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Biogeographic barriers and environmental gradients reveal distribution limits in *Hynobius* salamanders

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This study investigates the biogeographic barriers and environmental gradients that influence the distribution and niche segregation of seven *Hynobius* salamander species in South Korea. Using Ecological Niche Models (ENMs) developed with the Maxent software and 48 environmental variables, we conducted four niche overlap tests for each species pair: niche identity tests and background similarity tests to assess niche differentiation, lineage-breaking tests to evaluate species boundaries in relation to environmental shifts, and ribbon tests to determine whether unsuitable habitats serve as distributional barriers. The results indicated that significant biogeographic barriers, including both physical features such as rivers and mountains and environmental gradients, influence the range segregation of these salamanders. The study also identified distinct ecological niches among the species, with environmental gradients and unsuitable habitats acting as key factors shaping their distributions. These findings highlight the crucial role of biogeographic barriers in the distribution and speciation of *Hynobius* salamanders, emphasizing the need to consider these factors in conservation strategies aimed at preserving their ecological diversity and evolutionary trajectories.

Keywords Amphibian, Biogeography, Conservation, Ecological niche models (ENM), MaxEnt, Niche overlap

Elucidating biogeographic barriers between closely related species is critical for refining our comprehension of species distribution patterns[1,2]. Specifically, biogeographic barriers are shaped by a combination of abiotic and biotic factors such as dispersal capacity, interspecific competition, biological factors, temperature, precipitation, and climate[3–7], often confining species' dispersal and gene flow, which play a critical role in determining species distributions and promoting divergence and speciation[8]. For instance, two subspecies of smooth newt, *Lissotriton vulgaris kosswigi*, restricted to the southern Black Sea coast, and *Lissotriton vulgaris vulgaris*, with a broader European range, exhibit significant genetic differentiation due to barriers restricting gene flow between them, as evidenced by variation within eight nuclear and mitochondrial DNA gene fragments across multiple populations[9]. Similarly, climate change can act as a biogeographic barrier, influencing the distribution of species like the terrestrial slug *Deroceras panormitanum*[10].

Due to the specific habitat requirements of many amphibian species during their adult life[11,12], and the resulting limited mobility[12], amphibians are suitable models for testing biogeographic barriers[13,14]. For instance, the closely distributed congeneric giant tree frogs (genus *Leptopelis*) in west and central Africa are segregated by both a riverine barrier and an environmental gradient[15]. To assess these barriers, an analysis of genome-wide single-nucleotide polymorphism data demonstrated that species-level divergence coincides with a prehistoric event. Furthermore, applying ecological niche modeling provided additional evidence highlighting the distinct environmental niches occupied by each of the three species of the giant tree frog[15].

Ecological Niche Models (ENMs) integrate environmental and occurrence data to define a taxon's environmental preferences and suitability[16]. In addition, ENMs can be used with occurrence data to identify the impact of ecological variables on the boundaries of species' distribution and possible biogeographic barriers[17,18]. For instance, it is a challenge to understand the distributions of marine species, as there are often no physical

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barriers delineating these species[8,19], and while ocean currents generally promote dispersal and connectivity among marine populations, they can also act as abrupt environmental gradients[20]. Besides marine ecology, distinct refugia of tailed frog populations of *Ascaphus montanus* in the Rocky Mountains were confirmed by combining ecological modeling and a nested clade analysis[21].

Together with niche overlap tests, ENMs serve as valuable tools for examining ecological distinctions among taxa and assessing the influence of ecological factors on range boundaries, especially on broad geographic scales[22]. ENMs, used with occurrence data, identified that the climatic factors such as the minimum temperature of the coldest month were the ecological determinants delineating the parapatric range boundaries of these two closely related species[2].

The *Hynobiidae* family is a diverse and ancient lineage of East Asian salamanders, comprising two main lineages distributed across the Eastern Palearctic: *Onychodactylus*, and *Salamandrella* together with *Hynobius* (Hynobiinae). Limited dispersal ability, likely linked to climatic variations over geological times, has fragmented the distribution of the Hynobiidae family across Eastern Asia[23,24]. Several factors have shaped the distribution of these salamanders, including the uplift of the Qinghai-Tibetan Plateau and climatic changes associated with the establishment of the monsoon system[24,25]. Differentiating *Hynobius* species based solely on morphological characteristics can be challenging[26], making it essential to understand the ecological factors influencing their distribution for accurately defining species identities and boundaries[23–28]. Although these insights have aided in developing salamander conservation strategies, our understanding of specific speciation events and biogeographic boundaries remains limited. This highlights the significance of studying *Hynobius* salamanders in South Korea, where diverse species and distinct range boundaries provide an ideal system for investigating speciation mechanisms and their implications for conservation.

This study employed ecological niche modeling (ENM) methods based on the framework proposed by Glor&Warren[29] to evaluate niche overlap between congeneric *Hynobius* salamander pairs and identify potential biogeographic barriers associated with their sympatric ranges. We answered these questions through four hypotheses: (1) Whether *Hynobius* species have identical niche; (2) Whether they exhibit background similarity; (3) Whether these species encounter biogeographic barriers linked to abrupt environmental changes or steep environmental gradients; (4) Whether biogeographic boundaries are associated with regions of notably unsuitable habitat that separate two or more suitable regions. We first assessed the niche identity and background similarity for the seven *Hynobius* species pairs in South Korea. Next, we investigated the presence of potential biogeographic barriers for the same species pairs by conducting lineage-breaking tests and unsuitable ribbon tests. Our findings are suitable as a model for determining species boundaries, given the ongoing debate and lack of consensus on a universal definition of species.

Materials and methods

Species data and environmental niche modeling

Within the family *Hynobiidae*, *Hynobius* stands as the most species-rich genus, encompassing over 60 recognized species[30,31]. In South Korea, seven *Hynobius* species have been identified: *H. leechii*, *H. unisacculus*, *H. notialis*, *H. perplicatus*, *H. quelpaertensis*, *H. yangi*, and *H. geojeensis*. Except for *H. leechii*, which boasts a wide distribution across the Korean peninsula and extends into China, the remaining six species are primarily concentrated in the southern region of the Korean peninsula[26]. A diagonal along the hills and mountains in the southern region of the Korean peninsula, starting west of Iksan and extending to Ulsan, serves as a habitat and contact zone for *H. leechii* and other *Hynobius* species[32,33]. In addition to *H. leechii*, this diagonal, from west to east, also includes *H. quelpaertensis*, followed by *H. unisacculus*, *H. perplicatus*, *H. notialis*, and *H. yangi.* Finally, *H. perplicatus* overlaps with *H. notialis* in its range's northern part and *H. geojeensis* on the southern edge, from which the latter species distributes further south (Fig. 1). Phylogenetic and phylogeographic studies show that *H. unisacculus* is deeply divergent from *H. leechii*, *H. yangi*, and *H. quelpaertensis*, with genetic distances near 10% across COI and cyt b[34,35]. In the case of *H. quelpaertensis*, the species shows a structure segregating Jeju and the mainland population[36,37], and *H. perplicatus* is deeply divergent despite its interesting geographic location among the other clades[38,39]. Broader East Asian analyses have outlined several clades within the *Hynobius* genus, with all South Korean species clustered within the same one. We use these phylogenetic hypotheses solely as context for our overlap comparisons[26,35].

We relied on the Global Biodiversity Information Facility database (GBIF, https://doi.org/10.15468/dl.trnntk3, accessed 24 January 2023) to retrieve occurrence data for all *Hynobius* species across the Korean peninsula (see Supplementary Data S1 online). Following the literature[26], the range of presence was delineated, and false localities due to geocoding errors and duplicates were discarded, resulting in 5,547 occurrence points. The number of occurrence points available for each species (minimum $n = 33$) exceeded the number of points typically considered sufficient for accurate ecological niche modeling[40] ($n > 18$).

In this study, we initially tested 48 variables for potential inclusion in Ecological Niche Modeling (ENM). We combined the bioclimatic variables to define the fundamental niche and the recent anthropogenic variables to define the realized niche[41]. The latter captures pressures such as habitat fragmentation that prevent occupancy of climatically suitable areas[42]. This approach improves model performance in human-impacted landscapes and provides a more realistic evaluation of current niche configuration[26,43,44].

We downloaded environmental layers at a spatial resolution of 30-arc-second (~ 1 km) resolution. The dataset included 19 bioclimatic variables, solar radiation, elevation, terrain derivatives from elevation, landscape variables, and anthropogenic layers such as distance to urban areas and distance to river. Bioclimatic variables and solar radiation data came from the Worldclim dataset (v. 2.0, www.worldclim.org). Elevation and terrain derivatives were obtained from the U.S. Geological Survey (USGS, https://www.usgs.gov/). Landscape variables were extracted from World Land Cover 30 m BaseVue 2013 (https://landscape6.arcgis.com/arcgis/rest/services/World_Forests_30m_BaseVue_2013/ImageServer). https://landscape6.arcgis.com/arcgis/rest/services/World

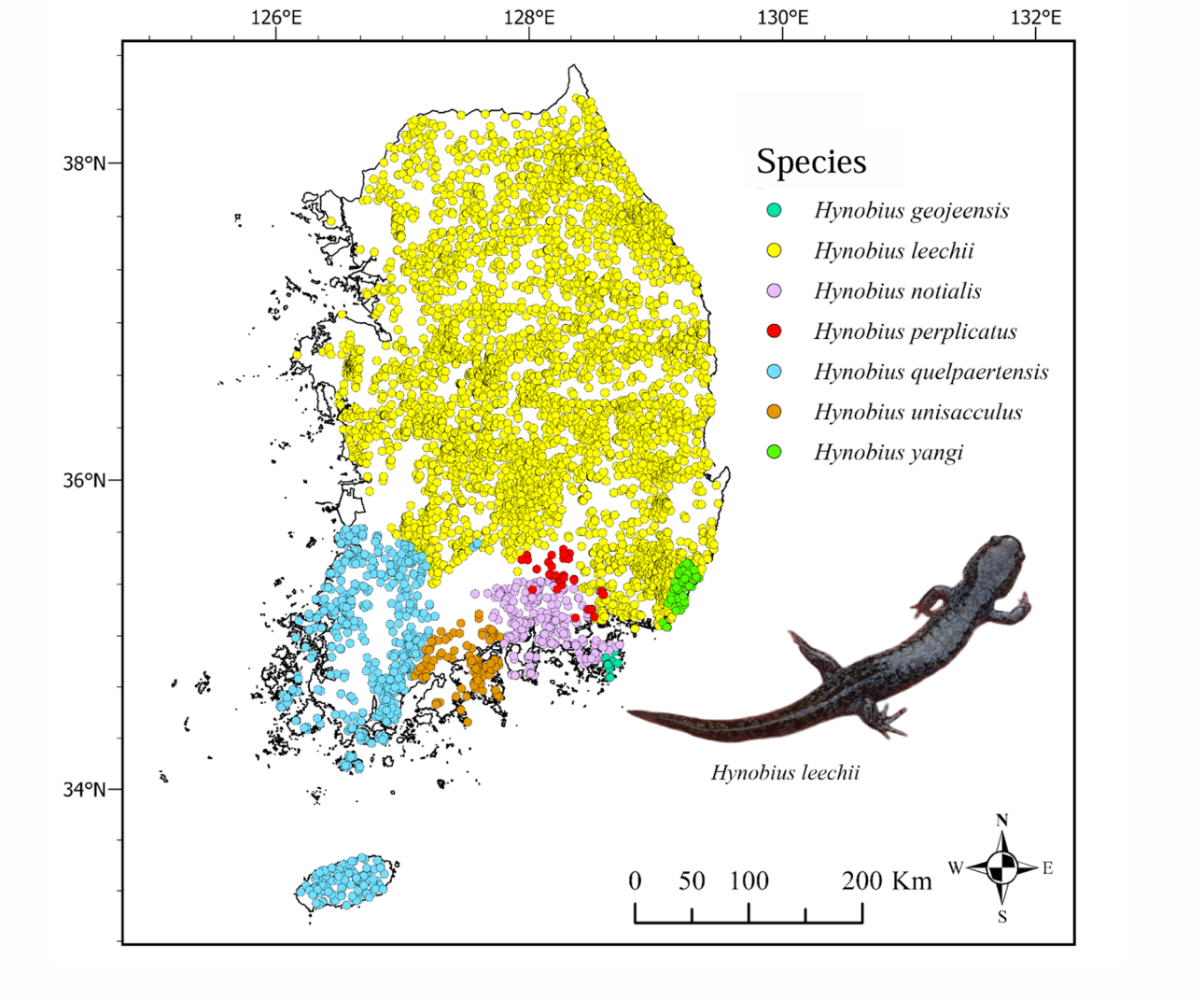


Fig. 1. Distribution of seven *Hynobius* species across the Korean peninsula, based on the occurrence data used for analysis. Each color corresponds to a species. The map was generated using ArcGIS Pro software (version 3.3, https://pro.arcgis.com/en/pro-app/3.3/get-started/release-notes.htm).

_Forests_30m_BaseVue_2013/ImageServer). For anthropogenic layers, we derived the distance by binarizing land cover and river raster (urban or river = 1, other = NoData), projecting to an equal-area coordinate system, computing Euclidean distance to the nearest target pixel and resampling the distance raster to the environmental grid. To reduce multicollinearity, we computed pairwise Pearson correlations and retained only variable with $r < 0.8$[45]. For highly correlated pairs with $r \geq 0.8$,, we retained the variable with a higher percentage of contribution in an initial full MaxEnt run across *Hynobius* species and removed the other[45]. Variable selection was guided by prior studies[46–49] and by the known behavioral and ecological traits of *Hynobius*[24]. The final model used 15 variables including bioclimatic, landscape, and vegetation (Table 1).

To develop the ecological niche models, we utilized the MaxEnt v. 3.4.3 software (available at http://biodiversityinformatics.amnh.org/open_source/maxent/, accessed on January 24, 2022). We constructed these models using occurrence records and environmental data with default settings[50]. This approach enabled us to assess the influence of individual bioclimatic variables on predictions as represented by the response curves. We evaluated the area under the receiver operating curve (AUC) metric and true skill statistic (TSS) to assess the performance of each model[51–53]. An AUC value greater than 0.5 indicates that the model outperforms a null model[51,52]. MaxEnt output raster files were classified into suitable and unsuitable habitats using the maximum training sensitivity plus specificity (MTSS) threshold, which is insensitive to pseudo-absences[54].

Testing whether species' pairs have different niches

To test for differing niche requirements between species pairs, we utilized niche identity tests, which compare ENM predictions of habitat suitability for each grid cell in the study area. Ecological niche differentiation can be assessed using ENMs, niche identity tests, and background similarity tests. We used I and Schoener's D metrics

Variables	Description
bio1	annual mean temperature (°C)
bio4	temperature seasonality (standard deviation x 100)
bio12	annual precipitation (mm)
bio14	precipitation of the driest month (mm)
bio16	precipitation of the wettest quarter (mm)
srad08	solar radiation of August (kJ m^{-2})
srad12	solar radiation of December (kJ m^{-2})
elevation	in meters (m)
average greenness within 1 km	area (ha)
human distance	distance to the combined urban/road network (m)
predominant landcover within 1 km	dimensionless
river coast distance	in meters (m)
river distance	in meters (m)
topographic wetness index	$\ln(a/\tan\beta)$
urban distance	in meters (m)

Table 1. List of environmental variables used for the ecological niche models to determine the habitat of the seven *Hynobius* species occurring in the Republic of Korea.

to measure niche overlaps, which range from (no overlap) to 1 (complete overlap) summarizing the amount of niche differentiation. Randomization tests comparing ENMs of paired species are employed to determine the degree of niche overlap between species, as outlined by Glor & Warren (2011) and Warren et al. (2008).

Testing whether species' pairs have background similarity

The niche identity test assesses whether the niches of two species are equivalent to each other, and the background similarity test assesses whether the niches of two species are more similar than expected by chance, based on their environmental backgrounds. The identity test analyzes whether the ENMs generated for two species differ from ENMs calculated from pairs of samples drawn at random from a pooled dataset of the two species' occurrence points. For the background similarity test, we compared models created using occurrence points of one species with models created using randomly placed points from the geographic range of the other species in the pairs. The observed niche overlap was then compared to the null distributions generated by the randomization tests. The test was run with 100 randomized pseudo-replicates using the R package 'enmtools'[55] (http://enmtools.blogspot.com.tr). If there were no significant difference between the ENMs and the randomized pseudo-replicates of each *Hynobius* pair, the pair was considered effectively identical in terms of niche.

Testing whether there is a sharp environmental transition or a significant environmental gradient between species pairs

We employed lineage-breaking tests to investigate whether species' range boundaries coincided with sharp environmental transitions[29]. We generated null distributions for similarity metrics I and Schoener's D by performing 100 random replicates. In the linear test, we pooled occurrences from both species and established a randomized dividing line within their shared range. The dividing line was positioned to ensure that the numbers of occurrences on either side of the line matched the numbers of respective occurrences for both species. We then generated ENMs for each side of the line. The null hypothesis (i.e., a sharp environmental gradient is not separating the two species) was rejected if observed I or Schoener's D values were statistically significant in the one-tailed *t*-test against the null distribution. Observed values significantly lower than the null distribution allowed us to reject the null hypothesis. In each pseudo replicate, a line separating the ranges of the two populations was randomly placed and adjusted until the number of individuals in each population matched the observed sample sizes for each species. If a starting angle failed to create appropriate partitions, it was replaced with another line. This method prevented bias in I and Schoener's D values due to sample size differences between pseudo-replicates. Duplicate partitions were excluded from the analysis.

Testing whether there is an unsuitable habitat between the species' pair

To assess whether the contact zones between the two species are separated by unsuitable habitats, we conducted the "ribbon" test[29]. Initially, a single ecological niche model was created using the pooled occurrence data of the two species along the ribbon. Then, a ribbon was created as a polygon between the two species ranges. This ribbon was populated with random points of the same number of the pooled species' occurrences. These random points were then used to create an ENM which was then tested against the pooled ENM for I and D. After the assessment of this overlap, we performed 100 replicates, each featuring a randomly generated ribbon within the combined occurrences of both species, with the same area as the original ribbon. By calculating I and D values from these pseudo-replicates, we generated a null distribution to determine whether the pooled niche for both species was more like the actual contact zone ribbon than a randomized ribbon.

Result

Habitat suitability model for hynobius species

The final performance of the ENMs for each species, based on the provided occurrence data, was highly satisfactory. In all cases, the models achieved or surpassed the established AUC and TSS standard. The AUC and TSS values for each species were as follows: *H. leechii* (AUC = 0.742 ± 0.002 and TSS = 0.428 ± 0.0188), *H. unisacculus* (AUC = 0.990 ± 0.006 and TSS = 0.818 ± 0.1346), *H. notialis* (AUC = 0.978 ± 0.002 and TSS = 0.845 ± 0.0613), *H. perplicatus* (AUC = 0.989 ± 0.006 and TSS = 0.625 ± 0.4145), *H. quelpaertensis* (AUC = 0.942 ± 0.002 and TSS = 0.822 ± 0.0223), *H. yangi* (AUC = 0.989 ± 0.001 and TSS = 0.428 ± 0.0188), and *H. geojeensis* (AUC = 0.999 ± 0.001 and TSS = 0.799 ± 0.1117). The habitat suitability results were thresholded using the maximum training sensitivity plus specificity (MTSS) value to classify MaxEnt output raster files into suitable and unsuitable habitats (Fig. 2). We found that the variables with the highest contribution to the model differed among *Hynobius* species: human distance for *H. leechii*, annual precipitation (bio12) for *H. unisacculus*, December solar radiation (srad12) for *H. notialis* and *H. perplicatus*, August solar radiation (srad08) for *H. quelpaertensis*, temperature seasonality (bio4) for *H. yangi*, and river distance for *H. geojeensis* (Table 2).

Niche identity test and background similarity test

The ecological niche differentiation test revealed that the predicted niches for the 21 *Hynobius* species pairs were not identical (Supplementary Table S1). However, the background similarity test indicated that the ENM projections from every species pair were not identical and differed by pairs. For example, of the 21 pairs, *H. leechii* vs. *H. perplicatus* showed significant overlap (I = 0.9067 and Schoener's D = 0.6702, both $p < 0.05$). In contrast, *H. notialis* vs. *H. unisacculus* was not significantly different (I = 0.9600 and Schoener's D = 0.8010, both $p > 0.05$), so we did not reject the null hypothesis. Furthermore, *H. yangi* versus *H. geojeensis*, and *H. unisacculus* versus *H. perplicatus* pairs significantly differed in only one direction. For example, *H. yangi* and *H. geojeensis* were not more similar than expected by chance (I = 0.0997 and Schoener's D = 0.022, $p < 0.05$/*H. yangi* vs. *H. geojeensis* background, I = 0.1252 and Schoener's D = 0.03, $p > 0.05$/*H. geojeensis* vs. *H. yangi* background; Supplementary Table S2). These tests revealed distinct ecological niches among *Hynobius* salamander species, rejecting the null

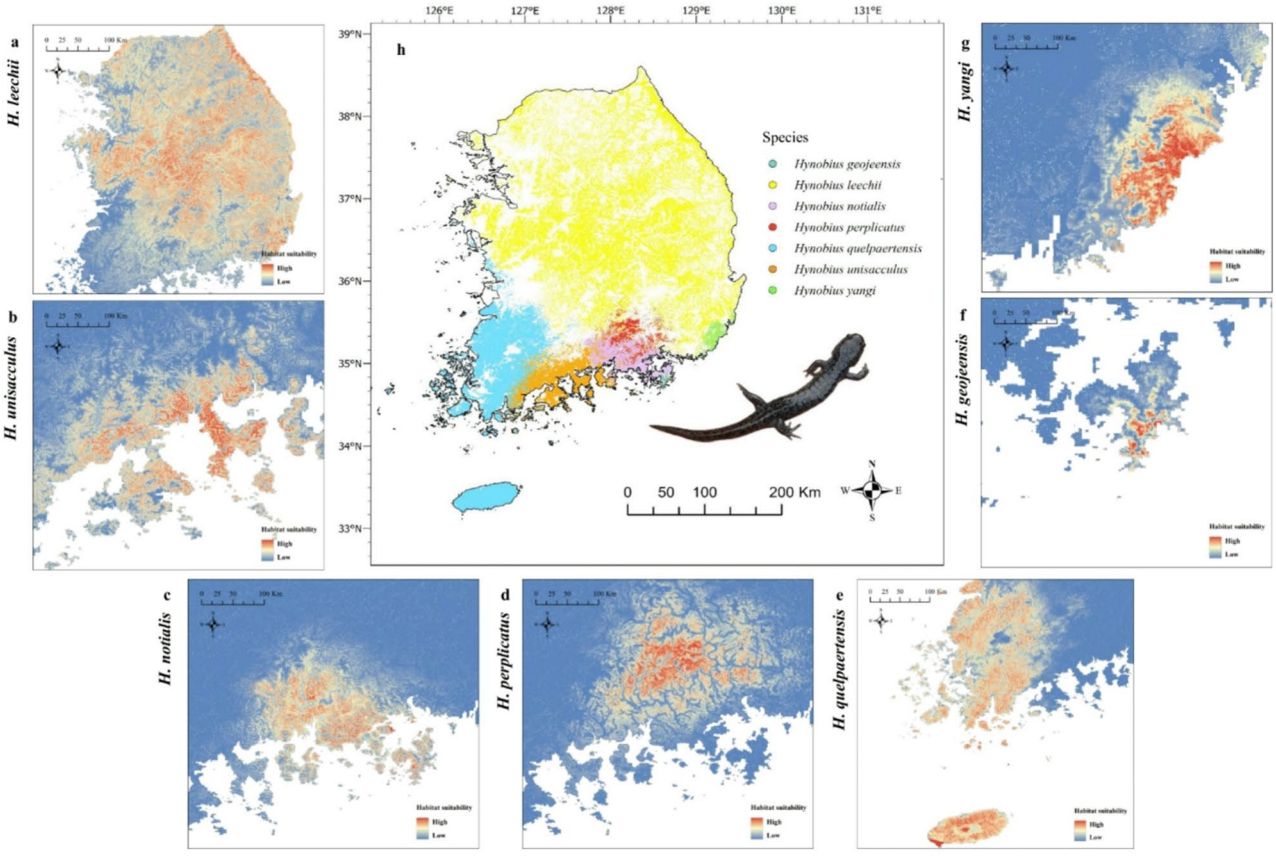


Fig. 2. MaxEnt habitat suitability models for seven *Hynobius* species. (**a**) *H. leechii*, (**b**) *H. unisacculus*, (**c**) *H. notialis*, (**d**) *H. perplicatus*, (**e**) *H. quelpaertensis*, (**f**) *H. geojeensis*, (**g**) *H. yangi*. Panel (**h**) shows predicted habitat suitability for all seven species across South Korea. In (**h**), areas above the MTSS threshold appear as colored regions. Each color indicates a different species, and all panels are labeled with species names. The map was generated using ArcGIS Pro software (version 3.3, https://pro.arcgis.com/en/pro-app/3.3/get-started/release-notes.htm).

Variables	H. leechii	H. unisacculus	H. notialis	H. perplicatus	H. quelpaertensis	H. yangi	H. geojeensis
bio1	1.9	0.1	1.1	0.1	0.5	0.1	0.1
bio4	1.9	17.7	3	2.6	8.9	60	0.3
bio12	1.4	25.8	11.6	8.2	0.1	6.9	10.8
bio14	1.2	6.1	0.2	17	0.2	2.7	0.5
bio16	2.3	0.7	4.1	0.1	0.1	6.6	0
srad08	19.7	25.1	5.1	1.9	68.5	15.6	0.9
srad12	1.2	10.5	64.8	42.6	12.5	3.2	38.2
elevation	19	2.5	0.6	0.1	2.1	1.9	0.9
average greenness within 1 km	12.3	1.2	0.2	0.7	2.2	0	0
human distance	22.4	0.9	2.7	1.2	0.9	0.6	0.2
predominant landcover within 1 km	2.1	0.2	2.6	9.8	0.5	0.6	1
river coast distance	1.6	0.1	1.1	5.7	0.5	0.1	0
river distance	0.9	1.4	0.1	1.1	0.6	0.7	42.6
topographic wetness index	11.2	4.5	1.3	3.9	2.2	0.3	0
urban distance	0.9	3.1	1.6	5.1	0.1	0.6	4.4

Table 2. Percentage contribution of 15 variables used for habitat suitability modeling for the seven *Hynobius* species in the Republic of Korea.

hypothesis stating that *Hynobius* pairs have identical niches. For some species pairs, we could reject the null hypothesis stating that species are more similar than expected by chance and have distinct biogeographic ranges (Fig. 3).

Lineage-breaking test

Testing for the presence of an abrupt environmental transition or steep environmental gradient through the lineage-breaking test showed that the environmental divergence between most of the pairs was greater than that observed between pseudoreplicated null distribution generated randomly. From the 21 pairs, we did not reject the null hypothesis for three pairs: *H. leechii* versus *H. notialis*, *H. leechii* versus *H. perplicatus*, and *H. notialis* versus *H. perplicatus* (Supplementary Table S3). For example, between *H. leechii* versus *H. notialis*, there is no significant environmental gradient between the two species since the I and D values are not significantly different ($I = 0.5216$ and Schoener's $D = 0.2307$, both $p > 0.05$). Overall, the lineage-breaking tests revealed a significant environmental divergence between most *Hynobius* species, supporting the existence of abrupt environmental transitions or steep environmental gradients that have contributed to their diversification (Fig. 3).

Ribbon test

The tests for the unsuitable habitat between two suitable regions through the ribbon test also differed by species pair. The null hypothesis was rejected if the flanking regions of the ribbon were significantly different from one another than expected by chance as indicated by the ribbon tests for these populations. From the 21 pairs, we did not reject the null hypothesis for two pairs: *H. notialis* versus *H. geojeensis*, and *H. leechii* versus *H. perplicatus* (Supplementary Table S4). For example, between *H. leechii* versus *H. perplicatus*, there was no perceived barrier between the two species since the I and D values are not significantly different ($I = 0.6542$ and Schoener's $D = 0.3309$, both $p > 0.05$ for *H. leechii* sampling vs. ribbon/$I = 0.8243$ and Schoener's $D = 0.5499$, both $p > 0.05$ for *H. perplicatus* sampling vs. ribbon). As such, the ribbon tests revealed unsuitable habitats between most pairs of *Hynobius* species (Fig. 3).

Discussion

Our approach successfully investigated four key hypotheses: (i) *Hynobius* species have distinguish niches. (ii) These species have different biogeographical backgrounds. (iii) Biogeographic barriers linked to abrupt environmental changes or steep gradients are present between species. (iv) These biogeographic boundaries coincide with regions of notably unsuitable habitat, effectively separating suitable regions for different *Hynobius* species. Applying a biogeographic barrier framework to the seven *Hynobius* species, we provide evidence supporting the presence of significant environmental biogeographic barriers to dispersal. These barriers come in the form of physical barriers and environmental gradients across the southern part of the South Korea, which are particularly unsuitable between some species pairs.

In this study, some species pairs, including *H. leechii*, *H. yangi*, *H. notialis*, *H. perplicatus* and *H. unisacculus*, were significantly segregated by existing physical barriers. The pairs *H. leechii* and *H. yangi*, and *H. notialis* and *H. perplicatus*, were separated by the Nakdong River, which has an average width of 500–600 m in both its upstream and downstream Sect[56]. The species pair *H. unisacculus* and *H. notialis* were separated by the Seomjin River, with an average width of up to 700 meters[57]. This segregation is similar to the one delineating the distribution of a Korean treefrog, *D. flaviventris*, by the Mangyeong River[58], despite an average width of only 300 meters[59]. As another example, major rivers in North Dakota act as barriers, shaping the regional population of the northern leopard frog (*Rana pipiens*)[60]. Such geographic segregations are also common among salamander species, for

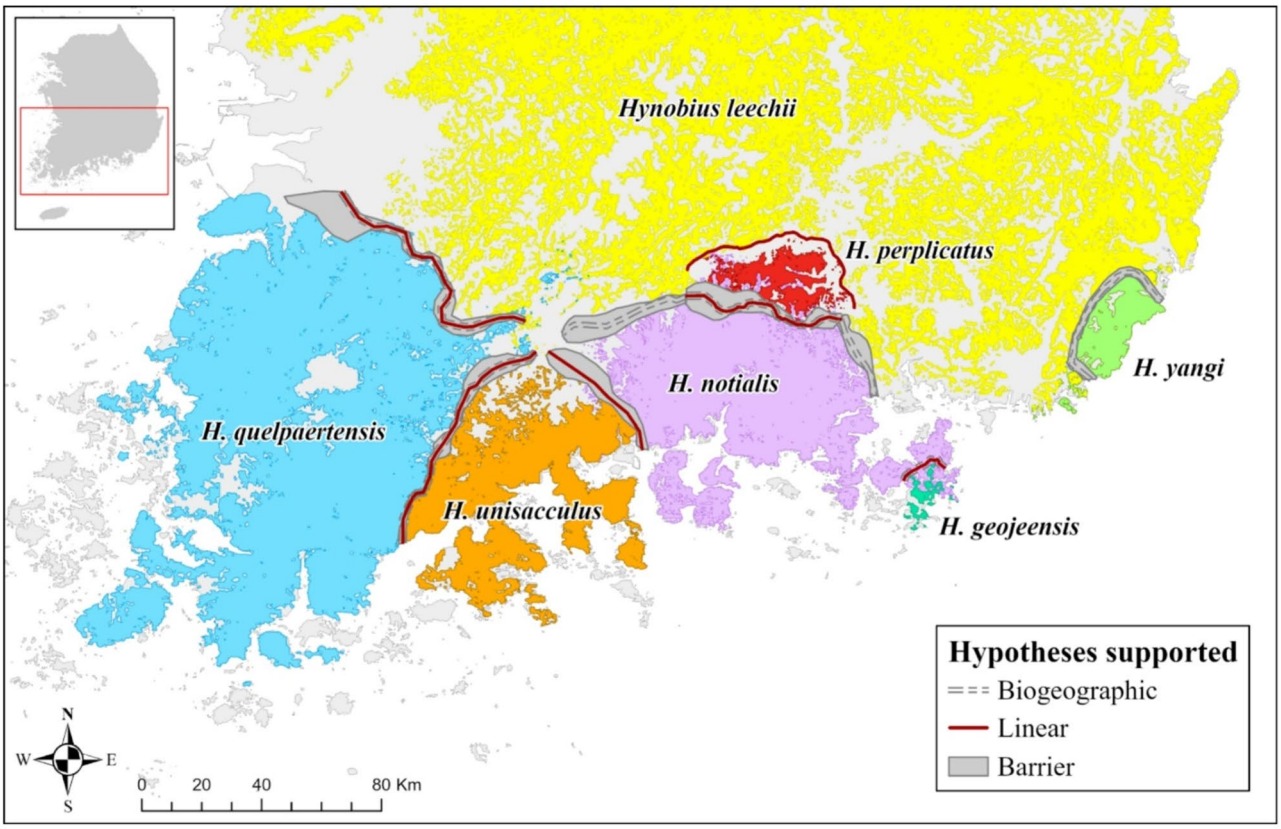


Fig. 3. Summarized map for four hypotheses testing biogeographic barriers on seven *Hynobius* species. Each line and polygons represent each hypothesis. The total biogeographic barrier is shown in dot lines, and the linear barriers are shown in straight line. The approximate barriers on the map are based on occurrence points used for modelling (grey polygon). The map was generated using ArcGIS Pro software (version 3.3, https://pro.arcgis.com/en/pro-app/3.3/get-started/release-notes.htm).

instance, moderate levels of genetic differentiation are observed between individuals of the salamander *Plethodon cinereus* located on opposite sides of small streams (width ≤ 7 m)[61].

Mountains, along with rivers and oceans, also serve as common barriers to the dispersal of species due to elevation and slope[62–64]. The Jiri Mountain range, with a peak of 1,696 m, separates *H. leechii* (mean elevation of 360 m) and *H. unisacculus* (mean elevation of 550 m)[65,66]. The Andes Mountain ranges, ranking as the second highest after the Himalayas and towering nearly 7000 m, have fostered lineage diversification[67]. They have also served as a conduit for species dispersal into adjacent biomes, a phenomenon supported by studies on lizards, emphasizing the role of Andean uplift in bolstering species diversity through allopatric fragmentation[68]. Not only do high-elevation mountains play a role, but lowland areas also contain isolated mountains with lower elevations, such as Bailing mountains, which have a peak of 546 m. These mountains, separated by deep valleys, can inhibit dispersal, and promote fragmentation, especially for taxa with limited dispersal abilities, like stream salamanders (Hynobiidae: *Batrachuperus*)[69].

Interpreting the results of the applied framework revealed that the presence of a biogeographic barrier either corresponds to a current physical barrier or historical events[70,71]. Many studies support riverine barriers as significant limits to amphibians' dispersal ability and barriers to gene flow[60,72]. For instance, the Amazon River separates the biogeographical regions in the north from those in the south, while the Madeira River divides the southeastern regions from the southwest, acting as strong barriers that impact the speciation of anuran species in these areas[73]. Similarly, the well-known ring species of salamanders, the *Ensatina eschscholtzii* complex, originated in northern California or southern Oregon and expanded southward along two paths: the coast ranges and the Sierra Nevada. Due to geographic variation acting as a barrier, these paths led to speciation, with the lineages reconnecting in southern California to form a ring around the Central Valley[74].

While some species pairs may be separated by physical barriers, others may be the result of historical events or abiotic factors that have influenced their distributions[2,75]. Competition for resources like basking sites, nesting areas, and refuges can limit the range of a species, particularly for amphibians and reptiles[2]. In the case of *Hynobius* salamanders, their similar breeding behaviors can have led to intense competition upon contact, resulting in the formation of niche partitions and biogeographic barriers[24]. Research on the agile frog (*Rana dalmatina*) in its refugial range in the Italian Peninsula highlights greater diversity and phylogeographic structure compared to the rest of the species' range in Europe[76]. Similarly, the Korean peninsula's mountainous terrain and

lack of glaciation during the Quaternary provided refuges for salamanders during periods of climatic upheaval, contributing to the peninsula's high salamander diversity and endemism[77]. These studies suggest that climate-driven microevolutionary processes during glacial and interglacial periods have significantly contributed to genetic diversity in these regions[76,78]. Consequently, these historical events likely led to increased population densities in the Korean peninsula's southern region, shaping salamanders' current distribution and establishing biogeographic barriers.

Unlike the delineated distributions of other salamander species, *Hynobius leechii* is widely spread across the peninsula[26]. This can be because *H. leechii* dispersed before encountering the highest mountain barrier of the peninsula, Baekdudaegan, thus avoiding its effects[79]. Alternatively, *H. leechii* may exhibit greater genetic divergence among populations separated by Baekdudaegan[26,34]. For instance, the lizard *Gonatodes humeralis* and the tree frog *Dendropsophus leucophyllatus*, distributed across South America, show significant genetic differentiation associated with all Amazonian rivers[80]. Our tests highlight two informative contrasts within the same analytical framework. For *H. leechii* and *H. perplicatus*, the identity test indicated a difference, with I equal to 0.9067 and Schoener's D equal to 0.6702 and both p values less than 0.05, yet the background, lineage break, and ribbon tests did not indicate strong environmental barriers between their distributions. These outcomes point to broadly similar environmental conditions and an absence of a pronounced contemporary barrier. The pair diverged about 5.3 million years ago[34], and phylogenetic evidence indicates they are distinct lineages within the South Korean assemblage[26,34–36,39]. This combination suggests that species limits are maintained by processes such as reproductive isolation and ecological interactions. Similar dynamics are reported for large terrestrial salamanders, and for instance, interspecific competition in *Plethodon glutinosus* and *P. jordani* system appears to drive isolation more strongly than differences in altitude preferences[81]. Closely related species that occur in sympatry can also show reproductive isolation, leading to genetic divergence and even sympatric speciation, as documented in multiple groups including plants and insects[82,83]. In contrast, *H. notialis* and *H. unisacculus* showed high overlap with an identity outcome that was not significant, with I equal to 0.9600 and Schoener's D equal to 0.8010 and both p values greater than 0.05, which aligns with niche conservatism among closely related or recently separated lineages[84]. A third pattern involves barrier-positive separations that coincide with spatial heterogeneity, for example *H. yangi* and *H. quelpaertensis* are separated by a corridor of low suitability, which is consistent with niche evolution in heterogeneous environments where divergence develops within varying conditions rather than across climatic dissimilarity[85]. Differences in microhabitat use along streams, breeding timing, and short-distance movements offer plausible mechanisms for these outcomes, and studies in frogs show that behavior can buffer environmental constraints in ways that shape contact and overlap[86].

Conclusion

This study employed the framework developed by Warren et al. (2008) and Glor & Warren (2011) to elucidate the biogeographic barriers separating the seven Korean *Hynobius* salamander species. In contrast to previous studies that focused on only two congeneric species of *Anolis* lizards across the Hispaniola plain, our investigation encompassed seven closely distributed species and integrated both frameworks into four hypotheses tested using R studio software. Specifically, the comparative framework employed in this study corroborates the underlying mechanisms of speciation and evolution in the seven *Hynobius* salamanders by supporting the distinct distribution patterns arising from dispersal barriers identified in our results. Our findings contribute to the overall understanding of the large-scale drivers influencing species' distribution patterns and evolution, enabling us to predict how environmental changes may affect distribution and conservation needs.

Data availability

All data generated or analyzed during this study are included in this published article and its Supplementary Information files.

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Author contributions

TEU: methodology, investigation, data curation, writing —original draft, visualization; DAB: methodology, investigation, writing —review, and editing, visualization; AB: methodology, investigation, writing—review, and editing; YKJ: investigation, writing—review, and editing. All authors edited and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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