for all species were standardized by SVL. The PGLS model was constructed with morphological traits as independent variables and RRS as the dependent variable, using the "caper" R package (Orme et al., 2023). The phylogenetic signal strength was assessed using lambda (λ), where values range from 0 (indicating trait evolution independent of phylogeny; weak phylogenetic signal) to 1 (indicating trait divergence comparable with Brownian motion; strong phylogenetic signal).

RESULTS

EOO and AOO of *Diploderma* species

Field surveys combined with literature data revealed significant variability in the geographic ranges of *Diploderma* species. Among the study taxa, *D. dymondi* exhibited the largest EOO, while *D. yangi* occupied the smallest range. Notable overlaps in distribution were observed between *D. qilin* and *D. aorun*, as well as between *D. vela* and *D. drukdaypo*. In contrast, *D. flaviceps* demonstrated a more isolated distribution range, with its northernmost limit located farther from other species (Supplementary Figure S1). By integrating empirical distribution data with predictions from the ensemble of small models, the estimated average AOO for each species was calculated. Results showed considerable variation across taxa: *D. aorun* had an average AOO of 553 km², *D. batangense* 399 km², *D. drukdaypo* 504 km², *D. dymondi* 737 km², *D. flaviceps* 312 km², *D. laeviventre* 477 km², *D. qilin* 346 km², *D. vela* 1 068 km², *D. yangi* 27 km², and *D. yulongense* 387 km² (Supplementary Figures S2–S11).

Dynamics of AOO under future climate and land-use change scenarios

Projected changes in the AOO under different climate scenarios reveal substantial variations in habitat loss across *Diploderma* species. Under the low-emission SSP126 scenario, *D. qilin* and *D. yulongense* experienced the highest AOO losses, at 73.47%±34.65% and 73.28%±32.67%, respectively, with corresponding losses of potentially suitable habitats of 70.76%±34.15% and 70.15%±32.59%. In contrast, *D. vela* showed the least impact, with AOO loss of only 12.38%±24.33%. Under the SSP370 scenario, the AOO loss for most species exhibited a substantial increase compared to the SSP126 scenario. For instance, the AOO loss of *D. flaviceps* increased dramatically from 48.27%±42.43% under SSP126 to 73.59%±47.66% under SSP370. Notably, *D. dymondi* was the only species to show a slight decrease in AOO loss, from 44.94%±61.47% under SSP126 to 42.31%±66.31% under SSP370. While *D. qilin* and *D. yulongense* continued to experience the highest losses, reaching 99.60%±0.01% and 97.16%±0.04%, respectively, *D. vela* showed a sharp increase in AOO loss, rising to 67.55%±30.49%. The high-emission SSP585 scenario demonstrated the most severe impact, with considerable loss of AOO and potentially suitable habitats across all species.

The empirical distribution ranges of *D. yangi* and *D. qilin* were completely eradicated. Most other species faced AOO reductions exceeding 90%, including *D. aorun*, *D. drukdaypo*, *D. dymondi*, *D. flaviceps*, *D. laeviventre*, *D. vela*, and *D. yulongense*. Even *D. batangense*, which exhibited the least loss, experienced a significant reduction of 79.45%±29.78%. These extensive losses in AOO predominantly resulted from reductions in potentially suitable habitats, as unsuitable habitats constituted a negligible proportion under most climate change scenarios. For example, suitable habitat loss for *D. yangi* was 50.40%±36.63% under SSP126 but 89.60%± 20.80% under SSP370 (Figure 3).

Risk assessment based on IUCN Red List criteria

Using the IUCN Red List criteria B1 and B2, EOO and AOO were analyzed to determine the conservation status of the 10 *Diploderma* species. According to the IUCN thresholds, a species is classified as "Critically Endangered" (CR), "Endangered" (EN), and "Vulnerable" (VU) based on EOO values of 100, 5 000, and 20 000 km², respectively, with corresponding thresholds for AOO of 10, 500, and 2 000 km², respectively. As shown in Figure 4, *D. yangi* met the criteria for EN under both EOO and AOO, indicating its position as the species facing the highest risk of extinction. Among the three species currently lacking formal IUCN Red List assessments, *D. aorun*, *D. drukdaypo*, and *D. qilin* fell within the VU category based on their EOO and AOO measurements. In addition, species previously assessed by the IUCN as 'Least Concern' (LC), including *D. vela*, *D. batangense*, and *D. laeviventre*, were found to meet the criteria for VU, warranting a reclassification to reflect their heightened vulnerability.

Risk assessment based on multi-scenario risk ranking scores

In most scenarios, *D. yangi* consistently exhibited the highest RRS, driven by significant losses in suitable habitats (S2), projected future distribution under climate change (S3), and future habitat changes (S4). Similarly, *D. qilin* ranked among the most at-risk species, with relatively high RRS values, ranking 10th in S5 and S3, 9th in S2 and S4, and 8th in S1 (Supplementary Table S7). Despite having relatively large EOO and AOO values, *D. qilin* is expected to lose nearly all its distribution range and habitat under future climate change scenarios, resulting in elevated extinction risk. In contrast, *D.*

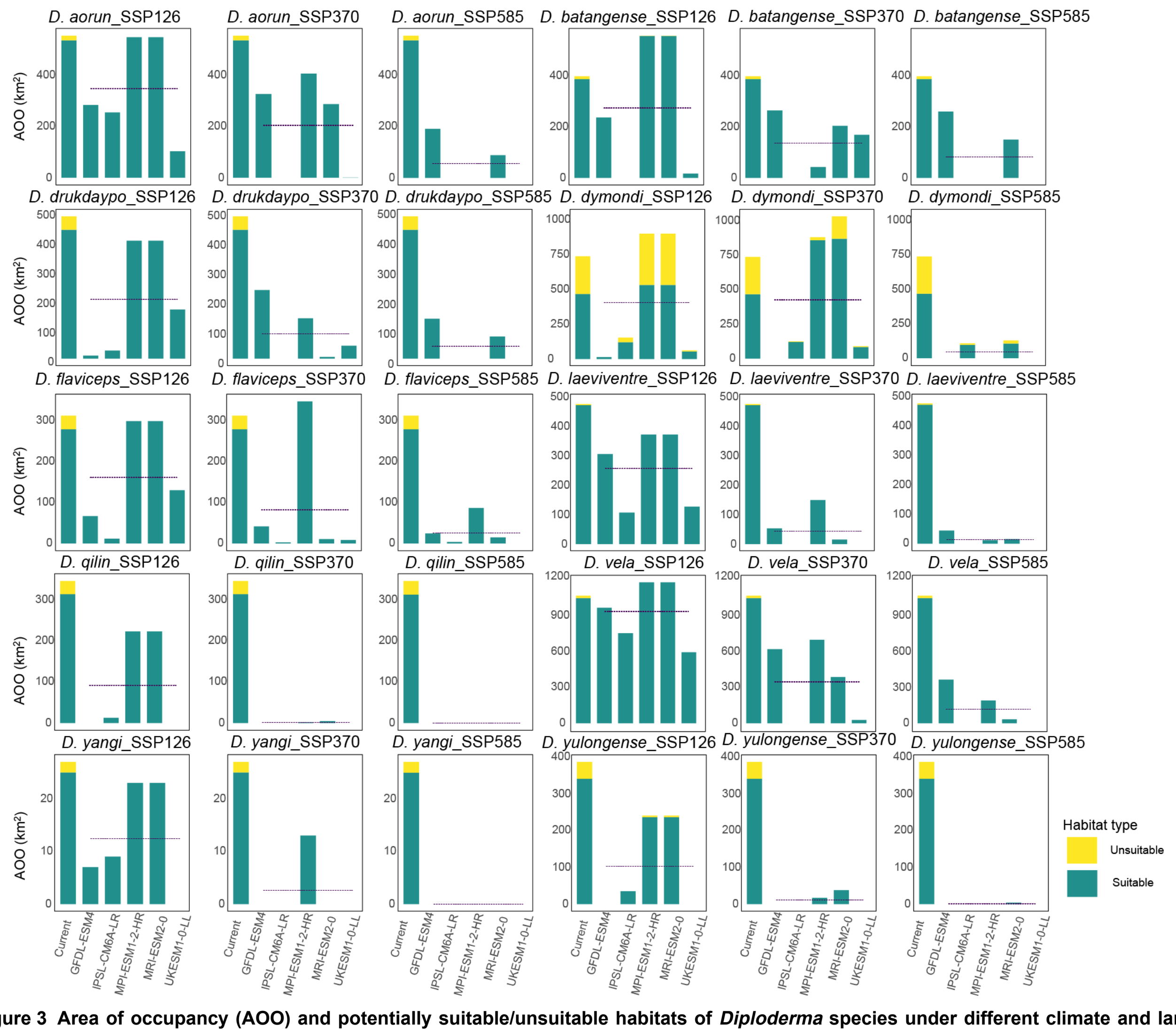

Figure 3 Area of occupancy (AOO) and potentially suitable/unsuitable habitats of *Diploderma* species under different climate and land-use change scenarios

SSP126, SSP370 and SSP585 represent future climate data under different emission scenarios. GFDL-ESM4, IPSL-CM6A-LR, MPI-ESM1-2HR, MRI-ESM2-0, UKESM1-0-LL represent different Earth system models.

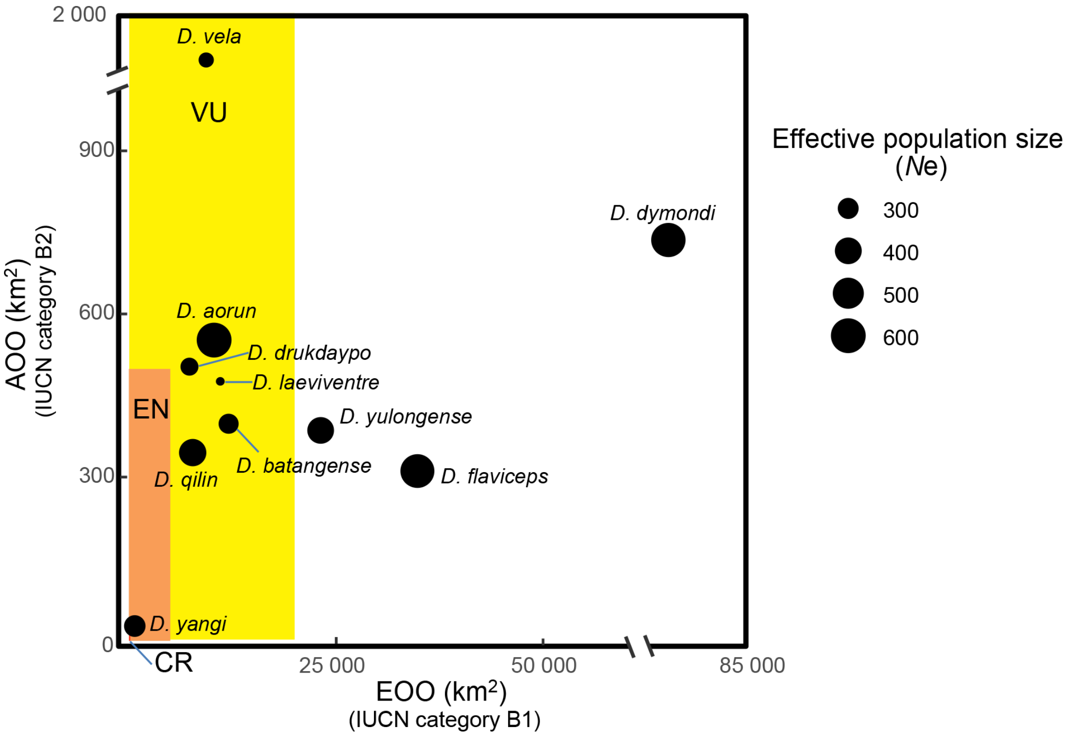

Figure 4 Assessment of current species threat status using IUCN criteria B1 and B2

Species were assessed based on extent of occurrence (EOO) and area of occupancy (AOO). For EOO, species with an EOO<100 km^2 were classified as Critically Endangered (CR), those with an EOO<5 000 km^2 were classified as Endangered (EN), and those with an EOO<20 000 km^2 were classified as Vulnerable (VU). For AOO: species with an AOO<10 km^2 were classified as CR, those with an AOO<500 km^2 were classified as EN, and those with an AOO<2 000 km^2 were classified as VU. Circle size represents effective population size (*Ne*), with larger circles indicating greater population sizes.

aorun and *D. dymondi* demonstrated lower extinction risks, achieving the lowest RRS ranking across three scenarios (S1, S2, and S3) and two scenarios (S4 and S5), respectively (Figure 5). Although *D. flaviceps* does not appear to be a threatened species based solely on EOO and AOO metrics, its risk rankings increased to 9th when potentially suitable habitats (S2) were considered. Its vulnerability further increased to 5th when habitat loss under future climate and land-use scenarios (S3 and S4) was accounted for. When all factors were combined (S5), *D. flaviceps* ranked at a moderate threat level, placing 5th overall.

Relationships between morphological characters and risk ranking scores

PGLS analysis revealed significant correlations between morphological traits and RRS across scenarios. SVL showed a strong association with the RRS of S1 and S2 (Figure 6), while relative diameter of the eye (RDE) was positively correlated with the RRS of S2. Notably, relative tail length (RTL) and relative head width (RHW) exhibited significant correlations across all five evaluation scenarios, although in opposite directions (Figure 6). These findings highlight the potential predictive value of specific morphological traits in extinction risk assessments.

DISCUSSION

Accurate, comprehensive, and predictive assessments of endangered species are essential for advancing biodiversity conservation efforts. In this study, an enhanced risk assessment framework was developed, integrating distribution dynamics, genetically inferred *Ne*, and morphological traits. By incorporating multiple climatic scenarios, land-use patterns, and species occurrence data, this framework enabled the construction of robust species distribution models to predict future changes in climatically suitable areas and potentially suitable habitat availability for 10 *Diploderma* species, which served as a model system for testing the effectiveness of the proposed framework. Compared to existing IUCN assessment approaches, this framework offers several key advantages: First, it explicitly accounts for the dynamic impacts of climate change and land-use modifications on species distributions, enabling more accurate risk evaluations. Second, it integrates multiple climate and land-use change scenarios, incorporating uncertainties to produce more robust predictions. Third, the inclusion of *Ne* as a metric provides insights into extinction risk from a genetic diversity perspective, addressing a critical gap in existing assessment systems.

Enhanced framework provided more accurate evaluations

The application of this enhanced risk assessment framework highlighted significant shortcomings in existing IUCN evaluations, particularly in underestimating the extinction risk of *Diploderma* species. One critical limitation of the IUCN Red List is its omission of genetic diversity patterns, which are essential for assessing long-term survival of species. Genetic diversity plays a pivotal role in species adaptability, with the post-2020 framework of the CBD prioritizing its preservation to prevent genetic erosion and safeguard the adaptive potential of both wild and domesticated populations (Laikre et al., 2020). Our framework integrates a *Ne* value greater than 500 as a critical indicator, aligning with this goal (Hoban et al., 2020). *Ne* reflects both the rate of random genetic drift and inbreeding levels, with smaller *Ne* values leading to increased

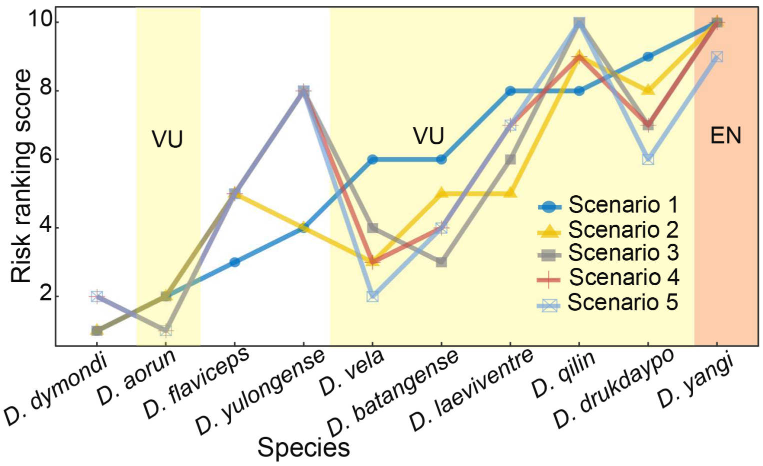

Figure 5 Risk ranking scores for 10 *Diploderma* species under different risk assessment scenarios

VU: Vulnerable; EN: Endangered.

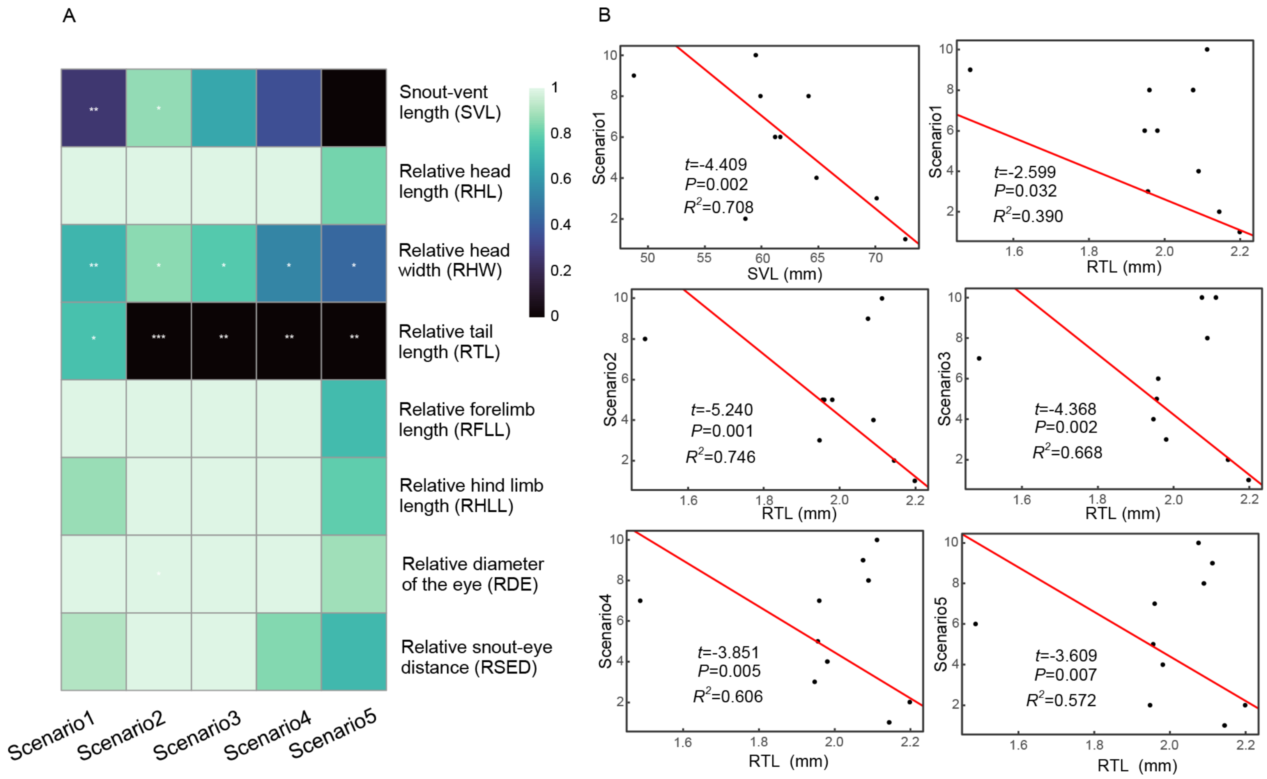

Figure 6 Correlations between morphological traits and risk ranking scores of each species

A: Lambda value of phylogenetic generalized least-squares (PGLS) analysis of traits and assessment indicators. Symbols indicating statistical significance, ns: Not significant; *: *P*>0.05; **: *P*≤0.01; ***: *P*≤0.001; ****: *P*≤0.0001. B: Correlations between morphological traits and risk ranking scores of each species.

inbreeding, reduced fitness, decreased population size, and increased susceptibility to extinction (Ren et al., 2022; Willi et al., 2022). According to the widely used 50/500 rule, none of the *Diploderma* species faces immediate threat of inbreeding depression, with all *Ne* above 50. However, *D. vela*, *D. batangense*, *D. drukdaypo*, *D. laeviventre*, *D. yulongense*, *D. yangi*, and *D. qilin* had *Ne* values below 500, suggesting insufficient genetic diversity for adapting to future environmental changes (Ryman et al., 2019). When evaluated against the more recent 100/1 000 minimum *Ne* threshold, all studied species fell below the recommended levels, highlighting the need for long-term conservation planning (Frankham et al., 2014; Hoban et al., 2024).

Additionally, the proposed framework also revealed that the endangered status of *Diploderma* species has been underestimated due to inadequate consideration of habitat loss under future climate and land-use changes. SDM and land-use change projections indicated alarming habitat reductions. For example, under the high-emission SSP585 scenario, the estimated AOO losses for *D. batangense* and *D. laeviventre* were 79.45%±8.86% and 96.93%±0.15%, respectively. Similarly, despite its low current distribution range and *Ne* ranking, *D. flaviceps* faces significant loss of habitat under future climate conditions, posing a significant threat to its survival. The extensive loss of habitat predicted under climate change scenarios emphasizes the urgent need for greater conservation attention and strategic planning to protect these species.

Enhanced framework highlighted higher risk for DD species underestimated by the IUCN

This study explored risk assessment for species lacking sufficient data from existing IUCN assessments. Species such as *D. drukdaypo*, *D. aorun*, *D. qilin*, and *D. yangi* are not recorded in the IUCN Red List, resembling DD taxa whose conservation needs are often overlooked. The absence of registration or critical ecological data for species can substantially influence conservation efforts. Research suggests that DD species face a higher extinction risk than many evaluated species, with 56% of unevaluated taxa in the IUCN Red List potentially at risk, suggesting potential biases in current conservation priorities (Borgelt et al., 2022). Based on our framework, *D. aorun*, *D. drukdaypo*, and *D. qilin* met the criteria for classification as VU, while the recently described *D. yangi* should be classified as EN. *Diploderma yangi* is restricted to the upper Salween River Valley in Zayu County in the Xizang Autonomous Region of China, extending to the Xizang-Yunnan border region. This species, identified as having the smallest surveyed distribution range, exhibited the highest relative threat level based on multi-scenario assessments (S2 to S5). Nevertheless, compared to other species, its limited distribution range makes it more susceptible to habitat destruction and environmental changes, underscoring the importance of habitat preservation, particularly shrub protection, within the Salween River basin.

These findings emphasize the conservation significance of many DD species that are at risk of extinction but remain unclassified as EN by the IUCN (de Oliveira Caetano et al., 2022). Enhanced and precise evaluations of unregistered or DD species are vital for refining conservation priorities and ensuring their inclusion in sustainable development goals and biodiversity preservation.

Enhanced framework identified morphological traits as preliminary risk indicators

The latest IUCN Red List has assessed the threat status of 10 254 reptile species, identifying 14.55% as DD. Addressing the conservation needs of these DD (including unassessed) species is critical, as their unknown status poses challenges to biodiversity preservation. To bridge this gap, studies have increasingly utilized key functional trait data to preliminarily predict extinction risk. Large body size is often associated with a higher extinction risk, as it is associated with ecological characteristics that heighten vulnerability, including low population densities, extensive home ranges, and slower recovery from rapid environmental disturbances (Reed & Shine, 2002; Zhong et al., 2022). Large lizards, for example, are particularly susceptible to predation by invasive species, further increasing their extinction risk (Tingley et al., 2013). However, within the genus *Diploderma*, a contrasting pattern emerged. Notably, SVL showed a negative correlation with endangered status, likely due to its positive relationship with species distribution range (R^2=0.493), with a smaller SVL associated with a reduced EOO, a critical predictor of extinction risk in the assessment framework. Limited range size has been consistently identified as a major predictor of extinction risk in birds, certain terrestrial mammals, and reptiles, showing a strong negative correlation with extinction risk (Ripple et al., 2017). In addition to SVL, other morphological traits such as RTL and RHW emerged as proxy indicators for extinction risk in the genus *Diploderma*. These traits may influence sensory abilities or locomotion capabilities, enabling species to navigate environmental disturbances. Although their precise roles remain unclear, further research is needed to explore the potential connections between these traits and species vulnerability.

In summary, this study proposes a new risk assessment framework based on AOO, EOO, *N*e, suitable habitat area, and projected changes in AOO due to future climate scenarios. Furthermore, this study proposes an automated assessment framework that incorporates morphological traits to predict extinction risk as an initial step prior to more detailed evaluations. This dynamic framework represents a significant advancement in assessing the risk of DD species, particularly in global biodiversity hotspots, ultimately bridging the assessment gaps for lesser-known taxa and offering a promising tool for global conservation efforts and effective prioritization of species at risk.

DATA AVAILABILITY

All sequences data generated in this study have been deposited in the NCBI (BioProjectID PRJNA1195238), Genome Sequence Archive (GSA) database with accession number CRA020990, National Genomics Data Center (NGDC) with accession number PRJCA029744, and Science Data Bank database (DOI: 10.57760/sciencedb.j00139.00148).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Q.X.: Conceptualization, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. X.D.S.: Visualization, Data curation, Resources, Writing – review & editing. L.S.: DNA extraction and Sequencing data analysis. Z.Y.Y.: Distribution records data curation. Y.H.C.: Writing – review & editing. W.Z.Y.: Methodology, Data curation, Resources. Z.Y.L.: Conceptualization, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing, Validation. Y.Q.: Conceptualization, Supervision, Writing – review & editing, Validation. All authors read and approved the final version of the manuscript.

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