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Conservation implications for endangered Euhadra murayamai, restricted to a large-scale limestone cliff: Insights from genome-wide SNP and morphological analyses

Shun ITO

s.ito.7330i@gmail.com

Shizuoka University

Osamu KAGAWA

University of Tsukuba

Ichiro YOSHIDA

Itoigawa UNESCO Global Geopark Natural Resources Conservation Committee

Satoshi CHIBA

Tohoku University

Takahiro HIRANO

University of the Ryukyus

Daishi YAMAZAKI

Toho University

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Original Research

Conservation implications for endangered *Euhadra murayamai*, restricted to a large-scale limestone cliff: Insights from genome-wide SNP and morphological analyses

Shun ITO (S.I.)[1*], Osamu KAGAWA (O.K.)[2], Ichiro YOSHIDA (I.Y.)[3,4], Satoshi CHIBA (S.C.)[5,6], Takahiro HIRANO (T.H.)[7] and Daishi YAMAZAKI (D.Y.)[8]

[1] Division of Biological Science, College of Science, Shizuoka University, Shizuoka, Japan

[2] Graduate School of Life and Environmental Sciences, Shimoda Marine Research Center, University of Tsukuba, Shizuoka, Japan

[3] Itoigawa UNESCO Global Geopark Natural Resources Conservation Committee, Niigata, Japan

[4] Niigata Shell Club, Niigata, Japan

[5] Center for Northeast Asian Studies, Tohoku University, Miyagi, Japan

[6] Graduate School of Life Science, Tohoku University, Miyagi, Japan

[7] Biology Program, Faculty of Science, University of the Ryukyus, Okinawa, Japan

[8] Department of Life Area Environmental Science, Faculty of Science, Toho University, Chiba, Japan

*Corresponding author. E-mail address: s.ito.7330i@gmail.com. Mailing address: Division of Biological Science, College of Science, Shizuoka University, Shizuoka, Japan. 836 Oya, Suruga-ku, Shizuoka, Shizuoka, 422-8529, Japan

ORCID

Shun ITO: 0000-0001-8830-8103

Osamu KAGAWA: 0000-0003-0040-5748

Satoshi CHIBA: 0000-0001-9273-0307

Takahiro HIRANO: 0000-0002-2422-0003

Daishi YAMAZAKI: 0000-0002-4568-8094

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ABSTRACT

Rapid biodiversity loss necessitates accurate taxonomic classification and the definition of conservation units for effective management. Here, we investigated the genetic and morphological distinctiveness of the endangered land snail *Euhadra murayamai*, endemic to Mt. Myojo, Japan, and its relationship with the closely related species *E. quaesita*. Genome-wide SNPs from double digest restriction site-associated DNA sequencing (ddRAD-seq) and detailed morphological measurements were obtained. Our analyses revealed clear genetic differentiation between *E. murayamai* and *E. quaesita*, challenging previous inferences based solely on mitochondrial markers. Moreover, we detected significant genetic differentiation within *E. murayamai* populations across low and middle elevations, as evidenced by distinct clustering in fastStructure, a phylogenetic network and principal component analysis, as well as a high F_{ST} value. Morphological results aligned with these findings, showing significant differences in shell width and morphology between elevational populations. Approximate Bayesian computation analysis supported a scenario without gene flow after divergence and estimated interspecific divergence at approximately 85,000 generations ago and intraspecific divergence at approximately 35,000 generations ago. These results indicate that limited dispersal and elevational isolation have promoted local adaptation, underscoring the necessity for elevation-specific conservation units. Furthermore, the urgency of incorporating comprehensive genetic and morphological data into conservation planning is emphasised, thereby providing crucial insights for resolving taxonomic uncertainties and guiding effective strategies. Overall, we underscore the importance of integrating high-resolution genetic markers with morphological data to inform conservation strategies for species with restricted distributions and complex evolutionary histories.

KEYWORDS

Conservation Units, ddRAD-seq, Elevational Divergence, Endemic Snail, Japan, Shell Morphology

1 | INTRODUCTION

Biodiversity is undergoing a rapid decline due to anthropogenic activities (Régnier et al. 2015). Although East Asia is recognised as a biodiversity hotspot (Ceballos and Brown 1995; Mittermeier et al. 2004), many species in this region are facing extinction and are critically endangered (Fonseca 2009). However, a significant proportion of these species remain taxonomically unresolved, with some still undescribed (Tedesco et al. 2014; Liu et al. 2022). Taxonomic uncertainties can hinder effective conservation efforts, as proper classification is essential for identifying conservation priorities. Additionally, establishing conservation units, even within a species, is equally important to reflect evolutionary and demographic histories (Funk et al. 2012; Coates et al. 2018). To address these challenges, integrating morphological and genetic analyses is considered indispensable (Mace 2004). This issue is particularly evident in non-marine molluscs, where numerous endangered species remain (Lydeard et al. 2004; Régnier et al. 2009).

Recent advancements in genetic techniques, particularly genome-wide SNPs analysis, have become a powerful tool in conservation genetics (Funk et al. 2012; Ghildiyal et al. 2023). These methods provide higher resolution than traditional genetic markers such as mitochondrial DNA, allowing us to uncover fine-scale genetic structure and trace evolutionary histories more accurately (Hohenlohe et al. 2021). They have been widely applied to identify cryptic species, define management units, and assess genetic diversity across various taxa (Hohenlohe et al. 2021). In molluscs, genome-wide SNPs are particularly valuable for addressing challenges such as introgressive hybridisation, incomplete lineage sorting, and recent divergence—issues that are difficult to resolve with single-locus markers (Yamazaki et al. 2022; Ishii et al. 2024). By providing high-resolution insights, genome-wide SNPs are transforming the design of conservation strategies, especially for non-marine molluscs experiencing complex evolutionary histories.

Euhadra murayamai is an endangered species (Fig.1). This species inhabits only Mount Myojo (hereafter Mt. Myojo), Itoigawa, Niigata, Japan, which is composed of limestone. This snail species was described in 1976 as an endemic species of Mt. Myojo due to its unique flattened shell morphology (Habe 1976). Because of this unique locality and morphology, the species has been over-collected, causing its population to rapidly decline (Murayama 2021). Today, this species is listed as critically endangered by the Ministry of Environment (Ministry of the Environment 2020) and as endangered by Niigata Prefecture, Japan (Niigata Prefecture 2021). Additionally, this species is designated as a “nationally rare

97 species of wild fauna and flora" under *the Act on Conservation of Endangered Species of*
98 *Wild Fauna and Flora* (Act No. 75 of 1992) and as a "specially designated rare and
99 endangered species of wild fauna and flora" under *the Itoigawa City Ordinance for the*
100 *Protection of Rare Wild Fauna and Flora*, prohibiting collection and requiring conservation.
101 Consequently, a field conservation project has been underway since 2019 (Ito 2024).

102 To conserve endemic species with a strictly limited distribution area such as *E.*
103 *murayamai*, it is essential to identify the evolutionarily significant units (ESUs) based on
104 their evolutionary history and population genetic structure. Previous phylogenetic analyses
105 of *E. murayamai* using a few mitochondrial DNA markers showed that this species forms a
106 polyphyletic group within the *E. quaesita* genetic clade (Ueshima and Asami 2003; Davison
107 et al. 2005). These studies suggested that there is no genetic evidence of reproductive
108 isolation between *E. murayamai* and *E. quaesita*, and *E. murayamai* is an intraspecific
109 morphotype of *E. quaesita.* However, phylogenetic analysis relying on only a single
110 mitochondrial locus may lead to misleading conclusions due to introgressive hybridisation
111 and incomplete lineage sorting (Harrison 1991; Funk and Omland 2003; Maddison and
112 Knowles 2006). Regarding genital morphology, *E. murayamai* has similar genital structures
113 to *E. quaesita* (Davison et al. 2005), but is distinct from other sinistral snails including *E.*
114 *quaesita* (Azuma 1978). Due to these factors, the taxonomic status of *E. murayamai* has been
115 controversial: while it is treated as a subspecies of *E. quaesita* (Davison 2020), it is also
116 regarded as an endemic species of Mt. Myojo (MolluscaBase 2025). Therefore, additional
117 genetic analyses using multiple loci, such as genome-wide SNPs, are required (Hirano et al.
118 2019b).

119 Furthermore, *E. murayamai* populations are scattered across Mt. Myojo at low,
120 middle, and high elevations. While the population size at low elevation is relatively large,
121 populations at middle and high elevations are sparse (Ito 2024). Their shell width also differs
122 among elevations (Murayama 2023). Funk et al. (2012) emphasised that morphological
123 differentiation along elevational gradients can inform the development of conservation units,
124 as phenotypic variation often reflects local adaptation. Palsbøll et al. (2007) argued that
125 demographically independent populations are typically treated as management units for
126 conservation. Understanding the genetic relationship between different elevations is thus
127 crucial for developing further conservation strategy.

128 From the above, this study aimed to clarify 1) the genetic relationship between *E.*
129 *murayamai* and *E. quaesita* within Mt. Myojo and 2) the population genetic structure across

different elevations of *E. murayamai*. To achieve these aims, we utilised double digest restriction site-associated DNA sequencing (ddRAD-seq; Peterson et al. 2012) and morphological analysis.

2 | MATERIALS AND METHODS

2.1 | Study sites

We conducted field surveys on Mt. Myojo, Niigata, Japan, to collect shells and DNA samples of *E. murayamai* from two sites: a low-elevation site (alt. 230 m) and a middle-elevation site (alt. 600 m), which are separated by a horizontal distance of approximately 1.0 km. To minimise the impact of sampling on the population, we clipped a small amount of foot tissue to collect DNA samples (low: $n = 29$, middle: $n = 5$). We also used DNA samples of *E. q. quaesita* at Mt. Myojo ($n = 6$) and Mount Kurohime (hereafter Mt. Kurohime; $n = 19$), which is approximately 8 km from Mt. Myojo (Fig.1), to elucidate the population genetic structure. For morphological analysis within *E. murayamai*, most samples were empty dead shells to further minimise population impacts: low ($n = 22$) and middle ($n = 14$). The field surveys were conducted with the permission of the Ministry of the Environment, Itoigawa UNESCO Global Geopark and Itoigawa City.

2.2 | Genetic analyses

We employed ddRAD-seq to elucidate the population genetic structure and trace recent gene flow between *E. murayamai* and *E. quaesita*. Genomic DNA was isolated using either the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) or the phenol-chloroform method described by Sokolov (2000). The molecular experimental protocol followed Ito et al. (2023), and sequencing was conducted on the DNBSEQ-G400 platform (MGI, Shenzhen, China), generating 150 bp paired-end reads at BGI (Hong Kong, China). Demultiplexing and *de novo* assembly of raw reads were performed following the method outlined by Ito et al. (2023). We employed ipyrad within a Python3 environment (Eaton 2014; Eaton and Overcast 2020). After SNP calling with ipyrad, SNPs were filtered based on minor allele frequency (<0.05), Hardy-Weinberg equilibrium (P value <0.001) and linkage disequilibrium (window size = 10, step size = 5 and $r^2 < 0.3$) using VCFtools (Danecek et al. 2011) and PLINK (Purcell et al. 2007). One SNP per locus was randomly selected from the remaining SNPs.

To estimate the population structure, we used 2,869 SNPs based on a minimum

sample size threshold of 54/59 (91.5%). FastStructure was performed with a simple prior, and the optimal number of clusters (*K*) was determined following Raj et al. (2014). We assumed a maximum of 10 clusters in this analysis. A non-rooted phylogenetic network was constructed using SplitsTree4 with uncorrelated_P distances (Huson and Bryant 2006). Additionally, we conducted principal component analysis (PCA) using the R package "adegenet" (Jombart 2008; Jombart and Ahmed 2011; R Core Team 2025).

We calculated the f_3-statistics for *E. quaesita* on Mt. Myojo since their genetic composition included *E. murayamai* (see Results), suggesting a hybrid origin. Negative f_3-statistics values support hybridisation. Two parental populations were assumed: *E. quaesita* from Mt. Kurohime and *E. murayamai* from the low elevation site. Calculations used 10,296 SNPs with a minimum sample size of 38/54 (70.4%), and analyses were performed using the R package "ADMIXTOOLS 2" (R Core Team 2025; Maier et al. 2023).

Approximation Bayesian computation (ABC) analysis was conducted to estimate divergence time and effective population sizes between *E. murayamai* and *E. quaesita*. Seven divergence scenarios were considered (Fig. S1): one with no gene flow after divergence, three with bidirectional or unidirectional gene flows after divergence but none occurring recently, and three others with directions or unidirectional gene flows after divergence. For each scenario, 500,000 simulations were performed with non-informative prior distributions (Table S1). Plausible scenarios were selected based on the top 10,000 scenarios using the "mnlogistic" method. Pairwise F_{ST} (Excoffier et al. 1992), Tajima's D (Tajima 1989), Fu's F_S (Fu 1997), and pairwise differences π (Tajima 1983) were used as statistical genetic summaries. Using the most plausible scenario, the top 100 parameters were selected with the rejection algorithm. Simulations utilised fastsimcoal2 (Excoffier and Foll 2011; Excoffier et al. 2013) and arlsumstat (Excoffier and Lischer 2010) within the ABCtoolbox framework (Wegmann et al. 2010). Results from the simulations were summarised using the R package "abc" (Csilléry et al. 2012; R Core Team 2025). To increase the number of informative SNP sites and avoid overestimation, 8,990 SNPs, before filtering based on both one SNP per one locus and linkage disequilibrium, were used in the ABC analysis.

We estimated recent gene flow between *E. murayamai* and *E. quaesita* on Mt. Myojo using BA3-SNPs (Mussmann et al. 2019). We used 4,727 SNPs with a minimum sample size of 28/34 (82.4%). Parameters were auto-tuned according to Mussmann et al. (2019), and the delta values (-m, -a, and -f) were 0.2125, 0.5500, and 0.0500, respectively. Simulations

included 50,000,000 Markov chain Monte Carlo (MCMC) iterations, sampling every 5,000 iterations, and discarding 10,000,000 iterations as burn-in steps. The effective sample size was confirmed to exceed 150 for all parameters using Tracer (Rambaut et al. 2018). Moreover, the significance of pairwise F_{ST} between all populations was assessed using 100 bootstraps in the R package "dartR" (Mijangos et al. 2022; R Core Team 2025).

2.3 | Morphological analysis

To investigate morphological differences between low- and middle-elevation populations of *E. murayamai*, we compared shell width and spire index, which is an index of flatness (Cain 1977). We measured shell width and height using a digital calliper (Mitutoyo Corp., Kanagawa, Japan). The spire index was calculated as the ratio of shell width to shell height. Differences in shell width and spire index were analysed using Student's *t*-test because all variables followed a normal distribution (Shapiro-Wilk test; $P = 0.70$ [Shell width, low], $P = 0.68$ [Shell width, middle], $P = 0.88$ [Spire Index, low], $P = 0.34$ [Spire Index, middle]) and the variance of both variables did not differ between elevations (F test; $P = 0.20$ [Shell width], $P = 0.22$ [Spire Index]). Statistical analyses were conducted using R software (R Core Team 2025).

3 | RESULTS

The ddRAD libraries yielded an average of 2,734,120 raw reads per sample. From *de novo* assembly, an average of 144,699 clustered loci were obtained with a mean depth of 18.1x. In total, 159,308 consensus loci were identified, with each sample possessing an average of 55,082 loci. Details of the sequencing and *de novo* assembly are provided in Table S2.

Population genetic structure analyses revealed clear genetic differentiation between *E. murayamai* and *E. quaesita* (Fig. 2a). Within *E. murayamai*, genetic structure varied between elevations (Fig. 2b). SplitsTree4 (Fig.2c) and PCA (Fig.2d–f) further confirmed the genetic distinctiveness of *E. murayamai* from *E. quaesita* and demonstrated genetic differences among elevations within *E. murayamai*. Although genetic components of *E. murayamai* were detected in *E. quaesita* from Mt. Myojo, f_3-statistic was significantly positive (0.023 ± 0.001; $Z = 20.27$; $P < 0.001$), indicating no admixture between the two species.

ABC analysis selected the no gene flow scenario with 99.98% probability (Fig. 3a;

Tables S3 and S4). Divergence between *E. murayamai* and *E. quaesita* was estimated at 85,480 generations ago [95% Bayesian confidence interval (BCI): 12,213–397,933] (Fig. 3a; Table S5). Divergence within *E. murayamai* occurred 35,205 generations ago [95% BCI: 7,366–220,146]. Divergence between *E. quaesita* at Mt. Kurohime and Mt. Myojo occurred 14,984 generations ago [95% BCI: 5,295–140,636]. ABC analysis strongly supported a population size increase at the divergence from the common ancestor of all nodes to the ancestor of *E. quaesita*, with 100% probability (Fig. 3b; Table S6). Similarly, population size increases were estimated at the divergence from the ancestor of *E. murayamai* to its current populations at low and middle elevations, with probabilities of 94% and 92%, respectively, although these values were slightly below the 95% threshold (Fig. 3b; Table S6). No significant changes in population size were detected at other divergence points (Fig. 3b; Table S6). Regarding current population sizes, *E. quaesita* at Mt. Kurohime exhibited a larger effective population size compared to *E. quaesita* at Mt. Myojo, *E. murayamai* at low elevation and *E. murayamai* at middle elevation. The probabilities for these comparisons were 92%, 93%, and 89%, respectively, although these values were slightly below the 95% threshold (Fig. 3b; Table S7). For other comparisons between current populations, no significant differences in effective population sizes were detected (Fig. 3b; Table S7).

BA3-SNPs analysis detected no recent gene flow between any population pairs (Table S8). Pairwise F_{ST} values were significantly greater than zero for all comparisons (Table 1). F_{ST} between *E. quaesita* and *E. murayamai* was 0.272–0.315. F_{ST} between *E. murayamai* at low and middle elevations was 0.226. F_{ST} between *E. quaesita* at Mt. Myojo and Mt. Kurohime was 0.103.

Morphological analyses revealed significant differences in shell width and spire index between low- and middle-elevation populations of *E. murayamai* (Fig. 4; shell width $P < 0.001$ and Spire Index $P < 0.05$). In other words, in the low-elevation population, shell width was smaller, and shell morphology was more flattened than in the middle-elevation population.

4 | DISCUSSION

Our study demonstrated that *E. murayamai* is genetically distinct from *E. quaesita*, contradicting previous studies that grouped the two species within a single clade based on a mitochondrial locus (Ueshima and Asami 2003; Davison et al. 2005). Phylogenetic analyses

using a single mitochondrial locus may yield misleading results due to introgressive hybridisation and incomplete lineage sorting (Harrison 1991; Funk and Omland 2003; Maddison and Knowles 2006). By using genome-wide SNPs, we confirmed the absence of gene flow and hybridisation between *E. murayamai* and *E. quaesita*. It is thus plausible that incomplete lineage sorting occurred and that these two species are reproductively isolated. These results support the perspective that *E. murayamai* is an endemic species on Mt. Myojo, according to the distinctness of its shell and genital morphologies (Habe 1976; Azuma 1978; MolluscaBase 2025).

The genetic components revealed by fastStructure, SplitsTree4 and PCA differed between elevations within *E. murayamai*. The magnitude of genetic differentiation (F_{ST}) between elevations was also greater than the differentiation observed among *E. quaesita* populations from different mountains. Previous studies on land snails reported that interspecific F_{ST} values were typically around or below 0.2 (Chueca et al. 2021; Oswald et al. 2022), whereas intraspecific comparisons sometimes yield F_{ST} values above 0.2 within the same species (Ramos-Gonzalez et al. 2022; Gretgrix et al. 2023), suggesting that the genetic differentiation between elevations in our study is relatively large for intraspecific variation. Morphological differences aligned with these genetic patterns: snails at middle elevations had larger and less flattened shells than those at low elevations. In land snails, elevation often plays a key role in shaping distribution patterns (Liew et al. 2010; Kagawa et al. 2024), with environmental factors such as temperature and humidity contributing to morphological variation along elevational gradients (Goodfriend 1986). In other words, an environmental gradient along elevation sometimes drives phenotypic change through differential selection pressures (Jones 1973). Similarly, the morphological divergence observed in this study may be a result of selection pressures associated with environmental changes along elevation gradients. These findings suggest that elevation acts as a geographical barrier. At Mt. Myojo, the elevational difference between low and middle populations is approximately 370 m, with a horizontal distance of about 1.0 km. Our preliminary mark-recapture survey showed that individuals moved only a few meters over the course of a year (Ito 2024). This limited mobility likely reinforces genetic isolation between elevations. Consistent with these ecological insights, neither gene flow after divergence nor recent gene flow was detected between elevations. These results suggest that divergence along elevation gradients, even at a small geographical scale, can occur in the same way as horizontal geographic isolation, which is common in land snail divergence.

Ongoing monitoring revealed that the number of *E. murayamai* individuals differed markedly between elevations (Ito 2024). Snails have rarely been found at middle elevations in recent years, whereas they remain abundant at low elevations. Taken together, these genetic and morphological analyses, combined with ecological monitoring, support the need for elevation-specific conservation strategies to preserve the distinct populations of *E. murayamai*. In addition, *E. murayamai* was described with its type locality at the summit of Mt. Myojo (Habe 1976). From our results, the population of the summit may be distinct from populations at other elevations. However, our recent field surveys did not find any live individuals, but only found two dead shells. Murayama (2023) stated that the morphology of the summit population was larger than that of other populations, highlighting that the necessity of further comprehensive surveys at the summit for future conservation.

ABC analysis revealed that the divergence between *E. murayamai* and *E. quaesita* occurred approximately 85,000 generations ago. Subsequently, divergence within *E. murayamai* and *E. quaesita* was estimated to have occurred 35,000 and 15,000 generations ago, respectively. Murayama (2022) clarified that *E. murayamai* reached maturity within one year. Based on this, the estimated divergence times can be converted to approximately 85,000, 35,000 and 15,000 years ago. These periods coincide with the Würm glacial period (Yasuda 1987). During this period, the early glacial phase (approximately 70,000–120,000 years ago) was characterised by decreasing temperatures. Around 35,000 years ago, a transition from interstadial to stadial occurred. Approximately 20,000 years ago, temperatures dropped, snowfall decreased, and then temperatures rose again (Ono 1984; Yasuda 1987). During the divergence from the most recent common ancestor to the common ancestor of *E. quaesita* (approximately 85,000 years ago) and the common ancestor of *E. murayamai* to its current populations (approximately 35,000 years ago), a significant increase in effective population size was also estimated. These increases align with the onset of glacial periods, suggesting that climatic changes may have contributed to the divergence of *E. murayamai* and *E. quaesita*.

However, what contributed to the speciation process between *E. murayamai* and *E. quaesita* remains unclear, partly because the precise timing of Mt. Myojo's formation is uncertain. The distribution of *E. murayamai* is restricted to the large-scale limestone cliffs at Mt. Myojo. It is plausible that ecological factors related to the limestone played a role in the speciation process. We hypothesise that ancestral populations, possibly lacking the characteristic flattened shell, colonised the limestone outcrops of Mt. Myojo. The selective

pressures in such habitats may have favoured a flattened shell morphology. Indeed, land snails inhabiting limestone areas often exhibit flatter morphologies, as selection for flatter shells benefits populations that exploit narrow spaces within or beneath limestone rocks (Goodfriend 1986). Given the current uncertainties in the geological history of Mt. Myojo, this scenario remains speculative and warrants further investigation.

In addition to these ecological insights, it is important to consider uncertainties in demographic parameters. For example, caution is warranted when assuming a generation time of one year, as other species in the same genus require 2.5 years to reach maturity (Ito et al. 2023), and the one-year estimate is based on observations from an outdoor rearing environment (Murayama 2022). Assuming a generation time of 2.5 years, the divergence time would be approximately 213,000, 88,000 and 38,000 years ago. Even under this assumption, the divergence of *E. murayamai* occurred within the last million years. Previous studies on Camaenidae snails in East Asia indicated that species divergence typically occurs on a million-year timescale (Hirano et al. 2019a; Hwang et al. 2021; Ito et al. 2023). Hence, the divergence of *E. murayamai* may be relatively rapid, although the reasons remain enigmatic.

ABC analysis also revealed differences in effective population size among the current populations. The effective population sizes of snails at Mt. Myojo, including *E. murayamai* and *E. quaesita*, were lower than those of *E. quaesita* at Mt. Kurohime, which had an estimated size of 2.2 million. Within Mt. Myojo, the effective population sizes of *E. murayamai* populations at low and middle elevations and *E. quaesita* were similar, with median values of approximately 100,000. In comparison, non-endangered snails of the same genus, *E. peliomphala simodae*, have estimated effective population sizes ranging from 1.7 to 4.4 million on various islands (Ito et al. 2023). Other non-endangered Camaenidae snails, *Bradybaena similaris* and *B. pellucida*, have estimated effective population sizes of 1.4–8.9 million on the Japanese mainland (Hirano et al. 2023). These comparisons suggest that the effective population size of *E. murayamai* is relatively small. However, given the scarcity of studies estimating effective population sizes in such small areas, further investigations into the effective population sizes of related snails—regardless of their conservation status—are needed to evaluate the population status of *E. murayamai*.

In conclusion, genome-wide SNPs analysis proved to be a powerful tool for informing conservation strategies for *E. murayamai*. Additionally, morphological data can complement genetic analyses in conservation planning, as highlighted in previous studies (Funk et al.

2012; Coates et al. 2018). The first implication is that ongoing conservation measures for *E. murayamai*, such as legal protection and periodic monitoring, should be continued, given its morphological and genetic distinctiveness. Second, populations at different elevations should be treated as separate conservation units, as *E. murayamai* exhibits distinct genetic structures and morphological traits. The *E. murayamai* populations, which have been genetically isolated for 35,000 years with no recent gene flow between elevations, warrant separate conservation efforts. In other words, conservation strategies should account for both species-level classification and evolutionary history. Taken together, this study clarifies not only taxonomic uncertainties but also the importance of defining proper conservation units within a species, underscoring the utility of genome-wide SNP analyses.

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STATEMENTS & DECLARATIONS

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Competing interests

The authors declare no conflicts of interest.

Author contributions

S.I., O.K. and D.Y. conceived the study. I.Y. obtained the necessary permissions. S.I., O.K., I.Y., T.H. and D.Y. conducted the field sampling. S.C. established the molecular experimental environment. S.I. conducted molecular experiments and data analyses, and wrote the initial draft. All authors reviewed, edited and provided comments on the drafts and approved the final version for publication.

Data availability

Demultiplexed fastq files are available from NCBI (DRA########; Table S2). SNP and morphological data are deposited in Dryad (https://doi.org/#######/dryad.#####). (We will deposit these data to NCBI and Dryad after acceptance.)

Figure 1

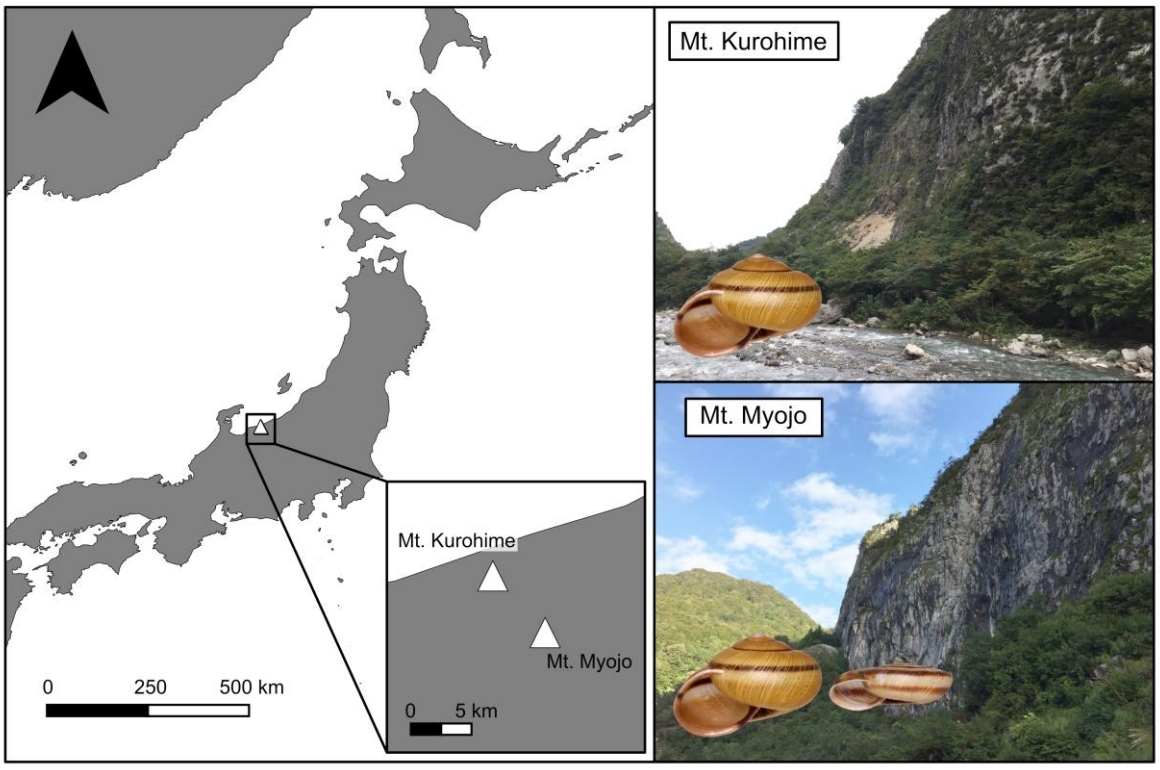


Figure 1. Maps and landscapes of the study sites: Mt. Myojo and Mt. Kurohime. Images of individual shells in each landscape image indicate whether the species inhabits the location. *Euhadra quaesita*, which has a globular shell morphology, inhabits both mountains; in contrast, *E. murayamai*, which has an extremely flattened shell morphology, inhabits only Mt. Myojo.

Figure 2

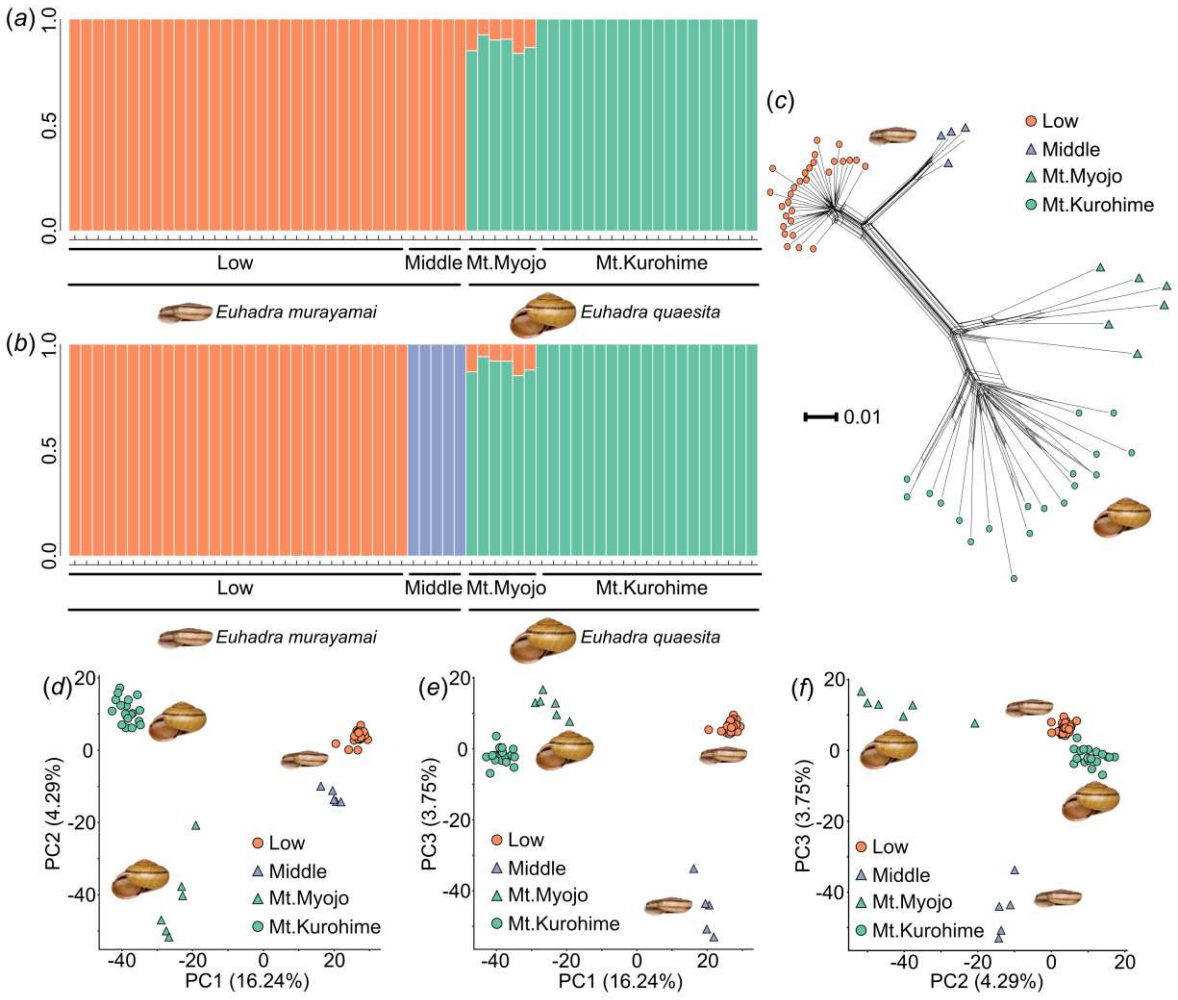
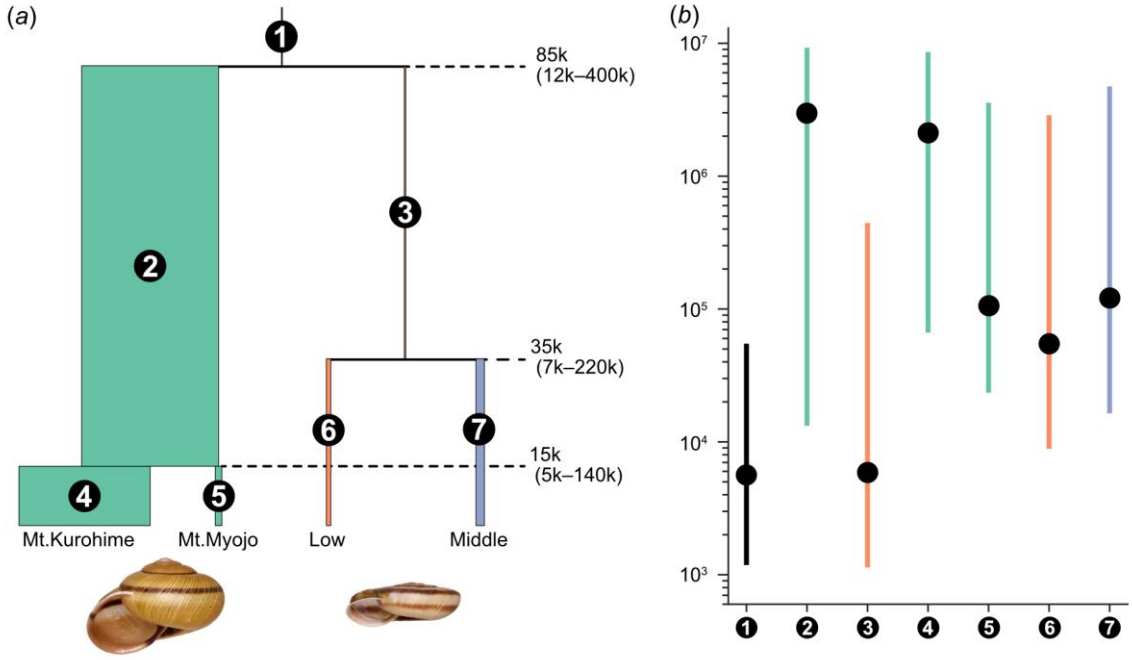


Figure 2. Results of population genetic analyses. (*a*) Population structure estimated from fastStructure analysis assuming $K = 2$. (*b*) fastStructure result assuming $K = 3$. (*c*) Phylogenetic network from Splitstree4. (*d, e, f*) Population genetic relationship estimated from principal component analysis (PCA).

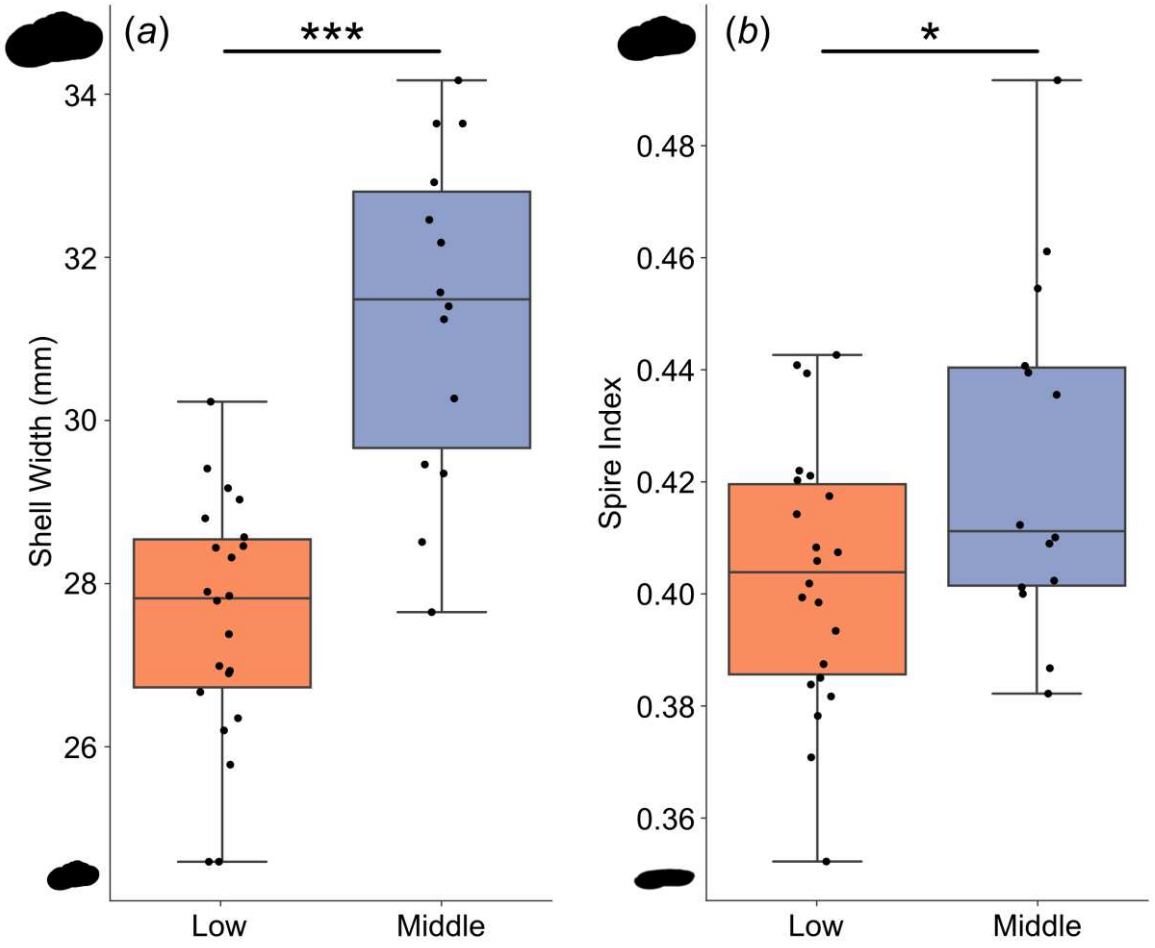
Figure 3



598

599 **Figure 3**. (*a*) The most plausible scenario based on Approximation Bayesian computation
600 (ABC) analysis. The width of each branch represents the median effective population
601 size. The number on each branch represents the branch number. (*b*) Effective
602 population sizes estimated from ABC analysis. Numbers on the *x*-axis indicate the
603 branch numbers in panel (*a*).

Figure 4



604

605 **Figure 4**. Boxplots depicting (*a*) shell width (mm) and (*b*) spire index of *Euhadra murayamai*
606 at middle- and low-elevation sites. "***" and "*" indicate that the *P* value was lower
607 than 0.001 and 0.05, respectively.

608 **Table 1**. Pairwise F_{ST} values between populations of *Euhadra murayamai* at different
609 elevations and *E. quaesita* at Mt. Myojo at Mt. Kurohime.

610

611

Figure S1. Illustration of the seven scenarios and parameters for Approximation Bayesian computation (ABC) analysis.

Table S1. Prior distribution for the simulation in Approximation Bayesian computation (ABC) analysis. N_{KQ} (④), N_{MQ} (⑤), N_{Low} (⑥), N_{Middle} (⑦), N_{ancQ} (②), N_{ancM} (③), and N_{anc} (①) indicate the effective population sizes of the *Euhadra quaesita* at Mt. Kurohime, *E. quaesita* at Mt. Myojo, *E. murayamai* at low elevation, *E. murayamai* at middle elevation, the ancestor of *E. quaesita*, the ancestor of *E. murayamai*, and the common ancestor of all, respectively. *G1*, *G2*, *G3* and *G4* indicate the divergence times at each node. *MIG1* and *MIG2* represent the migration rate between branches of *E. quaesita* at Mt. Kurohime and Mt. Myojo. *MIG3* and *MIG4* represent the migration rate between branches of *E. quaesita* at Mt. Myojo and *E. murayamai* at low elevation. *MAF* is the minor allele frequency. Details of the parameters are shown in Figures 1 and S1.

Table S2. Summary of sequencing and *de novo* assembly.

Table S3. Posterior model probabilities in Approximation Bayesian computation (ABC) analysis.

Table S4. Bayes factors in Approximation Bayesian computation (ABC) analysis. These represents the ratios of model probabilities in the rows to those in the columns. Models are shown in Figure S1.

Table S5. Posterior distribution of the parameters of Model 1, which is the most plausible model.

Table S6. Posterior distribution of the parameters for the rate of change in effective population size in Model 1, which is the most plausible model.

Table S7. Posterior distribution of the difference in effective population size between current populations. The differences represent the population size of the column minus that of row. Number outside brackets, numbers in round brackets and numbers in square brackets indicate the median, the probability of being greater than 0.0, and the 95% Bayesian confidence interval, respectively.

Table S8. Result of the estimation of recent gene flow using BA3-SNPs. Bold values indicate statistical significance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Appendixtablesfigs.docx