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Role of ancient lakes in genetic and phenotypic diversification of freshwater snails

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Abstract

Endemic organisms of ancient lakes have been studied as models to understand processes of speciation and adaptive radiation. However, it remains unclear how ancient lakes play roles in genetic and phenotypic diversity of freshwater mollusks. In the present study, we focus on viviparid freshwater snails in the ancient lakes of East and Southeast Asia (Japan and China) to address this question. Using molecular phylogenetic analyses based on mitochondrial (COI, 16S) and nuclear genes (18S, 28S, H3), we show that patterns of species diversification in viviparid lineages. Colonization to ancient lakes occurred independently in China and Japan at least four times, with subsequent diversification into more than two species within each lake group. Morphological analyses of fossil related viviparids suggest parallel phenotypic evolution occurred in the different lakes and ages. Each lake contained a single lineage which was phenotypically diversified relative to those from other sites. Using genome-wide SNPs obtained by MIG-seq, we also examined the genetic structure of three Japanese viviparids, including two endemic species of ancient Lake Biwa. The results suggest that these two species diversified from the population of the third species living in wetlands surrounding the lake. These findings suggest that rapid diversification of lineages and phenotypic divergence can occur in ancient lakes compared to other habitats. Formation of large lakes probably promotes speciation and phenotypic divergence as a result of adaptation into different microhabitats. High numbers of ancient lakes could be a driver of species diversity in Asian viviparid snails.

KEYWORDS
phenotype, phylogenomics, shell shape, Viviparidae

1 | INTRODUCTION

Topographical events play an important role in the process of species diversification (Blanco-Pastor, Fernández-Mazuecos, Coello, Pastor, & Vargas, 2019; Merckx et al., 2015). The establishment of closed systems such as oceanic islands provides opportunities for genetic diversification through geographical isolation and ecological diversification due to the creation of new niches. These systems have displayed speciation in association with ecological divergence and adaptation (Gavrilets & Losos, 2009; Rundle & Schluter, 2004; Schluter, 2000) and even repeatable adaptive radiation (Chiba, 1999).

In addition to oceanic islands, ancient lakes often provide excellent case studies addressing processes of speciation and phenotypic diversity because they have high species endemism and species diversity (Cristescu, Adamowicz, Vaillant, & Haffner, 2010). In this study, we define an ancient lake as one that has existed for at least one glacial cycle (i.e., present at, or before, 130 ka BP; Albrecht & Wilke, 2008; Cheng et al., 2009; Hampton et al., 2018; Lisiecki & Raymo, 2005). Various evolutionary phenomena such as adaptive radiation, parallel evolution, and hybrid speciation have been documented in some ancient lakes (e.g., Seehausen, 2006; Sturmbauer & Meyer, 1992). In these circumstances, mollusks are an excellent model to compare the relationships among morphology, phylogeny, and geographical distribution patterns (Glaubrecht, 2008; Miura, Urabe, Nishimura, Nakai, & Chiba, 2019; von Rintelen, Wilson, Meyer, & Glaubrecht, 2004; Van Bocxlaer, 2017; Van Bocxlaer & Hunt, 2013). Because mollusks are less mobile they are more likely to become geographically isolated (Miura et al., 2019). In particular, shell-bearing mollusks are an ideal model to investigate morphological evolution because of their high preservative quality of shell as a fossil (Matsuoka & Miura, 2018; Miura et al., 2019). These characteristics enable the information on both recent and past species histories to be effectively accessed (Miura et al., 2019). However, it remains unclear how ancient lakes play roles in genetic and phenotypic diversity of freshwater mollusks.

There are some ancient lakes in East and Southeast Asia that are hotspots of species diversity (Miura et al., 2019; Strong, Gargominy, Ponder, & Bouchet, 2008; Zhang, Chen, Yang, Jin, & Köhler, 2015). For example, Yunnan Province (Southwest China) has multiple ancient lake groups (e.g., Lakes Dianchi, Fuxian, Xingyun, and Qilu). Lake Dianchi was formed about 3.2 Ma (Jin, Wang, & He, 2006). Lakes Fuxian, Xingyun and Qilu are the remains of a large palaeolake (the Great Fuxian Palaeolake), which formed about 3.4 Ma (Wang & Dou, 1998). These lakes harbour a unique fauna of at least 241 fish and 124 gastropod species, most of which are endemic to the region (Chu & Chen, 1990; Zhang et al., 2015, 1997). In Japan, ancient Lake Biwa also harbours rich fauna and flora (>1,000 species/subspecies), including ~60 endemic species/subspecies (Nishino, 2003; Nishino & Hamabata, 2005). Among such freshwater organisms, snails show the highest species diversity (probably 20 species/subspecies; Strong et al., 2008). Lake Biwa is the oldest and largest lake in Japan formed approximately 4 Ma (Kawabe, 1989, 1994; Yokoyama, 1984). According to Tabata, Kakioka, Tominaga, Komiya, and Watanabe (2016), Paleo-Lake Biwa consisted of a series of lakes and marsh environments that existed from 4 to 0.4 Ma. After the Pliocene, the topographical and limnological features of Paleo-Lake Biwa and Lake Tokai, located east of Paleo-Lake Biwa successively changed: the paleo-lake experienced: (a) Shallow and large stages (Lake Ohyamada >3.2 Ma; Lake Ayama 3.0–2.7 Ma); (b) A deep and large stage (Lake Koka 2.7–2.5 Ma), similar to the northern basin of the present Lake Biwa; (c) A second shallow and large stage (Lake Gamo 2.5–1.8 Ma); (d) A swamp-like stage (1.8–1.0 Ma); and (e) A final shallow and large stage (Lake Katata 1.0–0.5 Ma) (Kawabe, 1989, 1994). Therefore, the present Lake Biwa is relatively newer than the other ancient lakes in East and Southeast Asia. Although there is an even newer lake, the recent lake expansion triggered the adaptive radiation of freshwater snails (the subgenus *Biwamelania*, Semisulcospiridae) in Lake Biwa (Miura et al., 2019).

In the present study, we focused on the freshwater snail family Viviparidae (Gray, 1847), which is endemic to all continents, except Antarctica and South America (Brown, 1994; Van Bocxlaer, Strong, Richter, Stelbrink, & von Rintelen, 2018); also absent from New Zealand and Madagascar and includes ~150 species and 31 genera which are considered valid (Strong et al., 2008). Many endemic species of Viviparidae are diversified in ancient lakes (e.g., Africa: Schultheiß, Van Bocxlaer, Riedel, von Rintelen, & Albrecht, 2014; Sengupta, Kristensen, Madsen, & Jørgensen, 2009; Southwest China: Du, Yang, von Rintelen, Chen, & David, 2013; Zhang et al., 2015; Japan: Hirano, Saito, & Chiba, 2015). In the case of Asian viviparid snails, Du et al. (2013) reported that the genus *Margarya*, endemic to the ancient lake groups in Yunnan Province, was polyphyletic. These authors also showed parallel morphological evolution in terms of shell ornamentation and thickness. Furthermore, this study documented complex patterns of genetic structuring of *Margarya*. Zhang et al. (2015) performed phylogenetic analyses of *Margarya* and the genus was divided into different three genera: *Anularya*, *Margarya*, and *Tchangmargarya*. In addition, Zhang et al. (2015) pointed out that endemic species of Yunnan ancient lake groups had a relatively larger juvenile size and fewer offspring (K-strategy), whereas closely related and widely distributed species adopted smaller juvenile and a larger number of offspring (r-strategy). Hirano et al. (2019) clarified the divergence times of several Asian viviparid species including *Margarya*, but the relationship between divergence times of these Chinese species and geological history of such ancient lakes has not been studied.

In Japan, modern viviparid fauna comprises five species in three genera (Hirano et al., 2015, 2019). One of these, *Cipangopaludina japonica* (Martens, 1861) is widely distributed in mainland Japan freshwater ecosystems, including ponds, gentle streams, and lake habitats. This species has also been recorded in South Korea (Min, Lee, Koh, & Je, 2004). By contrast, *Heterogen longispira* (Smith, 1886), known as an endemic species of Lake Biwa, can be found in approximately 5–15 m depth of this lake. Apart from five viviparid species, an undescribed species was also recently discovered in the northern part of the Lake Biwa (K. Nakai, unpublished data). *C. japonica* and

H. longispira are mud-burrowing species but this undescribed species is found only on the 7 m down of rocky shore of Chikubujima Island in Lake Biwa. These three species have different shell morphologies from each other, in terms of shell shape and ornamentation on the surface of the top of the shell. Certain fossil records have been recorded in *C. japonica* from Sakawa Clays of Katata Formation: 0.5 Ma (Matsuoka & Nakamura, 1981), in *H. longispira* from Nijigaoka Clays of Katata Formation: 1.2 Ma, Hiraen Clays of Katata Formation: 0.8 Ma, Kisen Clays of Katata Formation: 0.7 Ma, Sakawa Clays of Katata Formation (Matsuoka, 1986; Matsuoka & Nakamura, 1981).

In viviparid snails there are some taxonomic issues. For example, the type species of *Cipangopaludina*, Hannibal, 1912 is *C. malleata* (Reeve, 1863) described as from Japan, but the detailed type locality of *C. malleata* was not identified in the description (Hirano et al., 2019). Habe (1976), Pilsbry (1902), and Ponder, Hallan, Shea, and Clark (2016) suggested that this species is synonymous with *C. laeta* (Martens, 1861) or *C. chinensis* (Gray, 1834). Previous phylogenetic work using mtDNA found that *H. longispira* was nested within a clade of *C. japonica* (Hirano et al., 2015). Further taxonomic study is needed, but hereafter we use *Heterogen japonica* instead of *C. japonica* in this study. For convenience, we also refer to the undescribed species of Lake Biwa (K. Nakai, unpublished data) as *Heterogen* sp. However, the phylogenetic position of *Heterogen* sp. from Lake Biwa is still unclear.

Under these circumstances, the inference of species trees from multilocus data can be beneficial (Kubatko, 2009; Yu, Than, Degnan, & Nakhleh, 2011) in resolving phylogenetic and taxonomic problems. Molecular phylogenomics using a multilocus approach provides more detailed information than a single or a few genes (Razkin et al., 2016; Rivers, Darwell, & Althoff, 2016; Rubinoff & Holland, 2005; Takahashi & Moreno, 2015). Although prior studies focusing on phylogenetic relationships among viviparid species were performed using only a few genes (Du et al., 2013; Gu et al., 2019; Hirano et al., 2015; Schultheiß et al., 2014; Sengupta et al., 2009; Van Bocxlaer et al., 2018; Zhang et al., 2015) or mtDNA genome (Wang, Zhang, Jakovlić, & Wang, 2017), our previous study indicated that genome-wide single nucleotide polymorphism (SNP) was more useful for clarifying the relationships and population demographic history between viviparid species (Hirano et al., 2019).

In the present study, we investigated the molecular phylogeny and divergence time among viviparid species, mainly focusing on species of ancient Japanese and Chinese lakes, to address the question of how ancient lakes play roles in genetic and phenotypic diversity of viviparid snails. We estimated phylogenetic relationships of Asian viviparid species by sampling from wider areas and tested the hypothesis that the formation of the ancient lakes has promoted divergence of viviparid snails. Next, we examined genetic structure of three Japanese viviparid species (*H. japonica*, *H. longispira*, and *Heterogen* sp.) using population genetic analysis with genome-wide SNPs. In addition, we investigated how and when divergence of genetic and phenotypic traits occurred using the above three species of extant populations and fossils. In this study we aimed to elucidate the role of ancient lake in species diversification through understanding the evolutionary history of viviparid snails.

2 | MATERIALS AND METHODS

2.1 | Samples

Sampling was performed in the Japanese Archipelago, Vietnam, Russia, Poland, and the USA. In total, we collected 215 individuals comprising 22 viviparid species/subspecies (Table S1). In particular,

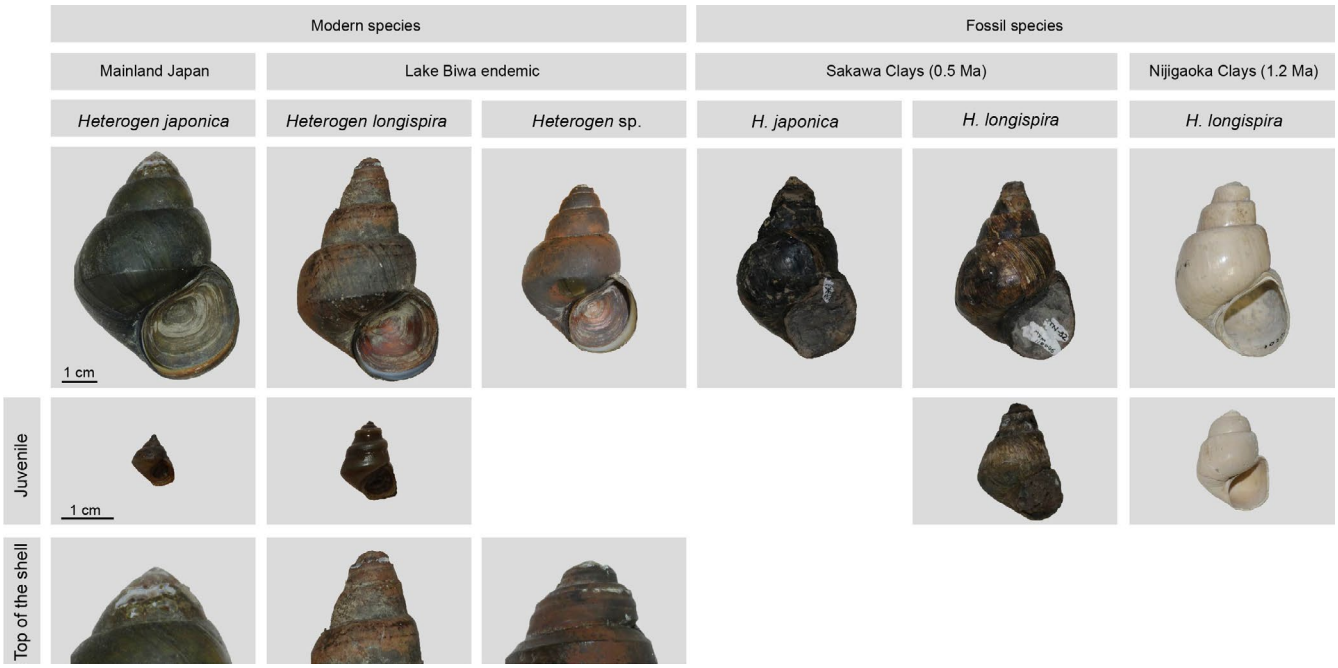

FIGURE 1 Adult and juvenile shell morphology of extant and fossil Japanese three viviparid species

we collected 151 individuals of *H. japonica*, 21 *H. longispira*, and six *Heterogen* sp. in the Japanese Archipelago (Table S1; Figures 1 and S1). A fragment of the foot muscle from each individual was stored in 99.5% ethanol prior to DNA extraction. In addition, sequences for 27 individuals of 25 viviparid species/subspecies were retrieved from GenBank (Table S1). For morphometric analysis, we also used 20 individuals of fossil specimens comprising four species deposited in the Toyohashi Museum of Natural History (Toyohashi, Aichi, Japan), as well as extant populations (Table S1; Figure 1: see Section 2.6).

2.2 | Phylogenetic analyses

Total DNA was extracted in accordance with the study by Hirano et al. (2015). To estimate the phylogenetic positions of Asian Viviparidae, we sequenced fragments of the mitochondrial cytochrome oxidase subunit I (COI), 16S rRNA, nuclear 28S rRNA, 18S rRNA, and Histone 3 (H3) genes. The polymerase chain reaction (PCR) primers are listed in Table S2. PCR products were purified using Exo-SAP-IT (Amersham Biosciences). Sequencing was performed using PCR primers and BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) followed by electrophoresis using an ABI 3130xl sequencer (Applied Biosystems). The newly generated sequences were deposited in the DDBJ, EMBL, and GenBank databases (Table S1).

Alignment of the COI and H3 sequences was straightforward and required no gaps. The 16S, 18S, and 28S sequences were aligned using MUSCLE version 3.8 (Edgar, 2004). GBLOCKS version 0.91b (Castresana, 2000) was used to select regions in the aligned sequences that were confidently aligned for analysis (Table S3). Phylogenetic analyses were conducted on the all combined data sets for all species and mtDNA for three Japanese species (*H. japonica*, *H. longispira*, and *Heterogen* sp.) using maximum likelihood (ML), and Bayesian methods. For ML and Bayesian analyses, Kakusan4-4.0.2011.05.28 (Tanabe, 2011) was used to select the appropriate models for sequence evolution (Table S4). Using the selected models, we performed ML analysis in RAXML HPC2 (Stamatakis, 2006). Nodal support for ML analysis was assessed using bootstrap analyses with 1,000 replications. Bayesian analysis was performed in MRBAYES version 3.1.2 (Ronquist & Huelsenbeck, 2003) using two simultaneous runs, consisting of four simultaneous chains for 30 million generations and sampling the trees every 1,000 generations. The first 20% of trees of each run were discarded as burnin. Both ML and Bayesian analyses were performed at the San Diego Supercomputer Center through the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010). One species of *Marisa* and two species of *Pomacea* were used as outgroups for phylogenetic analyses of all species and the below estimation of divergence time (Sengupta et al., 2009). For mtDNA phylogeny of Japanese three species, we used two species of *Ussuripaludina* as outgroups (see Section 3.1).

2.3 | Divergence time estimation

Approximate divergence times were estimated using the uncorrelated log-normal relaxed clock as implemented in BEAST 2.4.8 (Bouckaert et al., 2014) at the San Diego Supercomputer Center through the CIPRES Science Gateway (Miller et al., 2010). We used the default parameters except for step number (100 steps). Substitution models of each partition were set as follows: COI = HKY + Γ, 16S = HKY + Γ, 18S = JC69 + Γ, 28S = HKY + Γ, H3 = HKY + Γ. These models were decided upon using Kakusan4-4.0.2011.05.28 (Tanabe, 2011). In addition, the model of COI was chosen from models in which the molecular clock rate based on some fossil records of viviparid snails was considered in Schultheiß et al. (2014). A molecular clock rate (uniform prior) ranging from 0.0068 to 0.0118 (substitutions per site and My) was proposed for the COI gene of the family Viviparidae in Schultheiß et al. (2014). We set the parameters of the site models to Gamma category count = 4 and shape = 1.0 as described by Heath (2015). The Yule process was used to model speciation. The Monte Carlo Markov chain was run four times for 30 million generations with sampling every 1,000 generations to ensure that the effective sample size (ESS) values were above 1,000 for all parameters. MCC files were annotated in TREEANNOTATOR version 2.4.4 (BEAST package; burnin = 20%) to summarize the entire posterior distribution after log and tree files were checked by TRACER version 1.6 (Rambaut, Drummond, & Suchard, 2013).

2.4 | MIG-seq analysis and SNP detection

To obtain genome-wide SNP information of these species groups from total DNA, we conducted MIG-seq method (Suyama & Matsuki, 2015). MIG-seq is a type of reduced representation sequencing such as restriction site-associated DNA sequencing, and especially effective for low-quality DNA and small quantities of DNA (Tamaki, Yoichi, Matsuki, Suyama, & Mizuno, 2017), which was performed for viviparid snails in previous research (Hirano et al., 2019). Preparation of the MIG-seq library was executed under a method modified from Suyama and Matsuki (2015) using an Illumina MiSeq Sequencer (Illumina), and an MiSeq Reagent Kit version 3 (150 cycle; Illumina) (Hirano et al., 2019; Y. Tsunamoto et al., unpublished data).

SNPs selection were performed in accordance with the work of Suyama and Matsuki (2015). We used STACKS software package version 1.41 (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011) with the following parameters under a gapped option: maximum distance between stacks (*M*) = 1, maximum distance allowed to align secondary reads to primary stacks (*N*) = 1, and minimum depth option = 10 (-m 10). We selected only SNPs recorded at a rate of more than 50% among samples for MIG-seq, excluded individuals with a high deficiency rate (≥50%) from the analysis. As a result, 445 SNPs among all populations were detected.

2.5 | MIG-seq population genetic analyses

To compare the results of mtDNA with the genetic structure of nDNA among three Japanese species, we estimated individual genotypes of nDNA with STRUCTURE version 2.3.4 (Evanno, Regnaut, & Goudet, 2005; Falush, Stephens, & Pritchard, 2003), based on the MIG-seq data set (Table S1). The number of preassigned genetic

TABLE 1 Information on ABC analyses

	Pop1	Pop2	Pop3	Individual number	SNPs number	Scenario number
First ABC	1–8	20, 21	24, 26–28	51	198	6
Second ABC	1, 2	4	5	27	246	6
Third ABC	1	2	–	13	246	2

Note: Each scenario is shown in Figure 2. Number indicated in each Pop corresponds with site number.

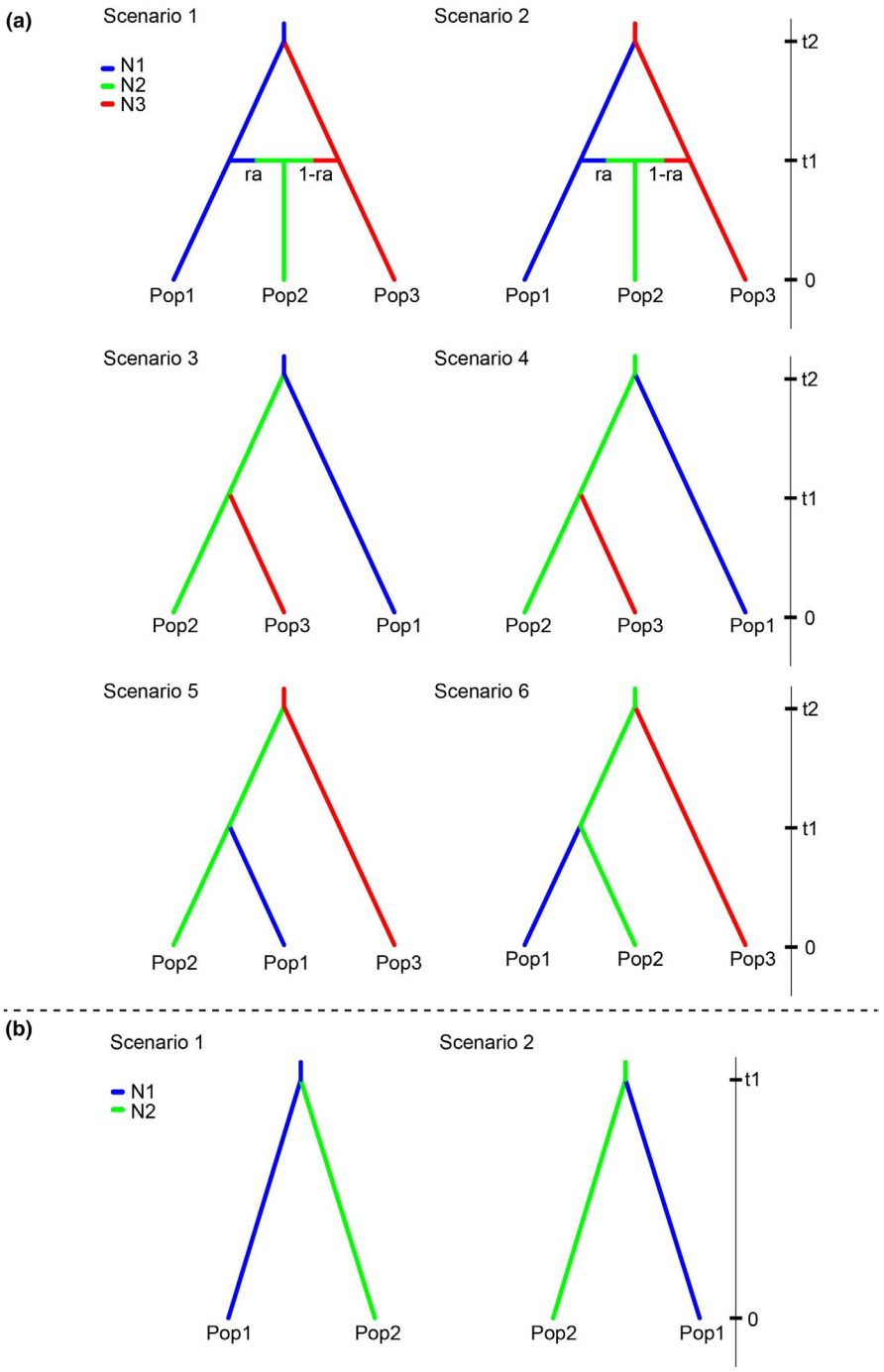

FIGURE 2 Simulated eight scenarios. N1, N2 and N3 are population size. t1 and t2 is generation time for merging of population. Information of each Pop is indicated in Table 1. (a) The first and second analyses. (b) The third analysis

clusters (*K*) was assumed to range from one to five. We performed 10 independent runs for each *K* value. Each run included 10,000 burnin iterations and 10,000 iterations. To help determine the optimal *K*, Δ*K* was calculated as described by Evanno et al. (2005) using STRUCTURE HARVESTER web version 0.6.94 (Earl & von Holdt, 2012). Bar charts for the proportions of the membership coefficient of each individual in STRUCTURE analysis in 10 runs for each *K* were summarised using CLUMPP version 1.1.2 (Jakobsson & Rosenberg, 2007) and visualised

TABLE 2 Information on summary statistics of ABC analyses

	Summary statistics
First ABC	Fst distances: Variance of nonzero values Nei's distances: Mean of nonzero values, Variance of nonzero values, Mean of complete distribution
Second ABC	Fst distances: Mean of nonzero values, Variance of nonzero values, Mean of complete distribution Nei's distances: Mean of nonzero values, Variance of nonzero values
Third ABC	Genetic diversities: Proportion of zero values, Variance of nonzero values Fst distances: Proportion of zero values, Mean of nonzero values, Variance of nonzero values, Mean of complete distribution Nei's distances: Mean of nonzero values, Variance of nonzero values, Mean of complete distribution

in DISTRUCT version 1.1 (Rosenberg, 2004). In addition, to determine the genetic structure of nDNA, we also conducted a principal component analysis (PCA) with GENODIVE version 2.0b27 (Meirmans & Van Tienderen, 2004) as well as STRUCTURE analysis. When we performed the first STRUCTURE and PCA, we used 148 specimens (445 SNPs). Next, we separately conducted the second STRUCTURE and PCA analyses using populations from Shiga Prefecture (42 specimens [377 SNPs]). This is because we investigated genetic structure within each genetic group determined by the first STRUCTURE and PCA.

To confirm the history of genetic diversification, we used an Approximate Bayesian computation (ABC) approach in DIYABC version 2.1 (Cornuet et al., 2014). ABC provides estimates of demographic and historical parameters and quantitative comparisons of an evolutionary scenario (Bertorelle, Benazzo, & Mona, 2010; Tsuda, Nakao, Ide, & Tsumura, 2015). On the basis of the structure analysis and PCA analysis on the SNPs, populations were grouped into three (the first and second analyses) and two (the third analysis) groups (Table 1; Figure 2). Because the results of STRUCTURE showed that some populations appeared to have a mixed genetic structure between the two different genetic groups, the above populations (Pop1–3) were used to investigate how the genetic structure was created for the first analysis. For the second analysis, we focused only on the populations of three species from Shiga Prefecture (including the populations of Lake Biwa). This was because these populations were not divided, even when compared hierarchically from $K = 2$ to $K = 5$ in the first STRUCTURE. The result of the second STRUCTURE showed two different genetic groups and a mixed genetic structure between the groups, so we investigated how the genetic structure was created for the second analysis (Pop1–3). Finally, we conducted the third analysis using two endemic species of Lake Biwa (*H. longispira*, and *Heterogen* sp., Pop1 and 2) because shell morphologies of the two species were clearly different to each other (see Section 3.5; Figure S6) despite their genetic relationships being relatively close in the second STRUCTURE (Figure 7). We conducted the pilot run with wide extensive parameters and all summary statistics values. We used the following parameters based on the results from the pilot run (Table S5). In total, we used four (first analysis), five (second analysis), and nine (third analysis) summary statistics, respectively (Table 2). We compared the different scenarios by calculating their relative posterior probabilities using a logistic regression method from the 1% of simulated data sets which most closely resembling the observed data set. We used the option "confidence in scenario choice" in DIYABC to evaluate the validity in scenario choice. We calculated each scenario specific prior based error using simulated 500 data sets per scenario with the same parameter setting. Under the most likely scenario as determined by the logit transformation of parameters, the posterior distributions of parameters were estimated on the 1% of simulated data sets most closely resembling the observed data set. We also used the option "model checking" with principal component analysis (PCA) using DIYABC to assess the goodness-of-fit of the scenarios. This option can be used to evaluate the consistency of the observed data with the posterior predictive distribution of the model for the best scenario.

2.6 | Morphological analyses for all species and clades

To investigate how habitat differences (lake endemic or widely distributed) affect shell morphologies of the snails in its evolutionary histories, we compared shell morphology among clades/fossil groups. Based on the phylogenetic relationships of the present study and previous studies, we decided how viviparid species belonged to each clade (Gu et al., 2019; Sengupta et al., 2009; Van Bocxlaer et al., 2018; Zhang et al., 2015). In the fossil species, we mainly focused on presumably lake endemic species (Japan: *Igapaludina* in the late Pliocene, and *Tulotomoides* in the late Pliocene-early Pleistocene [Matsuoka, 1985]; *Heterogen* in the early Pleistocene-present [Matsuoka & Nakamura, 1981; Matsuoka, 1986]; China: *Margarya* and *Macromargarya* in the early Oligocene [Ying, Fürsich, & Schneider, 2013]; Africa: *Bellamya* in the late Pliocene-early Pleistocene [Van Damme & Gautier, 2013]), which inhabited the same/close regions to the distribution areas of extant lake endemic species. In addition, we also used a fossil specimen of *H. japonica* (0.5 Ma: Matsuoka & Nakamura, 1981). In total, we used 179 individuals which represented 61 species (Table S6).

First, we took a photograph of the shell of each specimen obtained from the field and museum (Table S6). For the quantitative evaluation of the entire shell shape, we generated elliptic Fourier descriptors (EFDs; Kuhl & Giardina, 1982) using the same digital images we had taken and obtained images from the figures indicated

in the following references (Gu et al., 2019; Matsuoka, 1985, 1986; Matsuoka & Nakamura, 1981; Prozorova, Makarenko, & Balan, 2014; Sengupta et al., 2009; Van Bocxlaer et al., 2018; Van Damme & Gautier, 2013; Ying et al., 2013; Zhang et al., 2015). We used adult shells with strongly thickened outer lips. When using the figures from the references, we also only used the specimens which seemed to be adult. All images used for morphological analysis were placed so that the shell aperture faced forward. The parameter of harmonic amplitudes was set to $n = 40$. For the analysis of shell shape using Fourier coefficients obtained from the EFDs, we conducted a principal component analysis (PCA). We used SHAPE version 1.3 to process the digital images, obtain EFDs, and perform PCA (Iwata & Ukai, 2002). In the PCA, we treated the fossils as four distinctive groups (three Lake endemic groups: Japan [*Igapaludina*, *Tulotomoides*, and *Heterogen*], China [*Margarya* and *Macromargarya*], Africa [*Bellamya*]; one widely distributed group: Japan [*H. japonica*]).

Second, to reveal whether the clades and habitat reflected the shell morphology, we performed a Wilcoxon test for shell shape using the PCs. The Wilcoxon test was conducted in JMP 11 (SAS institute, North Carolina, USA). For the Wilcoxon test, some clades comprising only widely distributed species and a few individuals were removed before this analysis. In addition, a fossil of *H. japonica* was also not included in the analysis because of small sample size. Statistical significance was determined using Bonferroni corrections. The three effective PCs (PC1-3) were used in this and the next section for convenience.

Finally, to evaluate the influence of the lakes on the observed morphological pattern, we compared the maximum values of PCs (PC1-3) and the maximum values of uncorrected pairwise genetic distance (*p*-distance) among clades. We calculated the genetic distance between clades using the COI gene for the sampled snails using MEGA5 (Tamura et al., 2011). In addition, PC1-3 were converted into Euclidian distance using XLSTAT (Addinsoft).

2.7 | Morphological analyses for Japanese species

To determine whether the phylogenetic relationships of nDNA among the three extant Japanese species (*H. japonica*, *H. longispira*, and *Heterogen* sp.) and its evolutionary history corresponded with the differences in shell morphology, we analyzed the variation in its shell shape using additional specimens of these three species to the data set of the above first PCA. In addition, we used some fossil species as well as 2.6. In this analysis, shell width from the field specimens and museum was measured using digital calipers (D; Figure 3). In total, we analysed 165 specimens of extant populations (122 individuals of *H. japonica*, 16 individuals of *H. longispira*, and five individuals of *Heterogen* sp.) and 25 specimens of fossils (one individual of *H. japonica* [Sakawa Clays], two individuals of *H. longispira* [Sakawa Clays], one individual of *H. longispira* [Hiraen Clays], 15 individuals of *H. longispira* [Nijigaoka Clays], one individual of *I. stricta*, one individual of *T. sanaguensis*, and one individual of *T. japonica* [Tables S1 and S6]). Considering the results of population genetic analysis (first STRUCTURE analysis, see Section 3.3), we treated each population of *H. japonica* (Western Japan and Eastern Japan) as different groups. In addition, population demographic analyses showed that the fossil age of *H. longispira* (Nijigaoka Clays) was not consistent with the divergence time of the extant populations of *H. longispira* (the second DIYABC analysis, see Section 3.4), so we also treated *H. longispira* (Nijigaoka Clays) as a distinctive group.

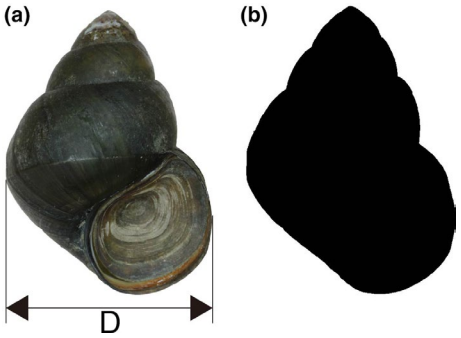

FIGURE 3 Examples of figure data for the morphological analysis. (a) Shell morphology and shell width (*D*). (b) The entire outline of the shell

We generated elliptic Fourier descriptors using the above method by SHAPE version 1.3, and also conducted PCA. Next, we also performed a Wilcoxon test for shell size (shell width [D]) and shape separately using the PCs and D in JMP 11. In particular, we clarified how and what shell morphology was different among the species and genetic groups of MIG-seq SNP. For the Wilcoxon test, fossils of *H. japonica* and *H. longispira* from Sakawa Clays, *H. longispira* from Hiraen Clays, *I. stricta*, *T. sanaguensis*, and *T. japonica* were excluded from the analysis because there were few sample sizes of each fossil species. Statistical significance was determined using Bonferroni corrections.

2.8 | Ancestral state reconstruction of shell morphology

To reconstruct the history of character evolution, ancestral character states of shell morphology at interior nodes were inferred from the BEAST tree using MESQUITE 3.6 (Maddison & Maddison, 2018). We reconstructed the ancestral state of shell surface pattern (ornamented/keeled or smooth) at each interior node using the MP analysis of "Trace character history" in MESQUITE. We then referred to some references in order to decide each stasis of shell surface of individuals from GenBank data (Lu, Du, Li, Chen, & Yang, 2014; Sengupta et al., 2009; Zhang et al., 2015), but some individuals were treated as unknown.

3 | RESULTS

3.1 | Phylogenetic relationships

The topologies of the trees from ML and Bayesian analyses were largely consistent at least regarding the relationships of the well

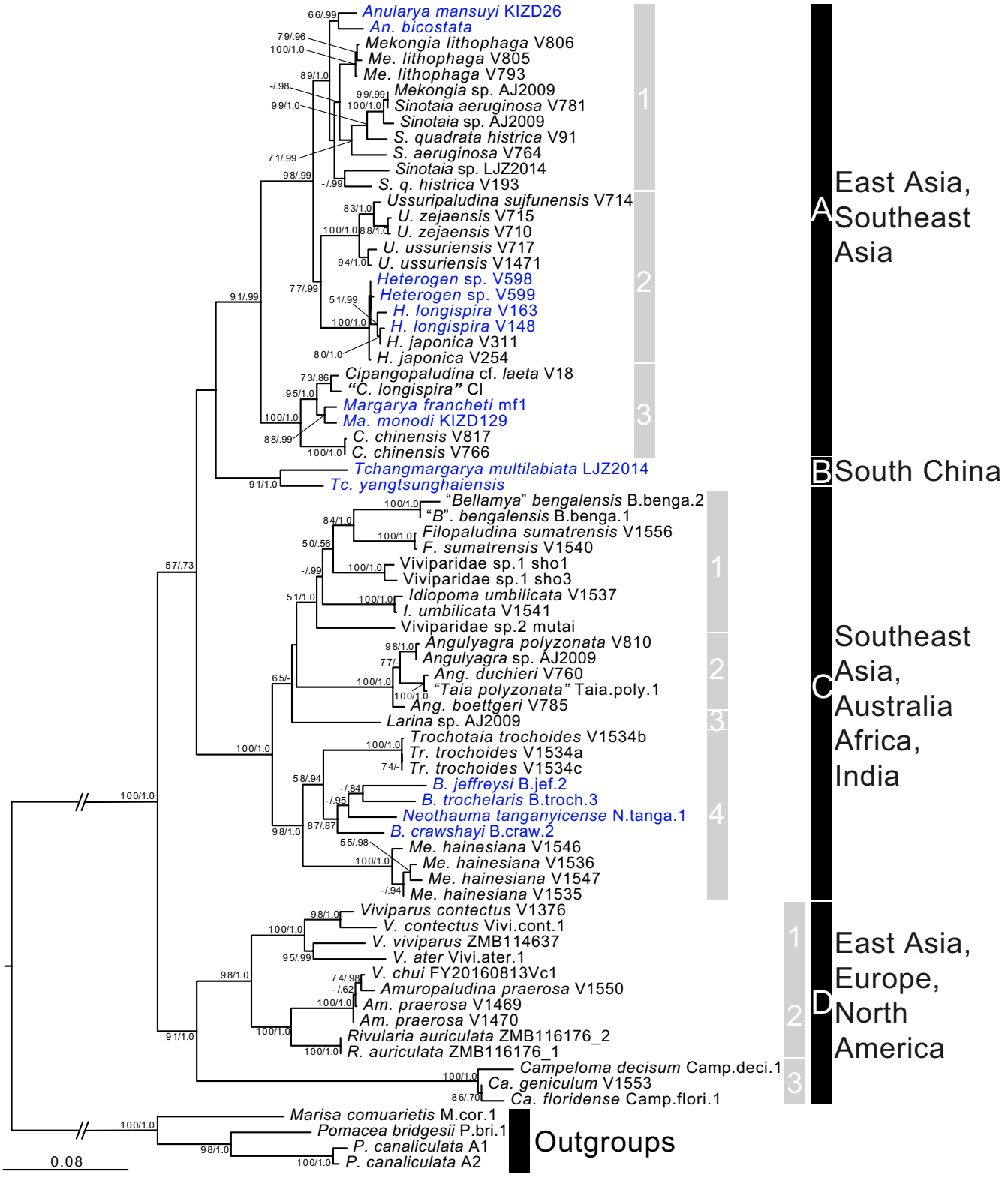

FIGURE 4 Maximum likelihood consensus tree of viviparids based on combined sequences from the COI, 16S, 18S, 28S and H3 genes. Each OTU label represents a species/subspecies name followed by the specimen ID. Each clade and subclade are differentiated by black and grey colours, respectively. The blue colour indicates lake endemic species. Numbers on branches indicate maximum likelihood bootstrap values followed by Bayesian posterior probabilities. Scale bar indicates 0.08 substitutions per site

supported clades (Figure 4). Only clades with high support (posterior probabilities ≥0.95 or bootstrap support ≥70%) were given further consideration.

The viviparid species we examined were largely separated into four major clades (Figure 4). Clade A was composed of *Anularya*, *Cipangopaludina*, *Heterogen*, *Margarya*, *Mekongia*, *Sinotaia*, and

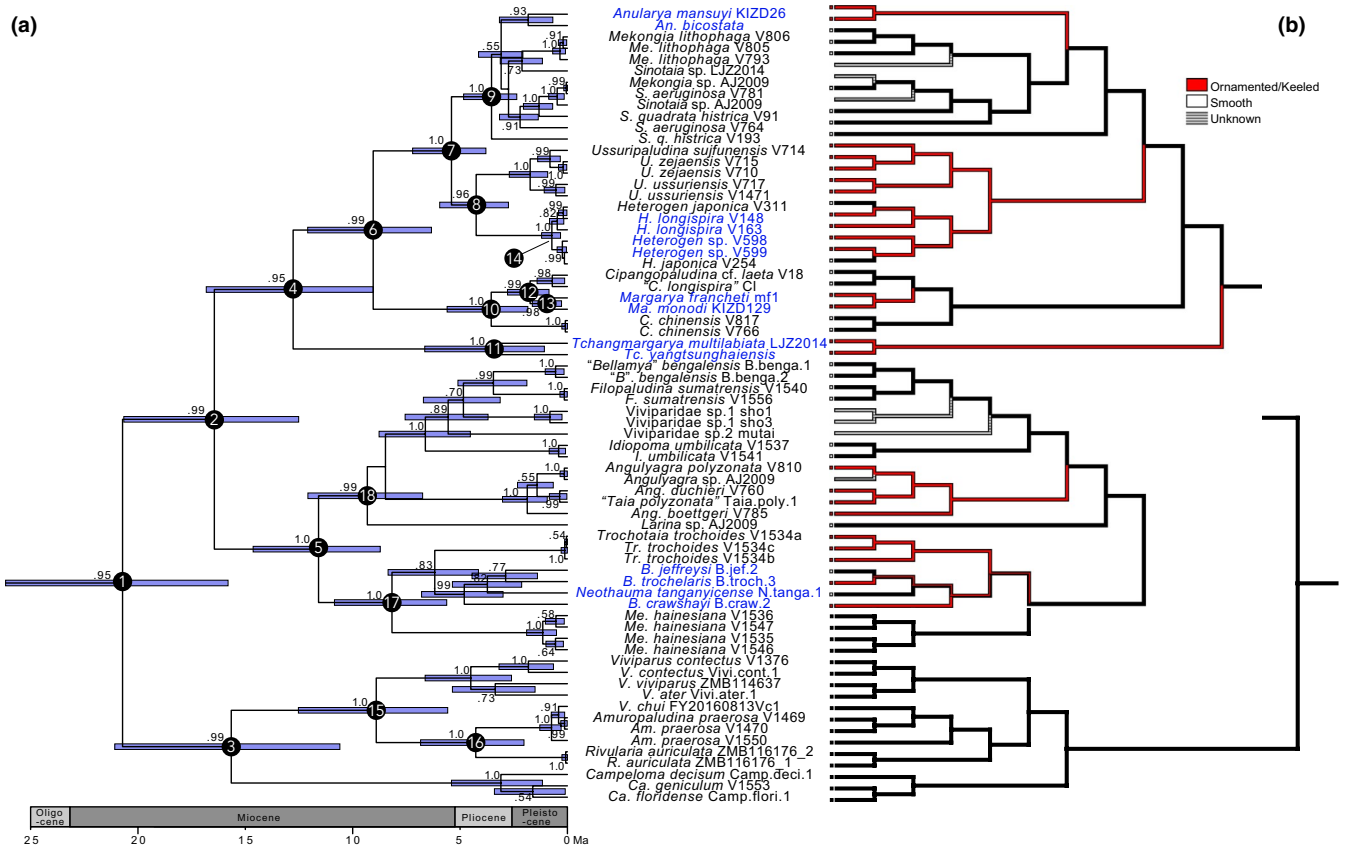

FIGURE 5 The results of divergence time estimation and ancestral reconstruction analysis. (a) Maximum clade credibility tree generated with the BEAST2 analysis from the combined sequences from the COI, 16S, 18S, 28S and H3 genes. Numbers on branches indicate Bayesian posterior probabilities. The principal nodes are named with nominal numbers. (b) The results of MESQUITE analysis mapping on the BEAST2 tree. The outgroups (*Marisa* and *Pomacea*) are not shown. Each OTU label represents a species/subspecies name followed by the specimen ID. The blue colour indicates lake endemic species

Ussuripaludina. Clade A was separated into three lineages: (a) a lineage composed of *Mekongia*, *Anularya*, and *Sinotaia*, (b) a lineage composed of *Ussuripaludina* sisters to the *H. japonica*/*H. longispira*/*Heterogen* sp. complex, and (c) a lineage composed of *C. chinensis*, *C.* cf. *laeta*, and *Margarya*. In particular, even considering the results of phylogenetic analyses focusing on only *H. japonica*/*H. longispira*/*Heterogen* sp. complex, each species could not be distinguished by mtDNA phylogeny (Figure S2). Clade B included only *Tchangmargarya*. Clade C was comprised of *Angulyagra*, *Bellamya*, *Filopaludina*, *Idiopoma*, *Larina*, *Mekongia*, *Neothauma*, "*Taia*", *Trochotaia*, and Viviparidae sp. Clade C was separated into four lineages: (a) a lineage composed of "*Bellamya*" *bengalensis*, *Filopaludina*, *Idiopoma*, and Viviparidae sp. (Thailand), (b) a lineage composed of *Angulyagra* and "*Taia*,", (c) a lineage composed of *Larina* only, and (d) a lineage composed of African *Bellamya*, *Mekongia hainesiana*, *Neothauma*, and *Trochotaia*. Clade D included *Amuropaludina*, *Campeloma*, *Rivularia*, and *Viviparus*. These genera are monophyletic and were separated into two lineages: (a) a lineage composed of *Viviparus* only, (b) a lineage composed of *Amuropaludina*, *Rivularia*, and *Viviparus*, and (c) a lineage composed of *Campeloma* only. In particular, the Asian linages inhabited in the ancient lakes may have occurred at least four times, and subsequently, species diversified to more than two species within each lake group.

3.2 | Divergence time estimation

For the molecular clock analyses, the ESS values visualized in TRACER version 1.6 were considerably higher than 1,000 in each analysis. First, we assigned numbers to the cardinal nodes from one to 18 for convenience. The topology of the tree almost completely coincided with that obtained for the Bayesian tree using MRBAYES and ML analyses (Figures 4 and 5a). The mean time of first divergence of the modern Viviparidae (node 1) was estimated to be 21 Ma (the lower and upper 95% HPD intervals were 16 and 26). The mean time of first divergence of clades A + B (the divergence time of the last common ancestor of *Tchangmargarya*: node 4) was estimated to be 13 Ma (the lower and upper 95% HPD intervals were 9 and 17). In addition, the mean time of first divergence of the last common ancestor of the modern *Anularya* (node 9) was estimated to be 4 Ma (the lower and upper 95% HPD intervals were 2 and 5). The mean time of first divergence of the last common ancestor of the modern *Margarya* (node 12) was estimated to be 2 Ma (the lower and upper 95% HPD intervals were one and three). For *H. japonica*, *H. longispira*, and *Heterogen* sp., the mean time of first divergence of these species was estimated to be 0.7 Ma (the lower and upper 95% HPD intervals were 0.3 and 1). The modern groups of such Chinese

and Japanese species diverged gradually since the beginning of the Miocene, particularly Pliocene. It seems that species diversification occurred mainly after Pleistocene at the intragenus level. Additional information is shown in Table 3.

3.3 | Morphological divergence among clades and habitat in all viviparid snails

Based on the results of PCA using the Fourier coefficients of all 179 specimens, 10 effective PCs were chosen by SHAPE version 1.3. The first, second, and third principal components explained 60.61%, 17.50%, and 4.58% of the total variance, respectively. The PCA results showed that there was overlap in shell morphology between lake endemic species and widely distributed species to some extent, but the scatter plots also indicated that these two habitats formed each cluster (Figure 6). In addition, these results showed that the presumably lake endemic fossil species and the extant lake endemic species clustered in each area group.

Significant differences in the morphological traits were found for the different combinations of groups for PC1, PC2, and PC3 (Table S7; Figure 6): PC1—between clade A3 of widely distributed and clades A1/A2/A3/B/C4/Japan (fossil) of lake endemic/A1 of widely distributed, between clade C4 of widely distributed and clades A1/A3/C4/Japan (fossil) of lake endemic/A1 of widely distributed, between Japan (fossil) of lake endemic and clade A2 of widely distributed; PC2—between Japan (fossil) and clades A2/A3 of widely distributed/C4 of lake endemic, between clade C4 of lake endemic and clades A1/C4 of widely distributed, between clade A1 of widely distributed and clades A2/A3 of widely distributed, between clade A2 of widely distributed and clade C4 of widely distributed; PC3—between clade A1 of lake endemic and clades A2/C4 of lake endemic/A1 of widely distributed, between clade A2 of lake endemic and clade A3 of lake endemic, between clade A3 of lake endemic and clades C4 of lake endemic/A1 of widely distributed, between clade A1 of widely distributed and clades B of lake endemic/A3 of widely distributed. In particular, results of PC1 showed that there were no significant correlations between lake endemic clades/fossils, and only a few correlations between widely distributed clades. However, there were some significant correlations between lake endemic clades/fossils and widely distributed clades.

Genetic distance showed that the lake endemic clades tended to have lower genetic distance within the clades than those of the widely distributed clades. However, morphological divergence of the lake endemic clades seemed to be similar or higher than that of the widely distributed clades.

TABLE 3 Detailed results of divergence time estimation

Node number in Figure 5	Divergence time mean (lower CI, upper CI: Ma)
1	20.72 (15.80, 26.16)
2	16.44 (12.52, 20.66)
3	15.66 (10.60, 21.08)
4	12.77 (9.04, 16.82)
5	11.59 (8.72, 14.64)
6	9.05 (6.33, 12.10)
7	5.40 (3.80, 7.21)
8	4.25 (2.75, 5.95)
9	3.53 (2.36, 4.83)
10	3.55 (1.84, 5.60)
11	3.45 (1.07, 6.65)
12	1.74 (0.86, 2.79)
13	0.88 (0.27, 1.63)
14	0.72 (0.32, 1.20)
15	8.90 (5.58, 12.53)
16	4.27 (2.02, 6.84)
17	8.17 (5.62, 10.85)
18	9.32 (6.74, 12.09)

3.4 | Morphological evolution of shell surface

Results of ancestral state reconstruction on the BEAST tree showed incongruence between the shell morphology and molecular phylogeny due to parallel evolution of pattern of shell surface (Figure 5b). In particular, lake endemic species tended to have an ornamented or keeled shell, but widely distributed species tended to have a smooth shell.

3.5 | Genetic differences of each population of Japanese species using nDNA

Likelihood (LnP [D]) was found to be greatest when K = 2, suggesting that three Japanese species could be divided into two major clusters of SNPs in the first STRUCTURE (Figure 7). In addition, at least in four populations from eastern Japan (Loc. No. 20–22, 29), there were mixed genetic structures between the two major clusters. These patterns of the clusters reflected geographical distribution (Western Japan including Shiga Prefecture and Eastern Japan), but were not associated with differences among species. A scatter plot of the first PCA also showed that these specimens could be divided into two major clusters reflecting the result of the first STRUCTURE (Figure 8). In the second STRUCTURE analysis was also found to be greatest when K = 2 (Figure 7). Two SNP clusters reflected species differences, in particular between *H. japonica* (besides Loc. No. 6) and *Heterogen* sp. In addition, the populations of *H. longispira* in the northern part of Lake Biwa (Loc. No. 2) were genetically similar to *Heterogen* sp., even when comparing hierarchically from K = 2 to K = 5. At least in three populations (*H. japonica*: Loc. No. 6; *H. longispira*: Loc. No. 3 and 4), there were mixed genetic structures between the two major clusters. A scatter plot of the second PCA showed that each species was divided into three major groups (Figure 8).

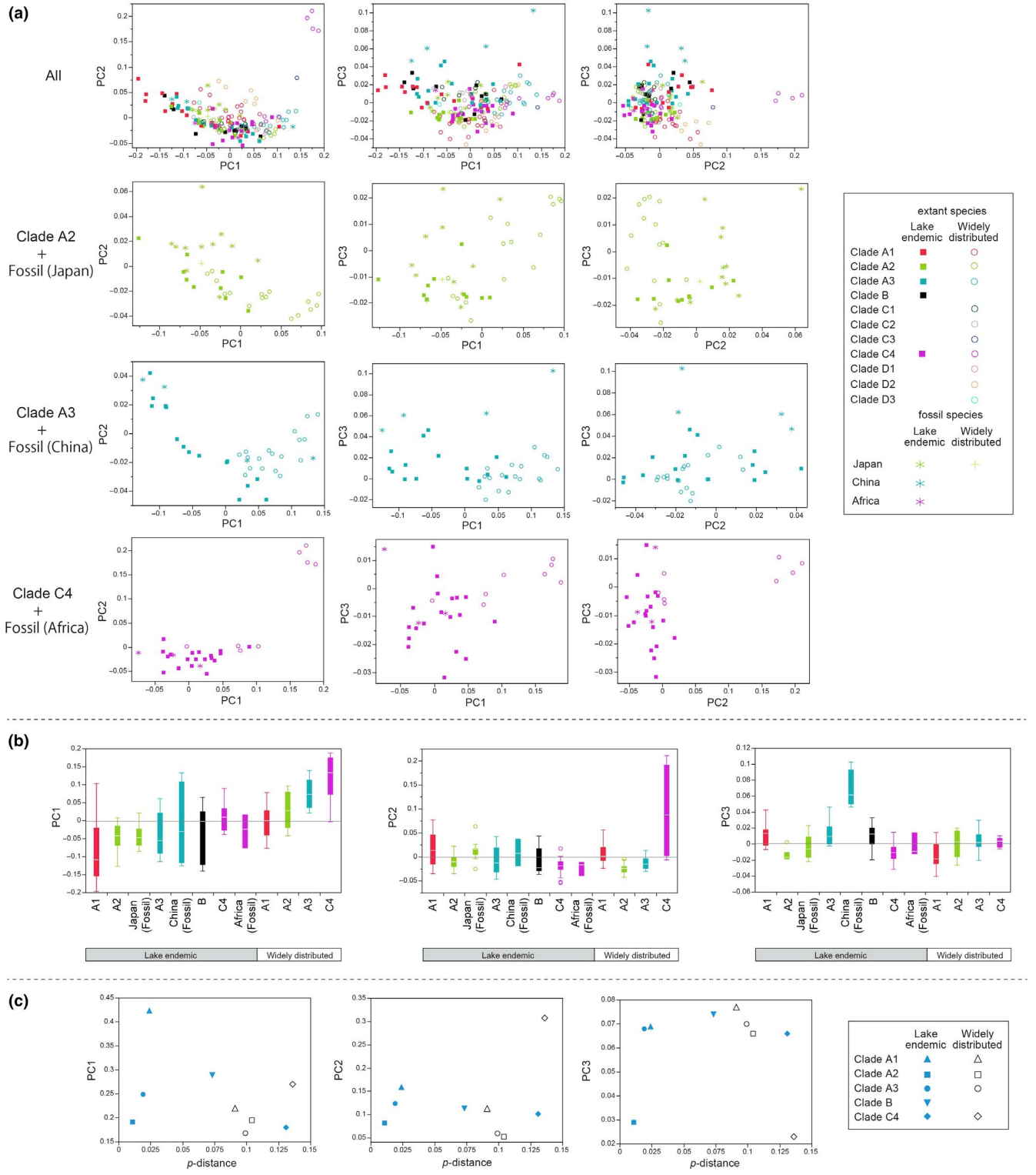

FIGURE 6 Results of the shell morphology statistical analyses between clades/fossils, and relationships between the principal components (PC) 1–3 and the uncorrected pairwise genetic distance of the COI gene (*p*-distance). (a) Scatter plots of the principal component analysis scores and (b) box plots for PC 1–3. (c) Scatter plots of the PCs and *p*-distance. *Y* axis of the box plot indicates each amount of PCs (point)

3.6 | Results of population demography analysis

Estimated values for each posterior probability of scenarios are listed in Figure S3. The results of the first population demography analysis by DIYABC using 198 SNPs showed that scenario 3 (Figure 2a) had the highest posterior probability (approximately 0.8; Figure S3). Based on this scenario, PopB initially separated from PopA, and subsequently PopC separated from PopB. In the case of scenario 3,

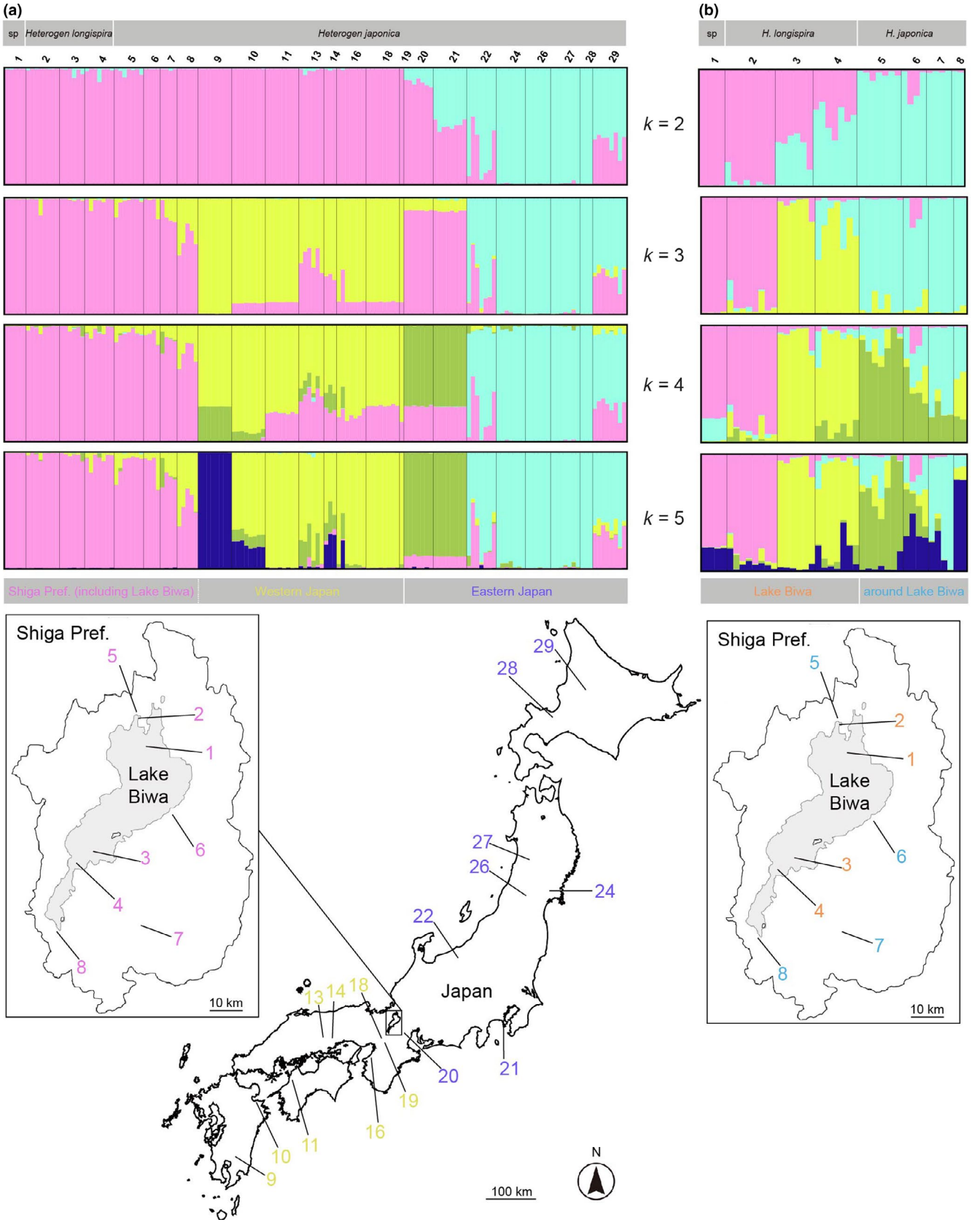

FIGURE 7 Results of the STRUCTURE analyses for K = 2–5. (a) The first and (b) the second analyses. The grey bars and numbers above the results of the STRUCTURE indicate each genetic group and distribution area, and sampling site number in the map, respectively

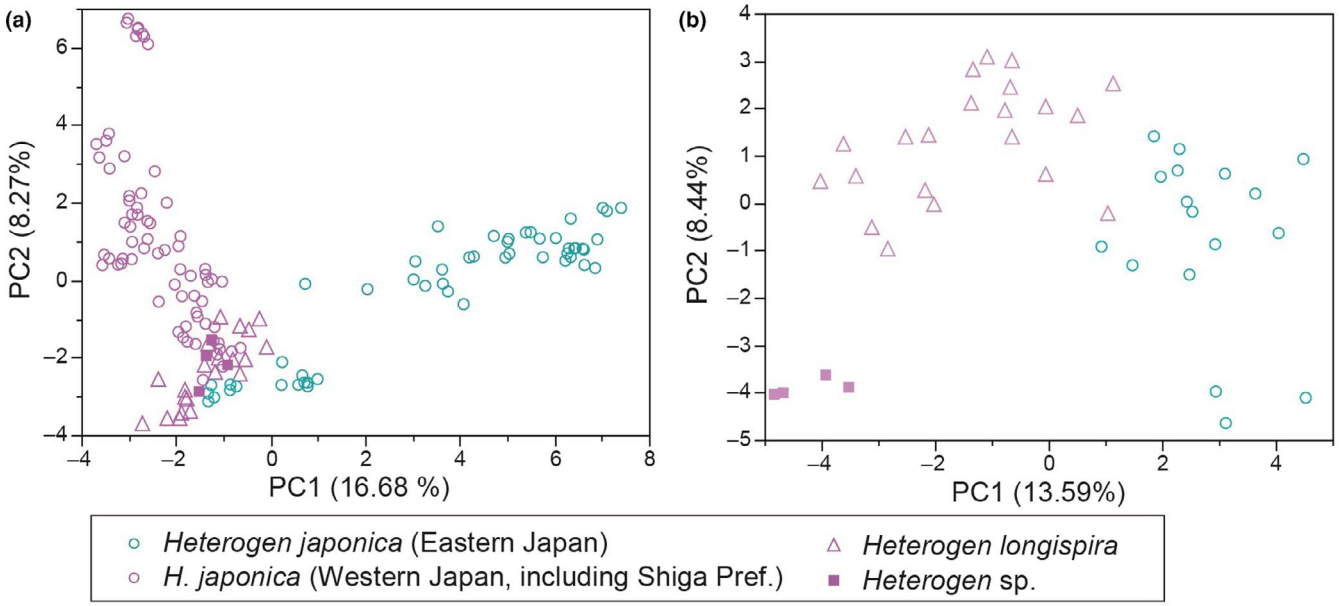

FIGURE 8 Plots of results from the principal component analyses (PCA) based on MIG-seq SNPs. (a) The first and (b) the second analyses. The purple and blue symbols under the plots indicate the two genetic clusters in the first and the second analyses of STRUCTURE for $K = 2$

the median values of divergence times were 6.37×10^5 generations ago for time t1 (95% CI: 2.10×10^5–1.25×10^6) and 9.85×10^5 generations ago for time t2 (95% CI: 3.08×10^5–1.81×10^6) (Table S8; Figure S4). Previous studies have suggested that the sexual maturation of *H. longispira* is likely to be when shell height is ~25 mm (Kondo, 2015), and *H. japonica* grows to be 20–30 mm at one years old (Kondo, Sato, Matsuo, & Yoshimura, 2016). Therefore, PopB was separated from PopA before 985,000 years ago (t2), and PopC was separated from PopB before 637,000 years ago (t1). The type I and type II errors for scenario 3 were 0.758 and 0.242, respectively (Table S9). In the second analysis, scenario 2 (Figure 2a) had the highest posterior probability (approximately 0.9; Figure S3). Based on this scenario, PopA initially separated from PopC, and subsequently PopB has been comprised by PopA and PopC. In the case of scenario 2, the median values of divergence times were 1.11×10^5 generations ago for time t1 (95% CI: 2.52×10^4–1.94×10^5) and 5.41×10^5 generations ago for time t2 (95% CI: 2.25×10^5–9.00×10^5) (Table S8; Figure S4). Therefore, *H. longispira*/*Heterogen* sp. was separated from *H. japonica* before 541,000 years ago (t2), and the introgressive hybridisation (the median values of ra: 2.17×10^{-1} [95% CI: 5.90×10^{-2}–6.08×10^{-1}]) between the two species and *H. japonica* occurred before 111,000 years ago (t1). The type I and type II errors for scenario 2 was 0.590 and 0.410, respectively (Table S9; Figure 8). In the third analysis, scenario 2 (Figure 2b) had the highest posterior probability (1.0) (Figure S3), indicating PopB separated from PopA. In the case of scenario 2, the median values of divergence times were 1.67×10^5 generations ago for time t1 (95% CI: 3.81×10^4–3.53×10^5; Table S8; Figure S4). Considering that, *Heterogen* sp. was separated from northern population of *H. longispira* before 167,000 years ago (t1). The type I and type II errors for scenario 2 was 0.966 and 0.034, respectively (Table S9). The result of PCA from each scenario on model checking option is shown in Figure S5.

3.7 | Morphological variation among Japanese species

Based on the results of PCA using the Fourier coefficients of all 161 specimens, 10 effective PCs were chosen by SHAPE version 1.3. The first, second, and third principal components explained 69.13%, 7.08%, and 4.41% of the total variance, respectively. The PCA results showed that there was little overlap in shell morphology (PC1 and 2) between *H. longispira* (including the fossils from Sakawa Clays)/*I. stricta*, *H. japonica* (including the fossil from Sakawa Clays)/*Heterogen* sp./*H. longispira* (including the fossils from Hiraen Clays and Nijigaoka Clays), *T. japonica*, and *T. sanaguensis*; nevertheless, *H. japonica* and *Heterogen* sp. largely overlapped (Figure S6). Each population of Western and Eastern Japan of *H. japonica* also largely overlapped each other. *Heterogen longispira* (Hiraen Clays and Nijigaoka Clays) was also distinctive from the other groups. In addition, these results showed that the presumably lake endemic fossil species and the extant lake endemic species clustered.

Significant differences in the morphological traits were found for the different combinations of groups for D (shell diameter), PC1 and PC2 (Table S10; Figure S6): D—between *H. japonica* (Eastern Japan) and the others, between *H. japonica* (Western Japan) and *H. longispira* (Nijigaoka Clays)/*Heterogen* sp., between *H. longispira* and *Heterogen* sp.; PC1—between *H. longispira* and both populations of *H. japonica*/*H. longispira* (Nijigaoka Clays); PC2—between *H. longispira* and both populations of *H. japonica*.

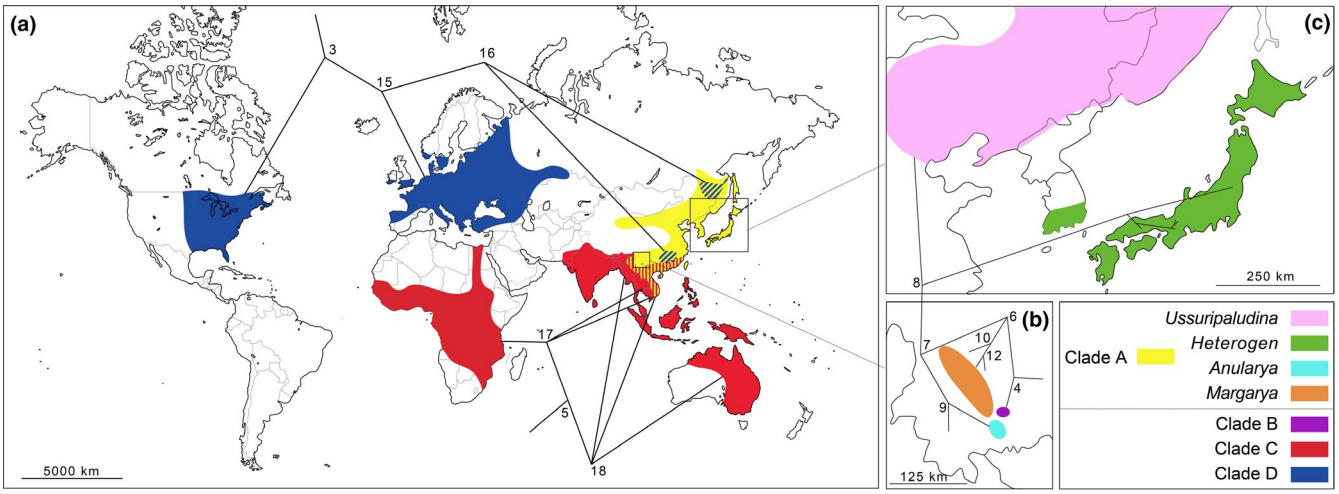

FIGURE 9 Maps showing estimated distributions of each clade and the patterns of colonization/diversification based on the results of the phylogenetic and population genetic analyses. (a) All clades, (b) clades A (Chinese species) and B, and (c) clade A (*Heterogen* and *Ussuripaludina*). Numbers on the maps correspond with node numbers of Figure 5. Distributions of widely distributed genera *Cipangopaludina*, *Sinotaia*, and *Mekongia* in clade A are not included in maps b and c. Distributions are based on Starobogatov Prozorova Bogatov and Saenko (2004), Anistratenko Degtyarenko Anistratenko and Prozorova (2014), Prozorova et al. (2014), Hirano et al. (2015), Hirano et al. (2019), Zhang et al. (2015), and Van Bocxlaer et al. (2018).

4 | DISCUSSION

The present analyses showed that endemic species in the same ancient lake/groups tended to be genetically closely related to each other (Figures 4 and S2). However, levels of morphological divergence among the lake endemic clades were equivalent or even greater than among more widely distributed clades (Figure 6). In addition, lake endemics tended to have similar shell morphology suggesting that parallel evolution had occurred independently in Viviparidae (Figures 4–6, and 9).

Divergence time estimation of Asian viviparid snails was performed (Table 3; Figure 5). Viviparidae has a rich fossil record that dates back to the Middle Jurassic (Hudson, Clements, Riding, Wakefield, & Walton, 1995; Tracey, Todd, & Erwin, 1993), and Cretaceous viviparids have been found on all continents, except Antarctica (Fischer, 1963; Hamilton-Bruce, Smith, & Gowlett-Holmes, 2002; Henderson, 1935; Kear, Hamilton-Bruce, Smith, & Gowlett-Holmes, 2003; Perea, Soto, Veroslavsky, Martínez, & Ubilla, 2009; Prashad, 1928; Van Damme, Bogan, & Dierick, 2015; Yen, 1950). Although some fossils might have been misplaced, the oldest divergence time of viviparid snails in our phylogenetic tree was much more recent than that estimated by the fossil record of this family. Therefore, our results imply that either the modern Viviparidae are a crown group rooted in a single lineage of fossil Viviparoidea (Viviparidae + Pliopholygidae), and all other lineages extinct, or all of the older Viviparidae fossils were identified erroneously as Viviparidae. However, the COI gene was saturated after about 10 million years because phylogenetically informative signal may be lost due to multiple point mutations at a single locus, due to the high mutation rate of this gene fragment (Brown, George, & Wilson, 1979), so the results of our study are potentially an underestimation of some of the observed divergence times (e.g., nodes 1–5). Nevertheless, divergence times of some terminal clades are consistent with the age of fossil record of some modern species (node 14: *H. japonica*, approximately 0.5 Ma; *H. longispira*, approximately 0.5 Ma [Matsuoka, 1987, see results]; node 13: *M. monodi*: early Pleistocene [Pan, 1984]). In addition, the divergence time of *Anularya* (node 9) is consistent with ages of formation of the lakes where *Anularya* species are distributed (lakes Fuxian, Xingyun, and Qilu: the Great Fuxian Palaeolake, about 3.4 Ma; Wang & Dou, 1998). Considering the results of the divergence times of *Margarya* (approximately 2.8 Ma) in Hirano et al. (2019) and the present study (node 12), this genus seems to have diversified after the formation of Lake Dianchi (about 3.2 Ma; Jin et al., 2006). Although the timing of formation of lakes Yangzonghai and Shilin are still unclear, divergence time of *Tchangmargarya* might be related to the histories of the lakes due to such correlations between the divergence times of lake endemic Viviparidae and the timing of formation of lakes.

In cases of *H. japonica*, *H. longispira*, and *Heterogen* sp., results of divergence time estimation and population demographic analyses corresponded with the topographical history of Lake Biwa in terms of the extant lake expansion, as well as fossil records of *H. japonica* and *H. longispira* (0.5 Ma; Matsuoka, 1987). The common ancestor of these species may have diversified from a continental lineage (e.g., far East Russia; Figure 9c). Next, the most recent common ancestor of these species diversified in the Miocene or Pliocene, and in Pleistocene, *H. japonica* spread from west to northern part of Japan and was diversified genetically in the west and central-northern Japan. In addition, *H. longispira* diverged from *H. japonica* of around the Lake Biwa, and *Heterogen* sp. derived from *H. longispira* in the present Lake Biwa. These two species diversified after at least emerging of paleo-Lake Biwa (Lake Katata). Thus, relatively rapid speciation occurred in the ancient lake, but hybridization also occurred in the present viviparid species (Figures 2 and S3). The three Japanese species could not be distinguished by the molecular

TABLE 4 Number and shell size of juveniles/embryos among closely related genera

Clade (Figure 4)	Lake endemic group (number/size)	Widely distributed group (number/size)
A1	*Anularya* 1–2/exceeding 10 mm: as juveniles (Zhang et al., 2015)	*Mekongia* 6–10/5.5–7.7 mm: as embryos (this study) *Sinotaia* 20–30/-: as embryos (Okada & Kurasawa, 1950) 30–40/-: as embryos (Okutani, 1986) -/approximately 4 mm: as embryos (Masuda & Uchiyama, 2004) Approximately 6–14/-: as embryos (Nishiwaki, Masu, & Hanawa, 2008) 30–100/small: as embryos (Zhang et al., 2015) 30/2.5–3.2 mm: as embryos (this study)
A2	*Heterogen longispira* 20–30/-: as embryos (Okada & Kurasawa, 1950) less than 20/approximately 10 mm: as embryos (Okutani, 1986) 5/approximately 10 mm: as juveniles (this study)	*Heterogen japonica* 30–40/small: as embryos (Okutani, 1986) 20–30/5–10 mm: as embryos (Kihira et al., 2003) 7–28/approximately 5.8–6.3 mm (calculated from Figure 2): as juveniles (Van Bocxlaer & Strong, 2016) 20–43/4.5–6.5 mm: as embryos (this study) *Ussuripaludina* 5–70/4.5–8.0 mm: as embryos (this study)
A3	*Margarya* 6–8/5 whorls: as embryos (indicated as *C. dianchiensis* in Lu et al., 2014) 2–8/exceeding 10 mm: as juveniles (Zhang et al., 2015)	*Cipangopaludina* 10–20/-: as embryos (Okada & Kurasawa, 1950) 30–40/small: as embryos (Okutani, 1986) -/6–9 mm: as embryos (Masuda & Uchiyama, 2004) 45–84/4 whorls: as embryos (only *C. chinensis* in Lu et al., 2014) 30–100/small as embryos (Zhang et al., 2015)
B	*Tchangmargarya* 1–2/exceeding 10 mm: as juveniles (Zhang et al., 2015)	-
C	*Neothauma* -/approximately 16 mm: as an embryo (Prashad, 1928)	*Filopaludina* -/5–7 mm: as juveniles (Piyatiratitivorakul & Boonchamoi, 2008) 5–25/6.2–7.0 mm: as juveniles (this study)
D	–	*Amuropaludina* 36–110/4.5–6.5 mm: as embryos (this study) *Viviparus* 20–30/4 whorls: as embryos (Simone, 2004) 15–50/4.5–6.0 mm: as embryos (this study) *Rivularia* 2 (from Figure 5b)/–5mm: as embryos (Van Bocxlaer et al., 2018)

phylogeny using mtDNA (Hirano et al., 2015; Figure S2). Rapid evolution among viviparid lineages of ancient lakes and introgressive hybridization may have affected such a complex pattern of mtDNA phylogeny.

Heterogen japonica inhabits rivers flowing into the Lake Biwa, providing an opportunity to come into contact with other lake-endemic species in Lake Biwa. In fact, some populations of *H. japonica* have previously been found in the estuary of rivers flowing into the lake (K. Matsuoka, personal observation). However, *H. japonica* was not actually found in Lake Biwa, so hybridization between *H. japonica* and lake-endemic species (see Section 3.6) is probably uncommon. This might indicate niche conservatism of these species and natural selection for the shell shapes in the habitat. In addition, our results showed multiple diversifications of shell morphology in Lake Biwa. Previous studies have suggested that shell morphology might be associated with habitat (Kagawa, Saito, Uchida, & Chiba, 2019; Miura et al., 2019), and the habitat of *Heterogen* sp. is clearly different from the habitat of *H. japonica* and *H. longispira*. To support this, our work demonstrates that *Heterogen* sp. has different shell ornamentation on the top of the shell than other species, but a similar shell shape to *H. japonica*. Enlarging the shell aperture might promote adhesion to the rock with a wider foot muscle (Yamamori & Kato, 2018), but functional differences of shell shape and details of microhabitat are still unclear. Further studies focusing on the microhabitat of each species are needed to clarify ecological speciation among these species.

Ancient lakes affected divergence in shell morphology and reproductive strategy for viviparid snails (Zhang et al., 2015). In fact, shell shape, hardness, juvenile size and number of offspring in *H. longispira* are different from *H. japonica* (Kihira, Matsuda, & Uchiyama, 2003; Okutani, 1986; Table 4). *Margarya* (including *Anularya* and *Tchangmargarya*) has been thought to be endemic to Pleistocene freshwater lakes of the Yunnan Province in southwest China (Li, 1987), but a recent report indicates that it was first present in the Oligocene of Guangxi in southeast China (Ying et al., 2013). However, our results show that the divergence of modern viviparid

species, including *Margarya*, occurred after the Miocene (Table 3; Figure 5). In Lake Biwa, some fossil viviparid species have similar shell shape to *H. longispira*, appearing at different times (*Igapaludina*: 3.4–2.4 Ma; *Tulotomoides*: 3.3 Ma and 2.5 Ma: Matsuoka, 1985). *Heterogen longispira* at 1.2 Ma may be different species from *H. longispira*, as our population demographic analyses showed that divergence time of extant *H. longispira* is younger than this fossil. But this fossil has similar traits (e.g., thick shell and large juvenile) to the extant species in addition to the overall shell shape and ornamentation of the top of the shell (Table S7; Figures 1 and 6). Moreover, *Sinotaia quadrata histrica* may be an alien species to Japan (Hirano et al., 2015; B. Ye, T. Hirano , T. Saito, Z. Dong, S. Chiba, unpublished data), but an individual of Lake Biwa has a larger and elongated shell than all other localities despite little genetic differentiation (Hirano et al., 2015). These results suggest that similar ecological factors (e.g., habitats and predators) may have caused diversification and parallel evolution of shell morphology.

Prey-predator interaction may be associated with the parallel evolution of these shell phenotypes. For example, endemic carp fishes (genus *Cyprinus*) are distributed in these ancient lakes (Nakajima, 2005), and diversified in the ancient lake groups of Yunnan. Carp use their pharyngeal teeth to eat food, and the morphology of pharyngeal teeth such as shape and number of grooves in A2 teeth is different among and within species (Nakajima, 2005). *Cyprinus* in Lake Biwa primarily eat viviparid snails (Mabuchi, personal communication), so thick shells and larger juvenile size might be an adaptation to counteract the physical pressure of biting carp as well as shell ornamentation (Klompmaker & Kelley, 2015; Vermeji, 1983). Preliminary experiments using juveniles of *H. longispira* and *H. japonica*, showed that juveniles of *H. japonica* were easily crushed and eaten by carp, but in many cases of juveniles of *H. longispira*, they were chewed, then spat out and were still alive without being destroyed (T. Hirano, unpublished data). Taking into account fossil records, ornamented (or keeled) and thick shells were found in paleo-Lake Biwa and paleo lakes in Yunnan with fossil teeth of carp embedded (Nakajima, 2005; Ying et al., 2013), implying coevolution might have occurred between carp and extant viviparid snails by prey-predator interaction. Another possibility, however, is that shell morphology such as ornamentation might be associated with the effect of wave action (Yamazaki & Goshima, 2012). Lake Biwa and Lake Dianchi are both very large lakes with strong waves. Calcium content in water also could affect components of shell morphology such as hardness and the existence of ornamentation (Charrier et al., 2012). These ancient lakes are surrounded by limestone areas, and these Asian viviparid snails can be considered good models for comparing biotic and abiotic effects to phenotypic divergence.

The present findings indicated that ancient lakes produce genetic and phenotypic divergence of viviparid snails. Diversification by geographical isolation (Figure 9), and ecological speciation might have driven the evolutionary history of viviparids. Similarly, ecological diversification might have occurred with the parallel evolution of shell morphology and reproductive strategy in case of some lineages of ancient big rivers like Mekong, Yangtze and Amur (e.g., shell shapes of *Mekongia* and *Amuropaludina*, Figure 4; reproductive strategies of *Mekongia* and *Rivularia*, Table 6). Diversification of viviparid lineages has also occurred in river systems, probably due to geographical isolation (Figure 9). However, divergence of viviparid snails might be accelerated in lake-endemic lineages than in more widely distributed lineages such as river species (Figure 6c). Further studies of ecological and phenotypic divergence of viviparid snails in river systems are needed to further test the hypothesis that ancient lakes are crucial environments in which to create phenotypic and genetic diversity of viviparid snails.

Many viviparid fossils are found outside of the family's current geographic range, including in South America (Ghilardi, Rodrigues, Simone, Carbonaro, & Nava, 2011), the far southwest of North America (Clench, 1962; Henderson, 1935), and the Middle East (Ashkenazi, Klass, Mienis, Spiro, & Abel, 2010; Sivan, Heller, & Van Damme, 2006). Modern viviparid snails show highly genus and species diversities in Asia (Figures 4 and 9), so it is probable that Asian ancient lakes might be able to be resources of genetic divergence even if extensive extinction of viviparid snails occurred in Asia.

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AUTHOR CONTRIBUTIONS

T.H. conceived and designed the study, collected samples, participated in laboratory work and data analysis, interpreted the data, and wrote the paper. T.S. collected samples and participated in data analysis. Y.T. and Y.S. participated in laboratory work and data analysis. J.K. participated in data analysis. L.P., D.V.T., K.N., and K.M. collected samples. S.C. participated in conceiving and designing the study and in interpretation of the data. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The alignment used in Dryad will be deposited (https://doi.org/10.5061/dryad.w9ghx3fjt). The original sequence data also will be deposited at the NCBI.

REFERENCES

Albrecht, C., & Wilke, T. (2008). Ancient Lake Ohrid: Biodiversity and evolution. *Hydrobiologia*, *615*, 103–140. https://doi.org/10.1007/s10750-008-9558-y

Anistratenko, V. V., Degtyarenko, E. V., Anistratenko, O. Y., & Prozorova, L. A. (2014). Modern distribution of gastropod mollusks of the family Viviparidae (Caenogastropoda) in continental water bodies of Eurasia. *Biology Bulletin*, *41*, 724–751. https://doi.org/10.1134/S1062359014090027

Ashkenazi, S., Klass, K., Mienis, H. K., Spiro, B., & Abel, R. (2010). Fossil embryos and adult Viviparidae from the Early-Middle Pleistocene of Gesher Benot Ya'aqov, Israel: Ecology, longevity and fecundity. *Lethaia*, *43*, 116–127. https://doi.org/10.1111/j.1502-3931.2009.00178.x

Bertorelle, G., Benazzo, A., & Mona, S. (2010). ABC as a flexible framework to estimate demography over space and time: Some cons, many pros. *Molecular Ecology*, *19*, 2609–2625. https://doi.org/10.1111/j.1365-294X.2010.04690.x

Blanco-Pastor, J. L., Fernández-Mazuecos, M., Coello, A. J., Pastor, J., & Vargas, P. (2019). Topography explains the distribution of genetic diversity in one of the most fragile European hotspots. *Diversity and Distributions*, *25*, 74–89. https://doi.org/10.1111/ddi.12836

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., … Drummond, A. J. (2014). BEAST2: A software platform for Bayesian evolutionary analysis. *PLOS Computational Biology*, *10*, e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Brown, D. S. (1994). *Freshwater snails of Africa and their medical importance* (2nd ed.). London, UK: Taylor & Francis.

Brown, W. M., George, M., & Wilson, A. C. (1979). Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, *76*, 1967–1971. https://doi.org/10.1073/pnas.76.4.1967

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Catchen, J., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. (2011). STACKS: Building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes, Genetics*, *1*, 171–182. https://doi.org/10.1534/g3.111.000240

Charrier, M., Marie, A., Guillaume, D., Bédouet, L., Le Lannic, J., Roiland, C., … Lebouvier, M. (2012). Soil calcium availability influences shell ecophenotype formation in the sub-Antarctic land snail, Notodiscus hookeri. *PLOS ONE*, *8*, e84527. https://doi.org/10.1371/journal.pone.0084527

Cheng, H., Edwards, R. L., Broecker, W. S., Denton, G. H., Kong, X., Wang, Y., … Wang, X. (2009). Ice age terminations. *Science*, *326*, 248–252. https://doi.org/10.1126/science.1177840

Chiba, S. (1999). Accelerated evolution of land snails Mandarina in the oceanic Bonin Islands: Evidence from mitochondrial DNA sequences. *Evolution*, *53*, 460–471. https://doi.org/10.1111/j.1558-5646.1999.tb03781.x

Chu, X. L., & Chen, Y. R. (1990). *The fishes of Yunnan, China, Part II Cyprinidae*. Beijing, China: Science Press.

Clench, W. J. (1962). A catalogue of the Viviparidae of North America with notes on the distribution of *Viviparus georgianus* Lea. *Occasional Papers on Mollusks*, *2*, 261–287.

Colgan, D. J., Ponder, W. F., & Eggler, P. E. (2000). Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta*, *29*, 29–63. https://doi.org/10.1046/j.1463-6409.2000.00021.x

Cornuet, J.-M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., … Estoup, A. (2014). DIYABC v2.0: A software to make Approximate Bayesian Computation inferences about population history using Single Nucleotide Polymorphism, DNA sequence and microsatellite data. *Bioinformatics*, *30*, 1187–1189. https://doi.org/10.1093/bioinformatics/btt763

Cristescu, M. E., Adamowicz, S. J., Vaillant, J. J., & Haffner, D. G. (2010). Ancient lakes revisited: From the ecology to the genetics of speciation. *Molecular Ecology*, *19*, 4837–4851. https://doi.org/10.1111/j.1365-294X.2010.04832.x

Du, L., Yang, J., von Rintelen, T., Chen, X., & David, A. (2013). Molecular phylogenetic evidence that the Chinese viviparid genus *Margarya* (Gastropoda: Viviparidae) is polyphyletic. *Chinese Science Bulletin*, *58*, 2154–2162. https://doi.org/10.1007/s11434-012-5632-y

Earl, D. A., & von Holdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–436. https://doi.org/10.1007/s12686-011-9548-7

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797. https://doi.org/10.1093/nar/gkh340

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, *164*, 1567–1587.

Fischer, J. C. (1963). Les gastéropodes du 'Continental Intercalaire' du Sahara. Les mollusques du 'Continental Intercalaire' (Mésozoïque) du Sahara Central. *Mémoires de la Société Géologique de France*, *96*, 41–50.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–299.

Gavrilets, S., & Losos, J. B. (2009). Adaptive radiation: Contrasting theory with data. *Science*, *323*, 732–737. https://doi.org/10.1126/science.1157966

Ghilardi, R. P., Rodrigues, S. C., Simone, L. R. L., Carbonaro, F. A., & Nava, W. R. (2011). Moluscos fósseis do Grupo Bauru. In I. D. Souza Carvalho, N. Kumar Srivastava, O. Strohschoen Jr, & C. Cunha Lana (Eds.), *Paleontologia: Cenários de Vida* (Vol. *4*, pp. 239–250). Rio de Janeiro, Brazil: Editora Interciência LTDA.

Glaubrecht, M. (2008). Adaptive radiation of thalassoid gastropods in Lake Tanganyika, East Africa: Morphology and systematization of a paludomid species flock in an ancient lake. *Zoosystematics and Evolution*, *84*, 71–122. https://doi.org/10.1002/zoos.200700016

Gu, Q. H., Husemann, M., Wu, H. H., Dong, J., Zhou, C. J., Wang, X. F., … Nie, G. X. (2019). Phylogeography of *Bellamya* (Mollusca: Gastropoda: Viviparidae) snails on different continents: Contrasting patterns of diversification in China and East Africa. *BMC Evolutionary Biology*, *19*, 82. https://doi.org/10.1186/s12862-019-1397-0

Habe, T. (1976). Type specimens of *Cyclophorus herklotsi* and *Cipangopaludina malleata*. *Chiribotan*, *9*, 46.

Hamilton-Bruce, R. J., Smith, B. J., & Gowlett-Holmes, K. L. (2002). Descriptions of a new genus and two new species of viviparid snails (Mollusca: Gastropoda: Viviparidae) from the Early Cretaceous (middle-late Albian) Griman Creek Formation of Lightning Ridge, northern New South Wales. *Records of the South Australian Museum*, *35*, 193–203.

Hampton, S. E., McGowan, S., Ozersky, T., Virdis, S. G. P., Vu, T. T., Spanbauer, T. L., … Fritz, S. C. (2018). Recent ecological change in ancient lakes. *Limnology and Oceanography*, *63*, 2277–2304. https://doi.org/10.1002/lno.10938

Heath, T. A. (2015). *Divergence time estimation using* BEAST *v2. Dating species divergences with the fossilized birth-death process*. Retrieved from http://phyloworks.org/workshops/DivTime_BEAST2_tutorial_FBD.pdf

Henderson, J. (1935). Fossil non-marine mollusca of North America. *Geological Society of North America*, *Special Papers*, *3*, 165–188.

Hirano, T., Saito, T., & Chiba, S. (2015). The phylogeny of freshwater viviparid snails in Japan. *Journal of Molluscan Studies*, *81*, 435–441. https://doi.org/10.1093/mollus/eyv019

Hirano, T., Saito, T., Tsunamoto, Y., Koseki, J., Ye, B., Do, V. T., … Chiba, S. (2019). Enigmatic incongruence between mtDNA and nDNA revealed by multi-locus phylogenomic analyses in freshwater snails. *Scientific Reports*, *9*, 6223. https://doi.org/10.1038/s41598-019-42682-0

Hudson, J. D., Clements, R. G., Riding, J. B., Wakefield, M. I., & Walton, W. (1995). Jurassic paleosalinities and brackish-water communities – a case study. *Palaios*, *10*, 392–407. https://doi.org/10.2307/3515043

Iwata, H., & Ukai, Y. (2002). SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity*, *93*, 384–385. https://doi.org/10.1093/jhered/93.5.384

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, *23*, 1801–1806. https://doi.org/10.1093/bioinformatics/btm233

Jin, X., Wang, L., & He, L. (2006). Lake Dianchi. *Experience and Lessons Learned Brief*, 159–178.

Kagawa, O., Saito, T., Uchida, S., & Chiba, S. (2019). Phenotypic divergence in viviparid snails in a recently converted freshwater lagoon. *Plankton and Benthos Research*, *14*, 189–196. https://doi.org/10.3800/pbr.14.189

Kawabe, T. (1989). Stratigraphy of the lower part of the Kobiwako Group around the Ueno basin, Kinki district, Japan. *Journal of Geoscience*, *Osaka City University*, *32*, 39–90.

Kawabe, T. (1994). Chapter 1. Formation of Lake Biwa. In Research Group for Natural History of Lake Biwa (Eds.), *The natural history of Lake Biwa* (pp. 24–72). Tokyo, Japan: Yasaka Shobo.

Kear, B. P., Hamilton-Bruce, R. J., Smith, B. J., & Gowlett-Holmes, K. L. (2003). Reassessment of Australia's oldest freshwater snail, *Viviparus* (?) *albascopularis* Etheridge, 1902 (Mollusca: Gastropoda: Viviparidae), from the Lower Cretaceous (Aptian, Wallumbilla Formation) of White Cliffs, New South Wales. *Molluscan Research*, *23*, 149–158. https://doi.org/10.1071/MR03003

Kihira, H., Matsuda, M., & Uchiyama, R. (2003). *Pisces ecological field guide series 1. Freshwater mollusks of Japan 1, freshwater mollusks of Biwako Lake and Yodogawa River*. Tokyo, Japan: PISCES Publishers co. Ltd.

Klompmaker, A. A., & Kelley, P. H. (2015). Shell ornamentation as a likely exaptation: Evidence from predatory drilling on Cenozoic bivalves. *Paleobiology*, *41*, 187–201. https://doi.org/10.1017/pab.2014.12

Kondo, K. (2015). Heterogen longispira. In Nature and Environmental Conservation Division, Department of the Environment, Kyoto Prefecture (Ed), *Red Data Book of Kyoto Prefecture 2015* (p. 465). Hikone, Japan: Sunrise.

Kondo, K., Sato, H., Matsuo, S., & Yoshimura, M. (2016). Habitat and Growth of Japanese Mystery Snail in a Pond. Memoirs of Osaka Kyoiku University. *Ser. III*, *Natural science and applied science*, *65*, 7–1.

Kubatko, L. S. (2009). Identifying hybridization events in the presence of coalescence via model selection. *Systematic Biology*, *58*, 478–488. https://doi.org/10.1093/sysbio/syp055

Kuhl, F. P., & Giardina, C. R. (1982). Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing*, *18*, 236–258. https://doi.org/10.1016/0146-664X(82)90034-X

Li, Y. T. (1987). *Margarya* and *Paraprosostheni*a (Gastropoda) from the Sheshan Formation in Lijiang Basin, West Yunnan. *Professional Papers of Stratigraphy and Palaeontology*, *22*, 313–321.

Lisiecki, L. E., & Raymo, M. E. (2005). A Pliocene-Pleistocene stack of 57 globally distributed benthic d18O records. *Paleoceanography*, *20*, PA1003. https://doi.org/10.1029/2004PA001071

Lu, H. F., Du, L. N., Li, Z. Q., Chen, X. Y., & Yang, J. X. (2014). Morphological analysis of the Chinese *Cipangopaludina* species (Gastropoda; Caenogastropoda: Viviparidae). *Zoological Research*, *35*, 510–527. https://doi.org/10.13918/j.issn.2095-8137.2014.6.510

Maddison, W. P., & Maddison, D. R. (2018). MESQUITE: *A modular system for evolutionary analysis. Version 3.6*. Retrieved from https://www.mesquiteproject.org

Masuda, O., & Uchiyama, R. (2004). *Pisces ecological field guide series 2. Freshwater mollusks of Japan 2, freshwater mollusks of Japan, including blackish water species*. Tokyo, Japan: PISCES Publishers co. Ltd.

Matsuoka, K. (1985). Pliocene freshwater gastropods from the Iga formation of the Kobiwako group, Mie Prefecture, central Japan. *Transactions and Proceedings of the Palaeontological Society of Japan. New Series*, *139*, 180–195. https://doi.org/10.14825/prpsj1951.1985.139_180

Matsuoka, K. (1986). Mollusca. In Research Group of Natural History of Lake Biwa (Eds.).*Freshwater fossil assemblages from the Pleistocene Katata Formation on the southwest side of Lake Biwa*. *Bulletin of Mizunami Fossil Museum* , *13*,82–89.

Matsuoka, K. (1987). Malacofaunal succession in Pliocene to Pleistocene non-marine sediments in the Omi and Ueno basins, central Japan. *The Journal of Earth Sciences*, *35*, 23–115.

Matsuoka, K., & Miura, O. (2018). Five new species of the genus *Semisulcospira* (Mollusca: Caenogastropoda: Semisulcospiridae) from the Pleistocene Katata Formation of the Kobiwako Group, Shiga Prefecture, central Japan. *Bulletin of Mizunami Fossil Museum*, *44*, 59–67.

Matsuoka, K., & Nakamura, T. (1981). Preliminary report of the freshwater Molluscs from the Pleistocene Katata formation of the Kobiwako group in Shiga prefecture, Japan. *Bulletin of the Mizunami Fossil Museum*, *8*, 105–126.

Meirmans, P. G., & Van Tienderen, P. H. (2004). GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, *4*, 792–794. https://doi.org/10.1111/j.1471-8286.2004.00770.x

Merckx, V. S. F. T., Hendriks, K. P., Beentjes, K. K., Mennes, C. B., Becking, L. E., Peijnenburg, K. T. C. A., … Schilthuizen, M. (2015). Evolution of endemism on a young tropical mountain. *Nature*, *524*, 347–350. https://doi.org/10.1038/nature14949

Miller, M., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Gateway Computing Environments Workshop (GCE)*, 1–8.

Min, D. H., Lee, J. S., Koh, D. B., & Je, J. G. (2004). *Mollusks in Korea*. Seoul, South Korea: Min Molluscan Research Institute.

Miura, O., Urabe, M., Nishimura, T., Nakai, K., & Chiba, S. (2019). Recent lake expansion triggered the adaptive radiation of freshwater snails in the ancient Lake Biwa. *Evolution Letters*, *3*, 43–54. https://doi.org/10.1002/evl3.92

Nakajima, T. (2005). How is various external shape of fish formed? – In the case of the pharyngeal teeth in cyprinids. In K. Matsuura (Ed.), *The shape in fish* (pp. 69–113). Tokyo, Japan: Tokai University Press.

Nishino, M. (2003). The problem of endemics in Lake Biwa. *Oumia*, *76*, 3–4.

Nishino, M., & Hamabata, E. (2005). *The message from "Naiko (lagoon around Lake Biwa)"*. Hikone, Japan: Sunrise Press.

Nishiwaki, S., Masu, H., & Hanawa, T. (2008). Seasonal and regional variations in the number of shelled embryos of *Sinotaia quadrata histrica* in Lake Kasumigaura, Japan. *Venus*, *67*, 73–80. https://doi.org/10.18941/venus.67.1-2_73

Okada, Y., & Kurasawa, H. (1950). Study for viviparid snails in Japan. *Aquatic Organisms Research*, 141–165.

Okutani, T. (1986). *Encyclopedia of shellfish*. Tokyo, Japan: Sekaibunkasha.

Palumbi, S. R., Martin, A. P., Romano, S. L., McMillan, W. O., Stice, L., & Grabowski, G. (1991). *The simple fool's guide to PCR*. Honolulu, Hawaii:

Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Pan, Y. T. (1984). The relation of the environment to the evolution of fossil Gastropoda from Quaternary sedimentary strata in Dianchi Lake area of Kunming. *Bulletin of the Chengdu Institute of Geology and Mineral Resources, Chinese Academy of Geological Sciences*, *5*, 111–124.

Perea, D., Soto, M., Veroslavsky, G., Martínez, S., & Ubilla, M. (2009). A Late Jurassic fossil assemblage in Gondwana: Biostratigraphy and correlations of the Tacuarembó Formation, Parana Basin, Uruguay. *Journal of South American Earth Sciences*, *28*, 168–179. https://doi.org/10.1016/j.jsames.2009.03.009

Pilsbry, H. A. (1902). Revision of Japanese Viviparidae, with notes on Melania and Bithynia. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *54*, 115–121.

Piyatiratitivorakul, P., & Boonchamoi, P. (2008). Comparative toxicity of mercury and cadmium to the juvenile freshwater snail, *Filopaludina martensi martensi*. *ScienceAsia*, *34*, 367–370. https://doi.org/10.2306/scienceasia1513-1874.2008.34.367

Ponder, W. F., Hallan, A., Shea, M., & Clark, S. A. (2016). *Australian freshwater molluscs*. Retrieved from http://keys.lucidcentral.org/keys/v3/freshwater_molluscs/

Prashad, B. (1928). Recent and fossil Viviparidae. A study in distribution, evolution and palaeogeography. *Memoirs of the Indian Museum*, *8*, 153–252.

Prozorova, L. A., Makarenko, V. P., & Balan, I. V. (2014). *Distribution of gastropods Viviparoidea (Caenogastropoda, Architaenioglossa) in the Amur River basin*. Paper presented at the Vladimir Ya. Levanidov's Biennial Memorial Meetings, Issue 6.

Rambaut, A., Drummond, A. J., & Suchard, M. (2013). TRACER *v1.6*. Retrieved from http://tree.bio.ed.ac.uk/software/tracer/

Razkin, O., Sonet, G., Breugelmans, K., Madeira, M. J., Gómez-Moliner, B. J., & Backeljau, T. (2016). Species limits, interspecific hybridization and phylogeny in the cryptic land snail complex Pyramidula: The power of RADseq data. *Molecular Phylogenetics and Evolution*, *101*, 267–278. https://doi.org/10.1016/j.ympev.2016.05.002

Rivers, D. M., Darwell, C. T., & Althoff, D. M. (2016). Phylogenetic analysis of RAD-seq data: Examining the influence of gene genealogy conflict on analysis of concatenated data. *Cladistics*, *32*, 672–681. https://doi.org/10.1111/cla.12149

Ronquist, F., & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rosenberg, N. A. (2004). DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, *4*, 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Rubinoff, D., & Holland, B. S. (2005). Between two extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Systematic Biology*, *54*, 952–961. https://doi.org/10.1080/10635150500234674

Rundle, H. D., & Schluter, D. (2004). Natural selection and ecological speciation in sticklebacks. In U. Dieckmann, M. Doebeli, J. A. J. Metz, & D. Tautz (Eds.), *Adaptive speciation* (pp. 192–209). Cambridge, UK: Cambridge University Press.

Schluter, D. (2000). *The ecology of adaptive radiation*. New York, NY: Oxford University Press.

Schultheiß, R., Van Bocxlaer, B., Riedel, F., von Rintelen, T., & Albrecht, C. (2014). Disjunct distributions of freshwater snails testify to a central role of the Congo system in shaping biogeographical patterns in Africa. *BMC Evolutionary Biology*, *14*, 42. https://doi.org/10.1186/1471-2148-14-42

Seehausen, O. (2006). African cichlid fish: A model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 1987–1998. https://doi.org/10.1098/rspb.2006.3539

Sengupta, M. E., Kristensen, T. K., Madsen, H., & Jørgensen, A. (2009). Molecular phylogenetic investigations of the Viviparidae (Gastropoda: Caenogastropoda) in the lakes of the Rift Valley area of Africa. *Molecular Phylogenetics and Evolution*, *52*, 797–805. https://doi.org/10.1016/j.ympev.2009.05.007

Simone, L. R. L. (2004). Comparative morphology and phylogeny of representatives of the superfamilies of Architaenioglossans and the Annulariidae (Mollusca, Caenogastropoda). *Arquivos do Museu Nacional, Rio de Janeiro*, *62*, 387–504.

Sivan, N., Heller, J., & Van Damme, D. (2006). Fossil Viviparidae (Mollusca: Gastropoda) of the Levant. *Journal of Conchology*, *39*, 207–219.

Stamatakis, A. (2006). RAXML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Starobogatov, Y. I., Prozorova, L. A., Bogatov, V. V., & Saenko, E. M. (2004). Molluscs. In S. Y. Tsalolikhin (Ed.), *Key to freshwater invertebrates of Russia and adjacent lands. Vol. 6. Molluscs, polychaetes, nemerteans* (pp. 10–491). Sankt-Petersburg, Russia: Nauka.

Stothard, J. R., Bremond, P., Andriamaro, L., Loxton, N. J., Sellin, B., Sellin, E., & Rollinson, D. (2000). Molecular characterization of the freshwater snail *Lymnaea natalensis* (Gastropoda : Lymnaeidae) on Madagascar with an observation of an unusual polymorphism in ribosomal small subunit genes. *Journal of Zoology*, *252*, 303–315. https://doi.org/10.1111/j.1469-7998.2000.tb00625.x

Strong, E. E., Gargominy, O., Ponder, W. F., & Bouchet, P. (2008). Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. *Hydrobiologia*, *595*, 149–166. https://doi.org/10.1007/s10750-007-9012-6

Sturmbauer, C., & Meyer, A. (1992). Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature*, *358*, 578–581. https://doi.org/10.1038/358578a0

Suyama, Y., & Matsuki, Y. (2015). MIG-seq: An effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Scientific Reports*, *5*, 16963. https://doi.org/10.1038/srep16963

Tabata, R., Kakioka, R., Tominaga, K., Komiya, T., & Watanabe, K. (2016). Phylogeny and historical demography of endemic fishes in Lake Biwa: The ancient lake as a promoter of evolution and diversification of freshwater fishes in western Japan. *Evolution and Ecology*, *6*, 2601–2623. https://doi.org/10.1002/ece3.2070

Takahashi, T., & Moreno, E. (2015). A RAD-based phylogenetics for Orestias fishes from Lake Titicaca. *Molecular Phylogenetics and Evolution*, *93*, 307–317. https://doi.org/10.1016/j.ympev.2015.08.012

Tamaki, I., Yoichi, W., Matsuki, Y., Suyama, Y., & Mizuno, M. (2017). Inconsistency between morphological traits and ancestry of individuals in the hybrid zone between two *Rhododendron japonoheptamerum* varieties revealed by a genotyping-by-sequencing approach. *Tree Genetics & Genomes*, *13*, 4. https://doi.org/10.1007/s11295-016-1084-x

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739. https://doi.org/10.1093/molbev/msr121

Tanabe, A. S. (2011). Kakusan4 and Aminosan: Two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources*, *11*, 914–921. https://doi.org/10.1111/j.1755-0998.2011.03021.x

Tracey, S., Todd, J. A., & Erwin, D. H. (1993). Mollusca: Gastropoda. In M. J. Benton (Ed.), *The Fossil record* (pp. 125–130). London, UK: Chapman and Hall.

Tsuda, Y., Nakao, K., Ide, Y., & Tsumura, Y. (2015). The population demography of *Betula maximowicziana*, a cool-temperate tree species in Japan, in relation to the last glacial period: Its admixture-like genetic structure is the result of simple population splitting not

admixing. *Molecular Ecology*, *24*, 1403–1418. https://doi.org/10.1111/mec.13123

Van Bocxlaer, B. (2017). Hierarchical structure of ecological and non-ecological processes of differentiation shaped ongoing gastropod radiation in the Malawi Basin. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20171494. https://doi.org/10.1098/rspb.2017.1494

Van Bocxlaer, B., & Hunt, G. (2013). Morphological stasis in an ongoing gastropod radiation from Lake Malawi. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 13892–13897. https://doi.org/10.1073/pnas.1308588110

Van Bocxlaer, B., & Strong, E. E. (2016). Anatomy, functional morphology, evolutionary ecology and systematics of the invasive gastropod *Cipangopaludina japonica* (Viviparidae: Bellamyinae). *Contributions to Zoology*, *85*, 235–263. https://doi.org/10.1163/18759866-08502005

Van Bocxlaer, B., Strong, E. E., Richter, R., Stelbrink, B., & von Rintelen, T. (2018). Anatomical and genetic data reveal that *Rivularia* Heude, 1890 belongs to Viviparinae (Gastropoda: Viviparidae). *Zoological Journal of the Linnean Society*, *182*, 1–23. https://doi.org/10.1093/zoolinnean/zlx014

Van Damme, D., Bogan, A. E., & Dierick, M. (2015). A revision of the Mesozoic naiads (Unionoida) of Africa and the biogeographic implications. *Earth-Science Reviews*, *147*, 141–200. https://doi.org/10.1016/j.earscirev.2015.04.011

Van Damme, D., & Gautier, A. (2013). Lacustrine mollusc radiations in the Lake Malawi Basin: Experiments in a natural laboratory for evolution. *Biogeosciences*, *10*, 5767–5778. https://doi.org/10.5194/bg-10-5767-2013

Vermeji, G. J. (1983). Shell-breaking predation through time. In M. J. S. Tevesz, & P. L. McCall (Eds.), *Biotic interactions in recent and fossil benthic communities* (pp. 649–669). New York, NY: Springer.

von Rintelen, T., Wilson, A., Meyer, A., & Glaubrecht, M. (2004). Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. *Proceedings of the Royal Society B: Biological Sciences*, *271*, 2541–2549. https://doi.org/10.1098/rspb.2004.2842

Wade, C., & Mordan, P. (2000). Evolution within the gastropod molluscs: Using the ribosomal RNA gene cluster as an indicator of phylogenetic relationships. *Journal of Molluscan Studies*, *66*, 565–570.

Wang, J. G., Zhang, D., Jakovlić, I., & Wang, W. M. (2017). Sequencing of the complete mitochondrial genomes of eight freshwater snail species exposes pervasive paraphyly within the Viviparidae family (Caenogastropoda). *PLoS ONE*, *12*, e0181699. https://doi.org/10.1371/journal.pone.0181699

Wang, S. M., & Dou, H. S. (1998). *The records of Chinese lake*. Beijing, China: Science Press.

Yamamori, L., & Kato, M. (2018). Morphological and ecological adaptation of limpet-shaped top shells (Gastropoda: Trochidae: Fossarininae) to wave-swept rock reef habitats. *PLoS ONE*, *13*, e0197719. https://doi.org/10.1371/journal.pone.0197719

Yamazaki, T., & Goshima, S. (2012). Adaptive differences in morphology of the periwinkle *Littorina sitkana* between two habitats separated by an artificial structure. *Venus*, *70*, 1–10. https://doi.org/10.18941/venus.70.1-4_1

Yen, T. C. (1950). *Fresh-water mollusks of Cretaceous age from Montana and Wyoming. Geological survey professional paper 233-A*. Washington, DC: United States Government Printing Office.

Ying, T., Fürsich, F. T., & Schneider, S. (2013). Giant Viviparidae (Gastropoda: Architaenioglossa) from the Early Oligocene of the Nanning Basin (Guangxi, SE China). *Neues Jahrbuch für Geologie und Paläontologie*, *267*, 75–87. https://doi.org/10.1127/0077-7749/2012/0298

Yokoyama, T. (1984). Stratigraphy of the Quaternary system around Lake Biwa and geohistory of the ancient Lake Biwa. In S. Horie (Ed.), *Lake Biwa*, *Monographiae Biologicae* (Vol. *54*, pp. 43–128). Dordrecht, The Nederland: Dr W Junk Publishers.

Yu, Y., Than, C., Degnan, J. H., & Nakhleh, L. (2011). Coalescent histories on phylogenetic networks and detection of hybridization despite incomplete lineage sorting. *Systematic Biology*, *60*, 138–149. https://doi.org/10.1093/sysbio/syq084

Zhang, L. J., Chen, S. C., Yang, L. T., Jin, L., & Köhler, F. (2015). Systematic revision of the freshwater snail *Margarya* Nevill, 1877 (Mollusca: Viviparidae) endemic to the ancient lakes of Yunnan, China, with description of new taxa. *Zoological Journal of the Linnean Society*, *174*, 760–800. https://doi.org/10.1111/zoj.12260

Zhang, N. G., Hao, T. X., Wu, C. Y., Chen, Y. X., Zhang, W., Li, J. F., & Zhang, Y. (1997). Primary investigation of freshwater Gastropoda in Yunnan Province. *Studia Marina Sinica*, *39*, 15–26.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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