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Re-evaluation of extinction risk of four Korean land snails (Mollusca, Gastropoda) based on intensive field survey and genetic diversity of *COI* or *16S rRNA*

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ABSTRACT

This study aims to re-evaluate risk of extinction the Red List categories of four Korean land snail species, *Euhadra dixoni* (Pilsbry, 1900), *E. herklotsi* (E. von Martens, 1861), *Nesiohelix samarangae* (Kuroda and Miyanaga, 1943), and *Cyclophorus herklotsi* E. von Martens, 1861, based on field surveys and genetic diversity analyses. Genetic diversity was evaluated with *COI* or *16S rRNA*. The results suggested that the categorical rank of the Red List of *E. dixoni* be changed from Endangered (EN) to Critically Endangered (CR). On the other hand, we recommended that the categorical ranks of *E. herklotsi*, *16S rRNA* and *C. herklotsi* may be kept to the present status, Vulnerable (VU). This study raises the usefulness of the addition of genetic information in evaluating extinction risk of the molluscan species listed in the Red List, which would be helpful for successful management and conservation of land snails in Korea.

Keywords: *Euhadra*, *Nesiohelix*, *Cyclophorus*, *COI*, *16S rRNA*, Red List, land snail, Korea

INTRODUCTION

The International Union for Conservation of Nature (IUCN) publishes the Red Data Book to prevent the extinction of wildlife and conserve biodiversity by identifying species at high risk of extinction, documenting their distribution and habitat status. The IUCN Red List categories and criteria are developed to assess and classify the extinction risk of wildlife. These can be divided into the "Global Red List," which evaluates species on a global level, and the "Regional Red List," which compiles assessments for specific areas at local, national, or regional levels. Republic of Korea also publishes a national Red Data Book that includes a regional Red List, helping to assess the conservation status of native species, monitor changes in species distribution, and provide essential information for appropriate conservation

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Table 1. The four land snails registered in the National Red List in Korea

Scientific name	Criteria	Category of the Red List
Euhadra dixoni	Population decline caused by environmental changes and habitat reduction	Endangered (EN)
Euhadra herklotsi	A large land snail primarily found on the southern coast, with population decline due to overharvesting, predation by natural enemies, habitat loss from environmental changes and development	Vulnerable (VU)
Nesiohelix samarangae	Population decline caused by overharvesting, damage from predators (such as birds or small mammals), and habitat destruction due to forest development	VU
Cyclophorus herklotsi	A southern species with its northern range limit at Mt. Naejangsan, experiencing a decrease in distribution and population due to environmental changes and habitat destruction	VU

actions. Currently, the Red Data Book published by the National Institute of Biological Resources (NIBR, Incheon, Republic of Korea)lists 4,650 species (excluding NE (Not Evaluated) category), with 4,092 animal species designated in the Red List (NIBR, 2024).

To evaluate extinction risk, the IUCN uses information on species range, population size, habitat quality, fragmentation levels, and trends in abundance. However, most of the established criteria and assessment metrics focus on vertebrates. Among these, estimating population size through local surveys, assessing the number of mature individuals, and calculating occupancy areas are challenging for certain invertebrate taxa due to the following characteristics: (1) morphological traits for identification are often not clearly described for many species, (2) the distribution of most described species is not well understood, (3) changes in species richness over time and space are not well documented, and (4) ecological information regarding habitat sensitivity for each species is largely lacking (Cardoso *et al.*, 2011). Furthermore, numerous studies have reported that genetic traits are not reflected in the assessment criteria used for the Red List evaluations. These studies indicate that approaches incorporating genetic diversity must be included in the conservation strategy framework and highlight the need for population genetics and distribution prediction research in the Red List categorization process (Santamaría and Mendez, 2012; Daniels *et al.*, 2020; Garner *et al.*, 2020; Canteri *et al.*, 2021; Petit-Marty *et al.*, 2021; Schmidt *et al.*, 2022, 2023).

This study aims to reassess the extinction risk in the Red List categories of four Korean land snail species - *Euhadra dixoni* (Pilsbry, 1900), *E. herklotsi* (E. von Martens, 1861), *Nesiohelix samarangae* (Kuroda and Miyanaga, 1943), and *Cyclophorus herklotsi* E. von Martens, 1861 - through comprehensive information including field surveys and genetic diversity analyses, while also suggesting the possibility of genetic diversity analysis as an indicator for evaluating endangered species.

Euhadra dixoni (Stylommatophora, Bradybaenidae) is a large land snail known for having the most beautiful shell among native land snails in South Korea (NIBR, 2022). It is a southern species with its northern limit at Mt. Naejangsan, primarily inhabiting the preserved subtropical broadleaf forests of Jeollabuk-do and Jeju Island in Korea (Min *et al.*, 2004). Although the same species is also found in Japan, its habitat is shrinking and populations are declining due to environmental changes and development, assigning to its Red List categorical rank as Endangered (EN) (NIBR, 2022) (Table 1).

Euhadra herklotsi (Stylommatophora, Bradybaenidae) is a large southern land snail that inhabits the preserved broadleaf forests of the southern island regions and Jeju Island in South Korea (Min *et al.*, 2004) (Supplementary Fig. 1A). Due to recent

Fig. 1. Field observations of the four land snails. **A, B.** *Euhadra dixoni* (Haean, Jeju) taken on June 28, 2023; **C.** *E. herklotsi* (Nambu, Geoje) taken on August 29, 2019; **D.** *E. herklotsi* (Saekdal, Jeju) taken on June 28, 2023; **E.** *Nesiohelix samarangae* (Bonggae, Jeju) taken on June 27, 2023; **F.** *Nesiohelix samarangae* (Topyeong, Jeju) taken on June 29, 2023; **G.** *Cyclophorus herklotsi* (Nambu, Geoje) taken on July 13, 2023; **H.** *Cyclophorus herklotsi* (Bonggae, Jeju) taken on June 27, 2023. All photos were taken by JS Lee.

environmental changes and habitat loss caused by human activities, its population has been declining, assigning to its Red List categorical rank as vulnerable (VU) (NIBR, 2022) (Table 1).

Nesiohelix samarangae (Stylommatophora, Bradybaenidae) is a Korean endemic species and the

Supplementary Fig. 1. Habitat landscapes of two land snails *Euhadra herklotsi* and *Nesiohelix samarangae* ranked as Vulnerable. **A.** *Euhadra herklotsi* (Bonggae, Jeju) taken on June 27, 2023; **B.** *Nesiohelix samarangae* (Topyeong, Jeju) taken on June 29, 2023. All photos were taken by JS Lee.

largest land snail in the country (NIBR, 2022). It has a wide distribution range, found from Pyeongchang-gun in Gangwon-do to Jeju Island, primarily inhabiting preserved broadleaf forests with high humidity (Min *et al.*, 2004) (Supplementary Fig. 1B). However, due to predation by birds and small rodents, as well as habitat destruction from development, its population is declining, leading to its classification as vulnerable (VU) (NIBR, 2022) (Table 1).

Cyclophorus herklotsi (Architaenioglossa, Cyclophoridae) is a primitive land snail of the order Architaenioglossa, characterized by having both gills and a lid (NIBR, 2022). This southern species is the largest within the family Cyclophoridae, found primarily in southern regions and the southern islands of South Korea, as well as Jeju Island. It typically inhabits the spaces beneath stones or leaf litter in preserved shrub forest environments (Min *et al.*, 2004). However, recent environmental changes and habitat destruction have led to a reduction in both its distribution area and population, resulting in its classification as vulnerable (VU) (NIBR, 2022) (Table 1).

For animal population genetic studies, mitochondrial *COI* and *16S rRNA* makers have been used very frequently until now (Choi *et al.*, 2021a, b; Choi and Hwang, 2021, 2023; Shin *et al.*, 2021; Park and Hwang, 2022; Hong *et al.*, 2023; Kim and Hwang, 2023; Kim *et al.*, 2023, 2024; Yu *et al.*, 2024). Here, we employ *COI* and *16S rRNA* markers to re-evaluate the Red List categorical ranks of mollusks. Notably, this research identifies for the first time the genetic diversity of *E. dixoni* and *N. samarangae*. Additionally, it is the first study to incorporate genetic diversity analysis in the re-evaluation of the Red List categories for mollusks listed in the Red Data Book published by NIBR, Ministry of Environment, Korean government.

MATERIALS AND METHODS

1. Field observation and sample collection

A field survey was conducted focusing on habitats identified through literature research, and specimens of four land snail species were collected by hand. *E. dixoni* is a southern species with its northern limit at Mt. Naejangsan; however, it exhibits a very localized distribution, currently found only at a very low frequency in the Jeju Island. Based on the information gathered so far, a concentrated investigation was carried out in Jeju Island. Two land snails, *E. herklotsi* and *C. herklotsi*, were also studied primarily in their major occurrence areas, Jeju Island and Geoje Island. *N. samarangae*, while a widely distributed species found from central to northern South Korea and extending to Jeju Island, was surveyed in high-density habitats in the southern island regions, including Jeju Island, as well as in the central and northern regions. Four species are listed on Korean Red List as Endangered (EN) and Vulnerable (VU) due to their limited habitat size and low population density, with indiscriminate collecting poses a significant threat to ecosystems and

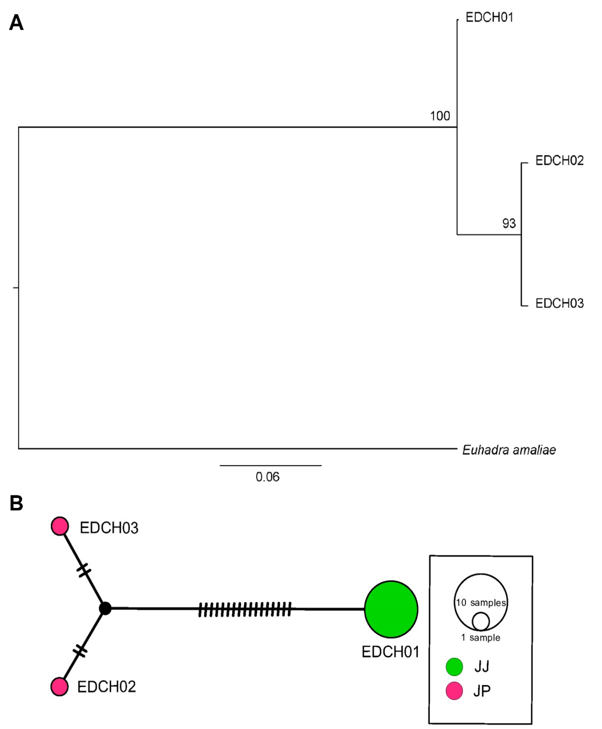

Fig. 2. Maximum likelihood tree (A) and TCS haplotype network (B) based on three *COI* haplotypes of *Euhadra dixoni*.

biodiversity.

2. Molecular experiments and genetic diversity analyses

The land snail samples collected during the field survey were used to extract genomic DNA following the DNeasy Blood and Tissue Kit protocol (Qiagen, USA). After measuring DNA concentration using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), the samples were stored at −70°C. To amplify the mitochondrial *COI* and *16S rRNA*, we used universal primers commonly employed in animal studies for PCR, followed by sequencing to obtain the final nucleotide sequences (Solgent, Daejeon). We used data derived from successful DNA extractions and *COI* or *16S rRNA* sequencing for our analyses. Based on these sequences, haplotypes were identified for genetic diversity analysis. This analysis included calculating haplotype diversity (*h*), nucleotide diversity (π), and the number of segregation sites (S) for each population using the program DnaSP 6.12.03 (Rozas *et al.*, 2017) to assess their Red List status. Additionally, haplotype networks were constructed using a statistical parsimony approach at the population level using PopART (Leigh and Bryant, 2015) and phylogenetic analyses were performed using the maximum likelihood (ML) method. In the ML tree, model selection in the IQ-Tree software package (http://www.iqtree.org) was tested and the phylogenetic tree was analyzed from 1,000 ultrafast bootstrap replicates using the IQ-Tree web server (http://iqtree.cibiv.univie.ac.at).

RESULTS AND DISCUSSION

1. Field survey and *COI*-based genetic analysis of *Euhadra dixoni* (Pilsbry, 1900)

As a result of field survey, the land snail *E. dixoni* has a limited occurrence in Jeju Island, where it is considered a low-occurrence frequency species, as multiple individuals are rarely observed in any single location. It appeared to be primarily observed on trees (Fig. 1A, B). Based on the information gathered so far, massive collection was conducted in Jeju Island, resulting in the collection of only five specimens from one site in Jeju Island (Table 2). Genetic diversity analysis was performed on these five individuals, yielding a 560 bp *COI* nucleotide sequence. *COI* haplotype analysis revealed only one haplotype (EDCH01) in Korea. We found two more Japanese *E. dixoni* haplotypes (EDCH02 and EDCH03) with addition of *COI* haplotypes from NCBI. *COI*-based ML tree reconstructed with the GTR+F+I+G4 model showed that the two Japanese haplotypes formed a clade with high bootstrap support value (BP 93) (Fig. 2A). Additionally, to examine the gene genealogy among the three *COI* haplotypes, we constructed TCS haplotype network, which revealed 19 distinct mutation steps between the Korean haplotype (EDCH01) and the Japanese haplotypes (EDCH02 and EDCH03) (Fig. 2B).

2. Field survey and *COI*-based genetic analysis of *Euhadra herklotsi* (E. von Martens, 1861)

Through intensive field surveys, numerous

Table 2. List of collection sites and the number of collected specimens of the four land snails in Korea

Scientific name	Collecting site	Population name	Number of collected individuals	Number of analyzed individuals
Euhadra dixoni	Haean-dong, Jeju-si, Jeju-do, Korea	JJ	5	5
Euhadra herklotsi	Tapo-ri, Nambu-myeon, Geoje-si, Gyeongsangnam-do, Korea	GJ	5	5
	Bonggae-dong, Jeju-si, Jeju-do, Korea	JJ	5	1
	Saekdal-dong, Seogwipo-si, Jeju-do, Korea	SG	5	3
Nesiohelix samarangae	Maha-ri, Mitan-myeon, Pyeongchang-gun, Gangwon-do, Korea	PC	10	10
	Tapo-ri, Nambu-myeon, Geoje-si, Gyeongsangnam-do, Korea	GJ	10	10
	Bonggae-dong, Jeju-si, Jeju-do, Korea	JJ	10	10
	Topyeong-dong, Seogwipo-si, Jeju-do, Korea	SG	10	10
Cyclophorus herklotsi	Songdan-ri, Baeka-myeon, Hwasun-gun, Jeollanam-do, Korea	JN	1	1
	Tapo-ri, Nambu-myeon, Geoje-si, Gyeongsangnam-do, Korea	GN	5	5
	Suwon-ri, Hanrim-eup, Jeju-si, Jeju-do, Korea	JJ	5	4

Table 3. Collection-site distribution of mitochondrial *COI* and *16S rRNA* haplotypes of *Euhadra herklotsi*, *Nesiohelix samarangae*, and *Cyclophorus herklotsi*.

		E. herklotsi			
Haplotype	GJ	JJ	SG	**Total**	
EHCH01	-	-	2	**2**	
EHCH02	-	1	-	**1**	
EHCH03	-	-	1	**1**	
EHCH04	5	-	-	**5**	
Total	**5**	**1**	**3**	**9**	
		N. samarangae			
Haplotype	PC	GJ	JJ	SG	**Total**
NSCH01	-	-	5	-	**5**
NSCH02	-	10	3	4	**17**
NSCH03	-	-	2	5	**7**
NSCH04	-	-	-	1	**1**
NSCH05	10	-	-	-	**10**
Total	**10**	**10**	**10**	**10**	**40**
		C. herklotsi			
Haplotype	JN	GN	JJ	**Total**	
CHCH01	-	5	3	**8**	
CHCH02	-	-	1	**1**	
CHCH03	1	-	-	**1**	
Total	**1**	**5**	**4**	**10**	

The full names of collection sites are shown in Table 2.

Table 4. Summary of the population genetic analyses from *Euhadra herklotsi*, *Nesiohelix samarangae*, and *Cyclophorus herklotsi*

Scientific name	Population name	*N*	Nh	*h*	π	S	k
E. herklotsi	GJ	5	1	0.000	0.00000	0	0.000
	JJ	1	1	nd	nd	nd	nd
	SG	3	2	0.667	0.00181	2	1.333
	Total	**9**	**4**	**0.694**	**0.03580**	**46**	**25.333**
N. samarangae	PC	10	1	nd	nd	nd	nd
	GJ	10	1	nd	nd	nd	nd
	JJ	10	3	0.689	0.01495	10	4.75556
	SG	10	3	0.644	0.01635	10	5.20000
	Total	**40**	**5**	**0.728**	**0.01453**	**13**	**4.61923**
C. herklotsi	JN	1	1	nd	nd	nd	nd
	GN	5	1	0.000	0.00000	0	0.00000
	JJ	4	2	0.500	0.01765	15	7.50000
	Total	**10**	**3**	**0.378**	**0.01313**	**17**	**5.57778**

N = the number of *COI* or *16S rRNA* sequences (individuals), Nh = the number of haplotypes, *h* = haplotype diversity, π = Jukes-Cantor corrected estimates of nucleotide diversity, S = the number of segregation sites, k = the average number of pairwise nucleotide differences, and 'nd' = not determined. The full names of collection sites are shown in Table 2.

individuals of *E. herklotsi* were observed living either on the trees of large broad leaf species or on the ground (Fig. 1C, D). Among the large land snails found in the southern island regions and Jeju Island of Korea, *E. herklotsi* has a relatively high occurrence frequency. In this study, we collected 15 individuals from three locations in Gyeongsangnam-do and Jeju Island, Korea (Table 2). From nine of these specimens, a total of 739 bp of the *COI* nucleotide sequence was determined, which results in four distinct *COI* haplotypes (EHCH01-04), each specific to the respective population (Table 3). The genetic diversity analysis across the entire population indicated *h* = 0.694 and π = 0.0358 (Table 4). Phylogenetic relationships among *E. herklotsi* haplotypes were analyzed using a total of 126 *COI* haplotypes with addition of *COI* data from NCBI. The ML tree reconstructed using the K3Pu+F+I+G4 best-fit model showed that there exist two distinct clades with high bootstrap support values, Clade N (BP 98) and Clade S (BP 99). They were named after their geographical distribution patterns first suggested by Nishi and Sota (2007) (Fig. 3A). The result of TCS haplotype network showed the separation of two genetic groups, Clades N and S (Fig. 3B), as in ML tree. Between the two clades, 46-49 mutation steps were observed, and genetic variation within Clade N was greater than that within Clade S. Regarding geographical distribution, the *COI* haplotypes (EHCH01, EHCH02, and EHCH03) of *E. herklotsi* from Korea were all included in Clade N, along with haplotypes from the Kyushu region of Japan. In contrast, no haplotype in this study was found in Clade S, which mainly contained individuals from the southern Kyushu region of Japan. Moreover, it was observed that some haplotypes centered around EHCH23 exhibited a star-like topology, suggesting that the population size may have recently expanded.

3. Field survey and *16S rRNA*-based genetic analysis of *Nesiohelix samarangae* (Kuroda and Miyanaga, 1943)

As a results of field survey, although *N. samarangae* is occasionally found on trees, it is primarily encountered on the ground (Fig. 1E, F). It often coexists with the *E. herklotsi*, showing slightly different occurrence frequencies depending on the habitat, but it is generally more frequently observed than *E. herklotsi*. For examining genetic diversity, we collected 40 individuals from four collection sites in

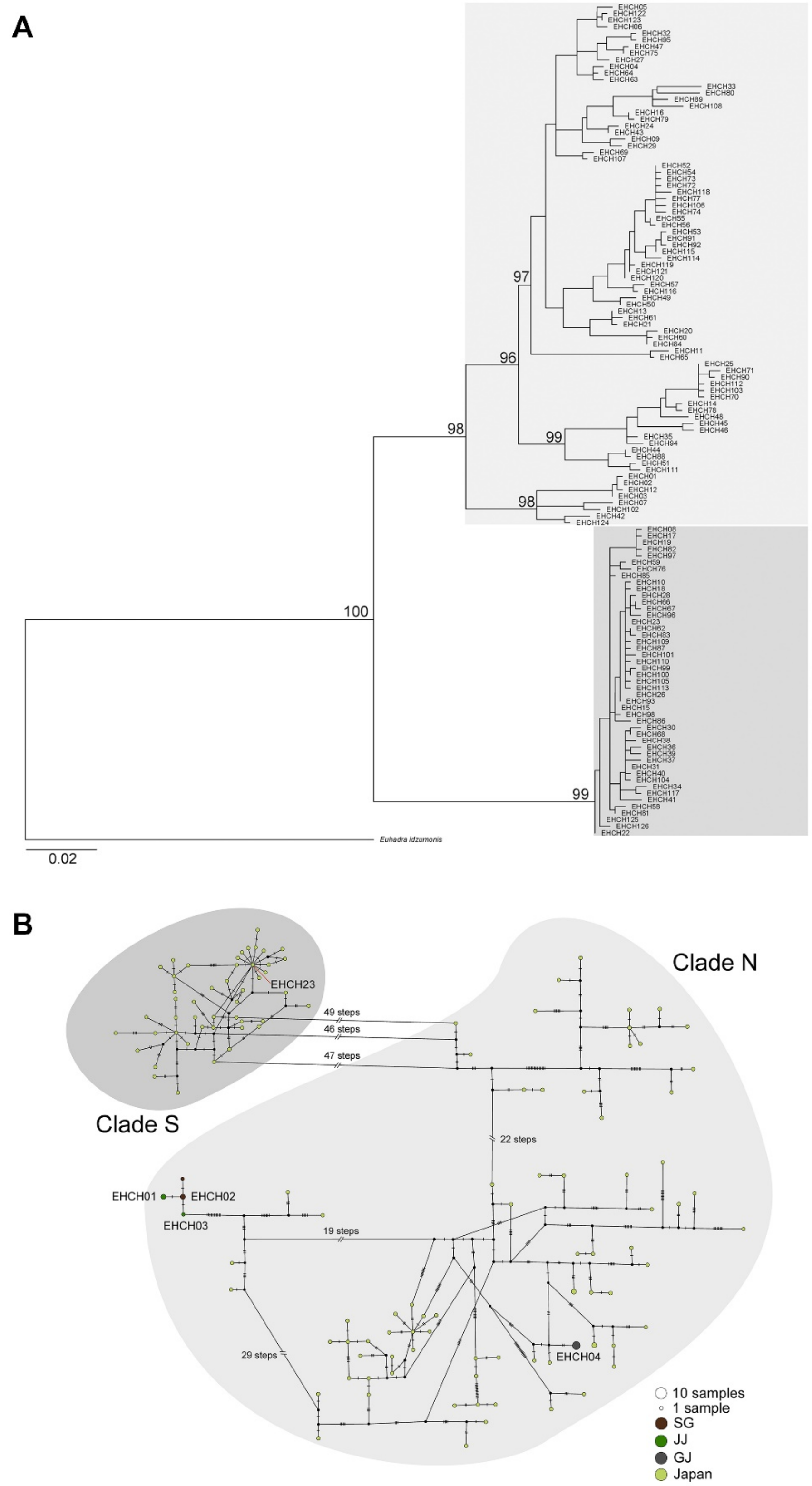

Fig. 3. Maximum likelihood tree (**A**) and TCS haplotype network (**B**) based on 126 *COI* haplotypes of *Euhadra herklotsi*.

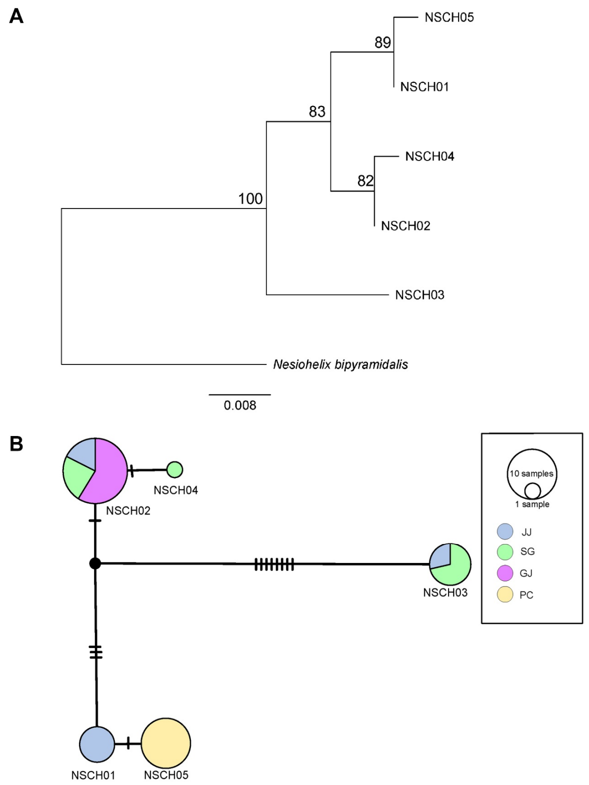

Fig. 4. Maximum likelihood tree (**A**) and TCS haplotype network (**B**) based on five *16S rRNA* haplotypes of *Nesiohelix samarangae*.

Gangwon-do, Gyeongsangnam-do, and Jeju Island (Table 2). A total of 318 bp of the *16S rRNA* nucleotide sequence was determined for these 40 individuals, revealing five distinct *16S rRNA* haplotypes (NSCH01–05). Two haplotypes of NSCH02 and NSCH03 were shared among multiple populations: NSCH02 was found in three populations of JJ, SG, and GJ, while NSCH03 was observed in two populations of JJ and SG. Additionally, the remaining three haplotypes were identified as population-specific, with no sharing with any other populations (Table 3). The analysis of genetic diversity across the entire population indicated h = 0.728 and π = 0.01453 (Table 4). Phylogenetic relationships among the five *N. samarangae* haplotypes were reconstructed with the five *16S rRNA* haplotypes. The resultant ML tree (Fig. 4A) was drawn with HKY+F best-fit model. NSCH01 + NSCH05 and NSCH02 + NSCH04 were grouped together. NSCH03 placed at the basal position. Additionally, TCS haplotype network (Fig. 4B) showed that the NSCH03 haplotype differed from the other haplotypes by 9-11 mutation steps, which is consistent with the result of ML tree (Fig. 4A).

4. Field survey and *COI*-based genetic analysis of *Cyclophorus herklotsi* E. von Martens, 1861

Unlike the other land snails such as *E. dixoni*, *E. herklotsi*, and *N. samarangae* examined in this study, *C. herklotsi* is a typical terrestrial species (Fig. 1G, H). According to intensive field survey, we found that it predominantly appeared in slightly drier areas of broad leaf forests and shrublands, with a relatively high occurrence frequency in suitable habitats compared to the other three species. Here, we collected 11 individuals from three collection sites in Jeollanam-do, Gyeongsangnam-do, and Jeju Island. From 10 among them, we determined partial *COI* sequences for genetic diversity analysis (Table 2). A total of 435 bp of the *COI* nucleotide sequence was determined, which revealed to be three distinct *COI* haplotypes (CHCH01–03). CHCH01 was shared between GJ and JJ populations, while CHCH02 and CHCH03 were identified specifically in JJ and HS populations, respectively (Table 3). The analysis of genetic diversity across the entire population indicated h = 0.378 and π = 0.01313 (Table 4). To examine phylogenetic relationships, ML tree was reconstructed for 27 *COI* haplotypes of *C. herklotsi* with additional NCBI data. The resultant ML tree drawn with TN+F+G4 best-fit model showed two distinct clades, Clades A and B, as suggested by Hirano *et al.* (2022). However, in this study, Clade A did not form a monophyletic group, but paraphyletic to Clade B (Fig. 5A). Like the phylogenetic tree, TCS haplotype network revealed the separation into two different genetic groups, Clades A and B (Fig. 5B). A minimum of 37 mutation steps were observed between the two clades. The degree of genetic variation within Clade A was higher than that within Clade B. Notably, CHCH26 within Clade A exhibited a minimum of 18 mutation steps from the nearest haplotype, showing the highest variation within the clade. The three *C. herklotsi*

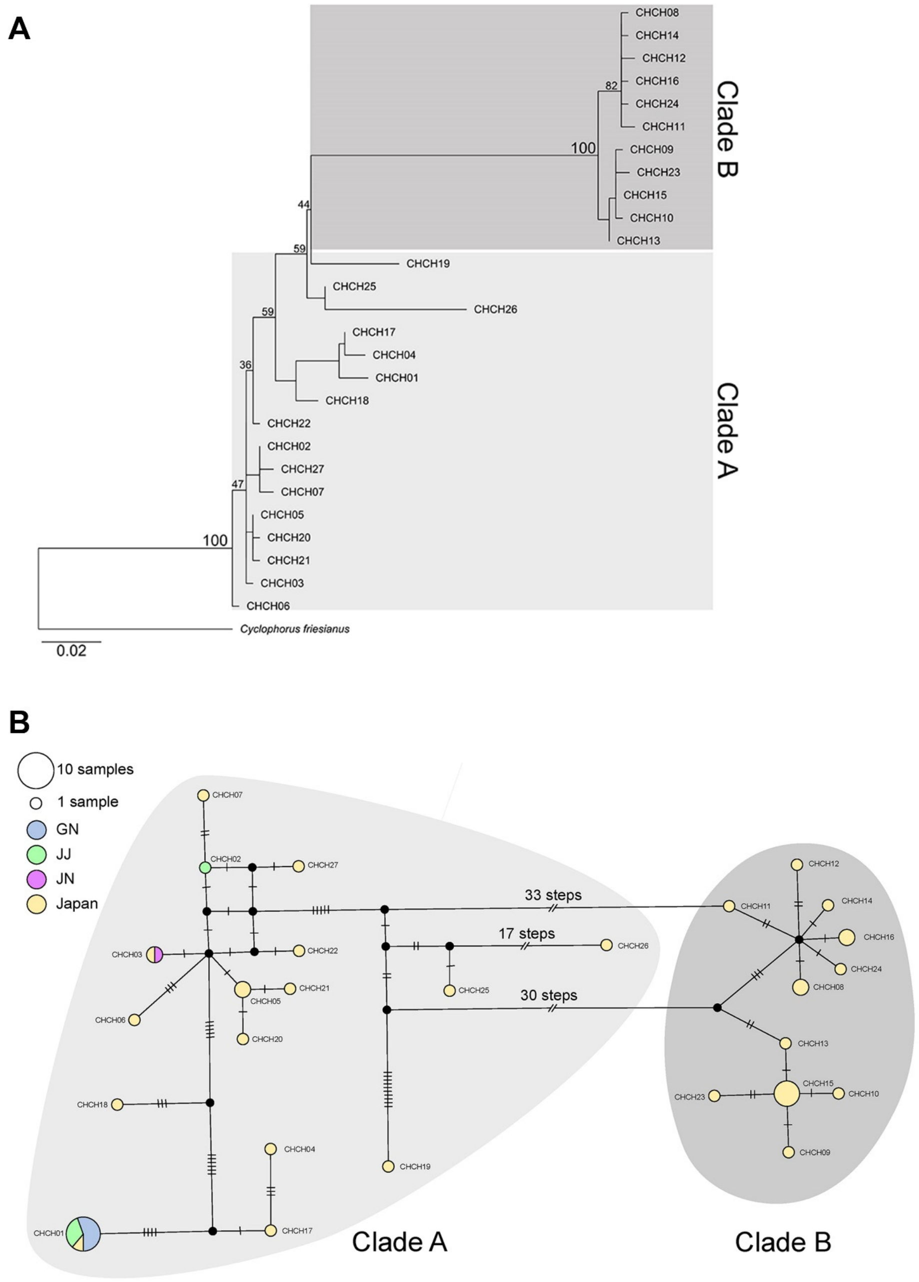

Fig. 5. Maximum likelihood tree (A) and TCS haplotype network (B) based on 27 *COI* haplotypes of *Cyclophorus herklotsi*.

haplotypes identified in this study were all included in Clade A, along with haplotypes from the southern Honshu and Kyushu regions of Japan. In contrast, no Korean *C. herklotsi* haplotype was found within Clade B, which mainly consisted of individuals from the Shikoku and eastern Honshu regions.

5. Re-evaluation of the Red List categorical rank of four land snail from Korea

This study conducted massive field surveys and genetic diversity analyses on four land snails to provide basic data for reassessing their existing Red List categories. Our study suggests that genetic research results should be reflected in species conservation planning, as indicated by previous studies (Santamaría and Mendez, 2012; Daniels *et al.*, 2020; Garner *et al.*, 2020; Canteri *et al.*, 2021; Petit-Marty *et al.*, 2021; Schmidt *et al.*, 2022, 2023). The present results showed that the distribution range of *E. dixoni* in Korea is strictly limited to narrow regions in Jeju Island, and the genetic diversity of *COI* is extremely low (only one haplotype in Korea). It indicates that the Red List categorical rank of *E. dixoni* should be reassigned from EN to CR. In the case of *E. herklotsi*, it was were observed with relatively high occurrence frequency in the southern islands and Jeju Island, and its genetic diversity based on *COI* is quite high (h = 0.694 and ð = 0.0358). In *N. samarangae*, it is more frequently observed than *E. herklotsi* in general. Also, its genetic diversity of *16S rRNA* is not lower (h = 0.728 and π = 0.01453). *C. herklotsi* has relatively higher occurrence frequently than the other three species mentioned before, and its genetic diversity of *COI* haplotypes (h = 0.378 and π = 0.01313) were slightly lower than those of *COI* of *E. herklotsi* and *16S rRNA* of *N. samarangae*. It may indicate that the Red List categorical rank of *E. herklotsi*, *N. samarangae*, and *C. herklotsi* may be kept to the present status, VU.

Euhadra dixoni is characterized by a very restricted occurrence and significantly low genetic diversity, prompting the recommendation for its category to be adjusted. However, further studies involving additional population assessments could yield more accurate classifications. In contrast, both *E. herklotsi* and *C. herklotsi* appear less threatened by extinction than *E. dixoni*, with both species also found to have a widespread distribution in Japan. The genetic diversity values identified for these two species were relatively higher. Previous studies have suggested considerable inter-individual variation in *E. herklotsi* and *C. herklotsi*, indicating the potential for subspecies differentiation (Minato, 1985; Azuma, 1995; Nishi and Sota, 2007; Lee *et al.*, 2008; Nantarat *et al.*, 2014, 2019; Hirano *et al.*, 2022). This suggests that the relatively sedentary nature of terrestrial snails may lead to population isolation, resulting in significant genetic variation accumulation. Therefore, accurate assessments of these species will require thorough understanding through classification and phylogenetic studies. For *N. samarangae*, a comprehensive category assessment was conducted based on the *16S rRNA* gene. Despite its widespread distribution in South Korea, its population and numbers appear to be low, indicating the need for further research. Future analyses using more data from each population of the four species may obtain additional meaningful results.

Our study emphasizes that the genetic diversity assessment of species and populations could be a key indicator. In particular, we showed that endangered species cannot be evaluated solely based on habitat size or distribution range. In addition, the short mitochondrial gene sequences used in the analysis provided information that could more easily identify populations and determine their genetic diversity. Therefore, molecular markers were considered to be quite useful for establishing Red List criteria.

It is well-known that most land snails face significant threats from indiscriminate overharvesting for ornamental purposes, predation, environmental changes, and habitat destruction due to forest development. Conservation measures that address these major threats should be implemented. Moreover, comprehensive genetic information is essential for assessing the conservation and management status of populations and species. A thorough evaluation of genetic diversity utilizing various markers such as microsatellites and SNPs is necessary. Based on these studies, long-term monitoring involving field surveys and genetic research should be conducted to draw comprehensive conclusions in the future.

In this study, field surveys confirmed the habitat environments of four Korean land snail species. Genetic diversity analysis revealed the extent of genetic variation and assessed the potential for population expansion. Based on these findings, we reassessed the extinction risk status of the four species as listed on the national Red List.

Our findings underscore that genetic diversity and population expansion are valuable complementary indicators, particularly when extinction risk cannot be accurately evaluated based solely on habitat size. This study provides a foundation for re-evaluating the Red List status of Korean mollusks, including the land snails analyzed here. Additionally, our results offer actionable insights for conserving and managing endangered species. We propose that genetic diversity analysis is a powerful tool for assessing the status of endangered species.

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