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# Diversification in ancient Lake Biwa: integrative taxonomy reveals overlooked species diversity of the Japanese freshwater snail genus *Semisulcospira* (Mollusca: Semisulcospiridae)

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RECEIVED: 17 MAY 2022 | REVISED AND ACCEPTED: 27 JULY 2022 | PUBLISHED ONLINE  
22 AUGUST 2022; PUBLISHED IN ISSUE 23 JANUARY 2023

EDITOR: D. HUANG

## Abstract

The freshwater snail genus *Semisulcospira* has adaptively radiated in the ancient Lake Biwa, Japan. Although significant geographical variations have been known in the shell morphology of *Semisulcospira niponica*, their systematics remain uncertain. We evaluated the systematic status of the geographical variations in *S. niponica* and its congeners *S. biwae* and *S. fuscata* through morphological and genetic investigations. Genome-wide SNPs were used to estimate their genetic relationships. Random Forest algorithms explored the morphological variation of the shells of the name-bearing types and newly collected specimens. Our results detected five genetic clusters and revealed the consistency of adult shell morphology with genetic relationships, reinforcing the robustness of shell morphology-based species delimitation of the genus. The present systematics synonymized *S. biwae* under *S. niponica*. The five genetic clusters corresponded to the two described species, *S. niponica* and *S. fuscata*, and three new species, *Semisulcospira watanabei* sp. nov., *Semisulcospira nakanoi* sp. nov., and *Semisulcospira salebrosa* sp. nov. discovered among the geographical variations of *S. niponica*. The observed similarities in substrate preference and differences in distribution patterns among species suggest that the diversification of the genus was accelerated by geographical isolation after the initial ecological niche differentiation. We suggest that the distributions of *Semisulcospira* species may have been influenced by competitive exclusion among them, and that multiple speciation events have occurred in similar patterns.

## Keywords

adaptive radiation – Caenogastropoda – classification – MIG-seq – next-generation sequencing

## Introduction

*Semisulcospira* Boettger, 1886 is a genus of freshwater snails widespread in Japan, Korea, Taiwan, and China (Davis, 1969; Du et al., 2019). Members of the genus share the synapomorphy of viviparous reproductive mode (Strong & Köhler, 2009). The genus contains 28 extant species, of which 16 are endemic to the Lake Biwa water system (Watanabe & Nishino, 1995; Sawada & Nakano, 2021; MolluscaBase, 2022).

It has been revealed that these lacustrine species consist of two phylogroups, *S. habei*- and *S. decipiens*-groups and that the past ecological niche differentiation caused by lake expansion accelerated their diversification (Nomoto, 2001; Kamiya et al., 2011; Miura et al., 2019).

The description of *S. niponica* (Smith, 1876) was the initial record of *Semisulcospira* in Lake Biwa. The species was discriminated from other known Japanese congeners by pronounced nodes on a conical shell, exhibiting variations in shell elongation and sculptures (Smith, 1876). The second oldest species, *S. biwae* (Kobelt, 1879), was described three years later based on a single specimen with fewer node numbers than in *S. niponica*. In the 1900s, snails with shell morphology similar to *S. biwae* were recorded in the lake on Chikubu-shima Island (Annandale, 1916). In addition, Kuroda (1929) treated the species as an intraspecific variation of *S. niponica*, and this was supported by later studies (e.g., Kuroda & Habe, 1965; Watanabe, 1984).

Geographical variations in the shell morphology of *S. niponica* were evaluated by Watanabe (1984). Subsequently, Watanabe

and Nishino (1995) identified three geographical variations of *S. niponica* based on the characteristics of shell sculpture (hereafter “sculpture type”): the “nodose” type common in the southern basin of the lake, the “ribbed” type common in the northern basin, and the “*biwae*” type distributed on lake islands. In addition, the work described *S. fuscata* Watanabe & Nishino, 1995, whose adult shells are almost smooth, despite having a similar embryonic shell morphology to the three morphotypes of *S. niponica*. In the 2000s, molecular phylogenetic approaches have detected a sister group formed by *S. niponica* and *S. fuscata* (hereafter “*S. niponica*-group”) and several divergent lineages in *S. niponica* (Kamiya et al., 2011; Miura et al., 2019). However, the relationships between these lineages, morphotypes and their systematic status remain unclear.

Taxonomic diagnoses of the lacustrine species were established by a comprehensive examination of adult and embryonic shell morphologies (Davis, 1969; Watanabe & Nishino, 1995; Sawada & Nakano, 2021). Recent genome-wide SNP analyses based on the restriction site-associated DNA sequencing (RAD-seq) have elucidated that the previous shell morphology-based species delimitation is concordant with the nuclear phylogeny (Miura et al., 2019). In contrast, significant incongruence was observed between the nuclear and mitochondrial phylogenies (Köhler, 2016; Miura et al., 2020).

In this study, we revisited the systematic status of the *S. niponica*-group members, focusing on the relationships among their geographical variations in shell characters and genetic relationships. In addition to the morphological

examination of the name-bearing types, we conducted investigations on adult and embryonic shells, radula, genitalia morphology, population genetic structure, and molecular phylogenetic relationships of the newly collected specimens. The present results clarified the genetic relationships among the three morphotypes of *S. niponica*, highlighting the overlooked species diversity within the group. The systematics of the *S. niponica*-group species were successfully elucidated, and new insights into their morphology, distribution, and evolutionary history are provided.

## Material and methods

### Samples

In total, 301 *Semisulcospira* specimens were newly collected by the first author via skin diving from 15 localities in Lake Biwa, central Japan, covering the distribution of *S. fuscata*

and the geographical variations of *S. niponica* (fig. 1). The specimens were identified following Davis (1969) and Watanabe and Nishino (1995). The following specimens were used for morphological examination: 80 females and 42 males of *S. niponica*, 38 females and five males of *S. fuscata*, 36 females and 15 males of *S. watanabei* sp. nov., 31 females and eight males of *S. nakanoi* sp. nov., 30 females and 14 males of *S. salebrosa* sp. nov., and one putative female hybrid between *S. fuscata* and *S. watanabei* sp. nov. (supplementary tables S1 and S2). Among them, 31 snails of *S. niponica*, 17 of *S. fuscata*, 13 of *S. watanabei* sp. nov., 10 of *S. nakanoi* sp. nov., 24 of *S. salebrosa* sp. nov., and one hybrid were used for the genetic analyses (table 1). In addition, one juvenile of *S. watanabei* sp. nov. collected from Oura was included in the genetic investigation. Except at Iso and Kitakomatsu Beach, all the snails were collected from piled rock, rocky bottoms, or harbours around the lakeside or islands at

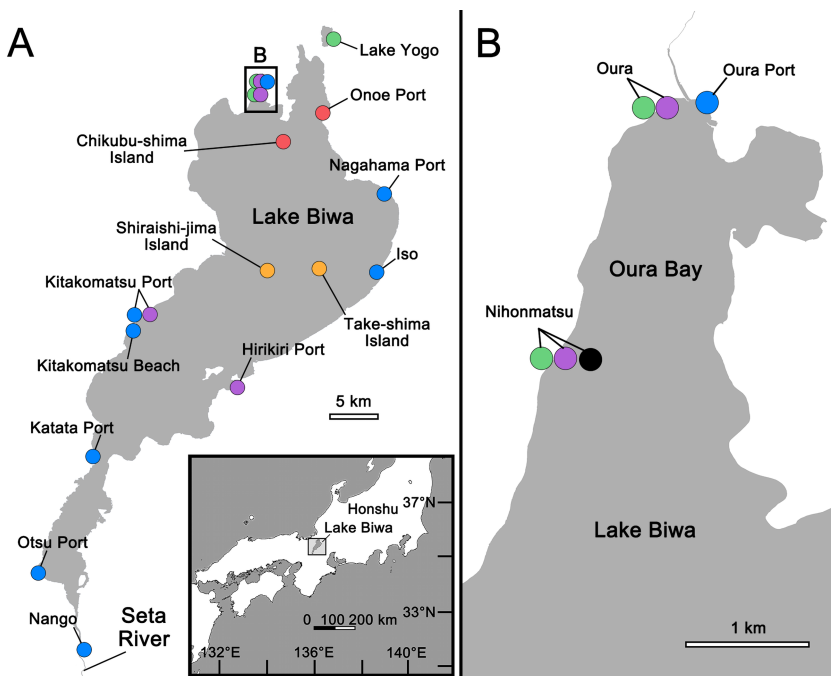


FIGURE 1 Map of Lake Biwa (A) and Oura Bay (B) showing 15 sampling localities: blue, *Semisulcospira niponica*; green, *S. fuscata*; purple, *S. watanabei* sp. nov.; red, *S. nakanoi* sp. nov.; orange, *S. salebrosa* sp. nov.; black, putative hybrid between *S. fuscata* and *S. watanabei* sp. nov.

TABLE 1 Specimen list of *Semisulcospira* species with voucher numbers, collection localities, and accession numbers of DDBJ Sequence Read Archive (DRA) for specimens used for the phylogenetic analysis

Voucher #	Collection locality	DRA accession #
<i>Semisulcospira niponica</i>		
NHM 1875.11.24.11/1 (lectotype)	Lake Biwa, near Kiyoto (Smith, 1876)	
NHM 1875.11.24.11/2, 1875.11.24.11/3 (paralectotypes)	Lake Biwa, near Kiyoto (Smith, 1876)	
KUZ Z3959, Z3766	Lake Biwa, Otsu Port, Otsu City, Shiga Prefecture, Japan	DRR360355– DRR360359
KUZ Z4091, Z4092	Lake Biwa, Oura Port, Nagahama City, Shiga Prefecture, Japan	DRR360360– DRR360362
KUZ Z4093, Z4094	Lake Biwa, Nagahama Port, Nagahama City, Shiga Prefecture, Japan	DRR360330– DRR360334
KUZ Z4095, Z4096	Lake Biwa, Iso, Maibara City, Shiga Prefecture, Japan	DRR360314– DRR360319
KUZ Z4097	Lake Biwa, Kitakomatsu Port, Otsu City, Shiga Prefecture, Japan	DRR360324, DRR360326
KUZ Z4098	Lake Biwa, Kitakomatsu Beach, Otsu City, Shiga Prefecture, Japan	DRR360329
KUZ Z4099, Z4100	Lake Biwa, Katata Port, Otsu City, Shiga Prefecture, Japan	DRR360320– DRR360323
KUZ Z4101, Z4102	Seta River, Nango, Otsu City, Shiga Prefecture, Japan	DRR360335– DRR360339
<i>Semisulcospira biwae</i>		
SMF 29151 (holotype)	Biwa-see (Kobelt, 1879)	
<i>Semisulcospira fuscata</i>		
LBM 13-1 (holotype)	Lake Biwa, Oura, Nagahama City, Shiga Prefecture, Japan (Watanabe & Nishino, 1995)	
KUZ Z4103, Z4104	Lake Yogo, Nagahama City, Shiga Prefecture, Japan	DRR360393– DRR360399
KUZ Z4105, Z4106	Lake Biwa, Oura, Nagahama City, Shiga Prefecture, Japan	DRR360363– DRR360367
KUZ Z4107, Z4108	Lake Biwa, Nihonmatsu, Nagahama City, Shiga Prefecture, Japan	DRR360340– DRR360344
<i>Semisulcospira watanabei</i> sp. nov.		
KUZ Z4109 (holotype)	Lake Biwa, Kitakomatsu Port, Otsu City, Shiga Prefecture, Japan	DRR360325
KUZ Z4110–Z4114 (paratypes)	Lake Biwa, Kitakomatsu Port, Otsu City, Shiga Prefecture, Japan	DRR360328
KUZ Z4115, Z4116	Lake Biwa, Kitakomatsu Port, Otsu City, Shiga Prefecture, Japan	DRR360327

TABLE 1 Specimen list of *Semisulcospira* species with voucher numbers, collection localities, and accession numbers of DDBJ Sequence Read Archive (DRA) for specimens used for the phylogenetic analysis (*cont.*)

Voucher #	Collection locality	DRA accession #
KUZ Z4117	Lake Biwa, Oura, Nagahama City, Shiga Prefecture, Japan	DRR360368
KUZ Z4118, Z4119	Lake Biwa, Nihonmatsu, Nagahama City, Shiga Prefecture, Japan	DRR360345, DRR360347– DRR360349
KUZ Z4120, Z4121	Lake Biwa, Horikiri Port, Omihachiman City, Shiga Prefecture, Japan	DRR360309– DRR360313
<i>Semisulcospira nakanoi</i> sp. nov.		
KUZ Z4122 (holotype)	Lake Biwa, Chikubu-shima Island, Nagahama City, Shiga Prefecture, Japan	DRR360304
KUZ Z4123–Z4126 (paratypes)	Lake Biwa, Chikubu-shima Island, Nagahama City, Shiga Prefecture, Japan	DRR360308
KUZ Z4127, Z4128	Lake Biwa, Chikubu-shima Island, Nagahama City, Shiga Prefecture, Japan	DRR360305– DRR360307
KUZ Z4129, Z4130	Lake Biwa, Onoe Port, Nagahama City, Shiga Prefecture, Japan	DRR360350– DRR360354
<i>Semisulcospira salebrosa</i> sp. nov.		
KUZ Z4131 (holotype)	Lake Biwa, Shiraishi-jima Island, Takashima City, Shiga Prefecture, Japan	DRR360369
KUZ Z4132–4135 (paratypes)	Lake Biwa, Shiraishi-jima Island, Takashima City, Shiga Prefecture, Japan	DRR360371
KUZ Z4136, 4137	Lake Biwa, Shiraishi-jima Island, Takashima City, Shiga Prefecture, Japan	DRR360370, DRR360372, DRR360373
KUZ Z4138, 4139	Lake Biwa, Take-shima Island, Hikone City, Shiga Prefecture, Japan	DRR360374– DRR360392
A putative hybrid between <i>S. fuscata</i> and <i>S. watanabei</i> sp. nov.		
KUZ Z4140	Lake Biwa, Nihonmatsu, Nagahama City, Shiga Prefecture, Japan	DRR360346

a water depth of 0–3 m. *Semisulcospira niponica* was obtained from stones on the sandy beach at a water depth of 0–1 m at Iso and Kitakomatsu Beach.

Sexual dimorphism and allometric growth have been known in the adult shell of *S. niponica* (Sawada & Nakano, 2022). Accordingly, the largest 1–16 fully mature adults of each sex in

each population were morphologically examined where possible. Adult and embryonic shells, radulae, and reproductive organs were separated, cleaned, and observed following the method described by Sawada and Nakano (2021). The foot tip was cut off and preserved in 99% ethanol for genetic analyses. The algae on the adult shell surface were removed with

3% sodium hypochlorite following Sawada et al. (2021). The newly collected specimens were deposited in the Zoological Collection of Kyoto University, Kyoto (KUZ). Morphological examinations of the syntypes of *S. niponica* preserved in the Mollusca Collections at Natural History Museum, London (NHM), the holotype of *S. biwae* in the Malacological Collection at Senckenberg Naturmuseum, Frankfurt (SMF), the holotype of *S. fuscata* in the Lake Biwa Museum, Kusatsu (LBM), and *S. niponica* collected from Otsu Port deposited in the KUZ were also conducted.

#### *DNA extraction, library preparation and sequencing*

Genomic DNA was extracted from 96 snails using the NucleoSpin DNA RapidLyse Kit (Macherey-Nagel GmbH & Co., Düren, Germany). The phylogenetic relationships of the *Semisulcospira* species were estimated using Multiplexed ISSR Genotyping by sequencing (MIG-seq) analyses (Suyama & Matsuki, 2015). We followed Onuki and Fuku (in press) for sequencing methods and primer sets, and Watanabe et al. (2020) and Suyama et al. (2022) for MIG-seq library preparation methods. Pooled libraries were outsourced for 150 bp paired-end sequencing by Illumina NovaSeq 6000 to Novogene. The raw MIG-seq data were deposited in DDBJ Sequence Read Archive (accession number: DRA013885).

#### *SNP detection*

The raw data were demultiplexed using the “process\_shortreads” programme in Stacks 2.59 (Rochette et al., 2019). Low-quality bases ( $< Q 30$ ) and the adapter sequences were removed using fastp 0.20.1 (Chen et al., 2018). The read length was trimmed to 109 bp to match the shorter Read 1. SNP detection was performed on quality-controlled reads using the “Denovo\_map.pl” pipeline of Stacks with

the following settings: “paired-end” mode; the minimum depth of coverage was set to five ( $m = 5$ ), and the maximum allowable number of substitutions between stacks was set to three ( $M = 3$ ). SNP filtering and output were performed using populations with the following settings: only one SNP from a locus (“--write-single-snp”) common to more than 50% of all samples ( $R = 0.5$ ) retained; SNPs with heterozygosity greater than 75% (“--max-obs-het” = 0.75) and minor alleles less than two (“--min-mac” = 2) excluded. All other parameters were set to the default setting.

#### *Population structure and phylogenetic analyses*

Principal component analysis (PCA) was performed to estimate population genetic structure using PLINK v1.90b6.24 (Purcell et al., 2007). We evaluated individual admixture proportions via the likelihood model-based clustering using ADMIXTURE v1.3.0 (Alexander et al., 2009) with the following setting: the number of genetic populations ( $K$ ) was set 1–10; the convergence criterion ( $C$ ) was set 0.0001. These analyses were repeated 100 times with random seeds, and the optimal  $K$ -value was estimated based on the lowest mean cross-validation error value for each  $K$  calculated in ADMIXTURE. Estimated admixture proportions were visualized using the seed value for  $K = 2–5$ .

Neighbour-net was reconstructed to estimate the phylogenetic relationships via Splitstree 4.15.1 (Huson & Bryant, 2006). The genetic distance was calculated by the “Uncorrected P” algorithm, and the handling of ambiguous sites was set as “MatchState”.

#### *Morphological examination*

The sample numbers of adult and embryonic shells, radulae and reproductive organs from each locality used for the morphological

analyses are shown in supplementary tables S1 and S2.

**Adult and embryonic shell:** Morphometric characters were measured following the methods in Sawada and Nakano (2021; fig. 2). However, the spire angle was measured as an angle between the lines connecting the most prominent points on the body to third whorls where the ridge created by the sculpture is absent (fig. 2A). In addition, the greatest distance in the swell on the aperture outer lip was measured as aperture swell length. The classification of Watanabe and Nishino (1995) was used to determine the sculpture types of the snails from the lakeside. The criteria for the classification were modified as follows (fig. 2B–C): nodose type, ribs granulated, or nodes connected by spiral cords on penultimate whorl; ribbed type, nodes completely or mostly longitudinally ribbed on penultimate whorl; *biwae* type, collected from three islands (Chikubu-shima, Take-shima, and Shirai-shima islands). Specimen with fine growth lines instead of sculptures on the penultimate whorl were also classified as the “smooth” type (fig. 2D).

Abbreviations of morphological characters examined are as follows: Adult shell: AH, aperture height; AL, aperture length; ASL, aperture

swell length; ASR, aperture slenderness ratio (the proportion of aperture length to fourth aperture width); AW, aperture width; BCN, basal cord number; BWL, body whorl length; FWL, fourth whorl length; PWL, penultimate whorl length; RN, longitudinal rib number of penultimate whorl; SA, spire angle; SCN, spiral cord number of penultimate whorl; SH, shell height; SW, shell width; TWL, third whorl length; WER, whorl elongation ratio (the proportion of aperture height to fourth whorl length); WN, whorl number. Embryonic shell: EN, number of embryos; RNE, longitudinal rib number on body whorl of the largest embryonic shell; SHE, shell height of the largest embryonic shell; SWE, shell width of the largest embryonic shell; WNE, whorl number of the largest embryonic shell.

**Operculum, radula, and genitalia:** Measurement of the operculum was conducted with the five largest females in each population. The number of dental cusps and the proportion of denticle lengths of radula were obtained. The external morphology of the reproductive organs was observed, focusing on the protrusion length in the seminal receptacle following Prozorova and Rasshepkina (2006) and Sawada and Nakano (2021).

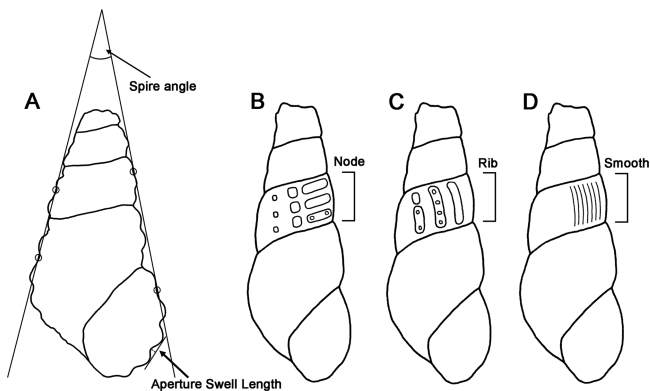


FIGURE 2 Schematic drawings representing shell measurements of *Semisulcospira* species in this study. A, Spire angle (SA) and aperture swell length (ASL) of adult shell. B–D, Criteria for types of sculptures

### Random Forest classification

Random Forest (RF) classification was used for exploring the morphological variation among the groups discriminated by the present genetic analyses. RF is a machine learning algorithm using tree predictors generated by bootstrap sampling and applicable to data with categorical variables, such as the current dataset (Breiman, 2001). Females and males were used separately for analyses to reduce the variation attributed to sexual dimorphism. Twelve characters were used for females (ASL, ASR, BCN, BWL, RN, SA, SCN, WER, WN, sculpture type, EN, SHE) and 10 for males (ASL, ASR, BCN, BWL, RN, SA, SCN, WER, WN, sculpture type). Sculpture types of the specimen from Chikubu-shima, Take-shima, and Shiraishijima islands were identified morphologically with the criteria mentioned above. The three sculpture types (node, rib, smooth) were identified as variants for the RF analyses.

Three variables were randomly sampled for each split as candidates in the RF classification. A total of 5000 trees was generated, given that the out-of-bag (OOB) error rate was fully decreased with a large number of trees. After training the RF, the morphological similarity of a putative hybrid to each species was predicted. The morphological

similarity of a lectotype of *S. niponica* and a holotype of *S. biwae* were also predicted with another trained RF because the exact type locality of the species could not be obtained from the type labels (see Systematic description). The prediction of the type specimens was conducted with the RF trees trained by all females and males of each species using nine characters (ASL, ASR, BCN, BWL, RN, SA, SCN, WN, sculpture type). The missing values were replaced by the population average. The proximities among individuals were converted to Euclidean distances to visualize the morphological relationships among the groups. Measurements of morphological characters were obtained with ImageJ v. 1.51 (Schneider et al., 2012). The RF analyses were conducted with the package randomForest v. 4.6–14 (Andy & Matthew, 2002) for R v. 3.6.1 (R Development Core Team, 2019).

## Results

### Genetic analyses

A total of 738 SNPs were obtained from the 96 snails sequenced. The PCA generated twenty PCs based on all SNPs, and the first five PCs explained 46.94% of the total variation (fig. 3).

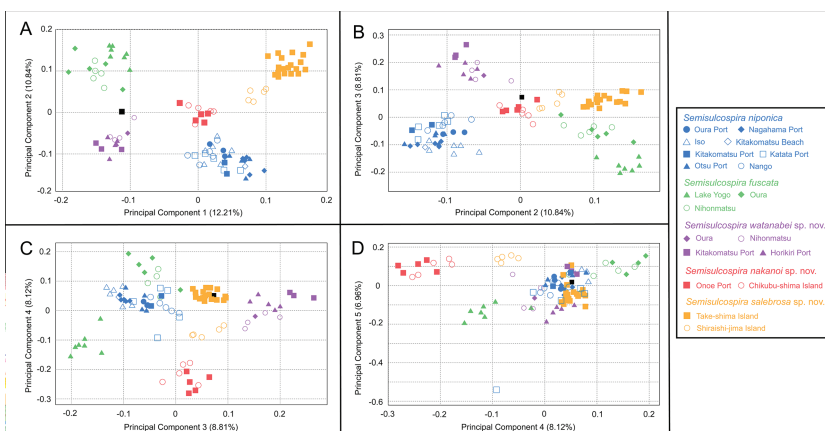


FIGURE 3 Results of principal components analysis based on 738 SNPs conducted for five *Semisulcospira* species. A, Principal component (PC) 1 vs PC2. B, PC2 vs PC3. C, PC3 vs PC4. D, PC4 vs PC5

PC1, PC2 and PC3 separated the specimen into five groups, *S. niponica*, *S. fuscata*, *S. watanabei* sp. nov., *S. nakanoi* sp. nov., and *S. salebrosa* sp. nov. The ADMIXTURE analysis found low mean cross-validation error values for 2–5 genetic populations while the optimal number of clusters was four (supplementary table S3). The clustering by the ADMIXTURE analysis at  $K=5$  corresponded to the five species discovered by the PCA (fig. 4). The reconstructed Neighbor-net showed the molecular phylogenetic relationships of five species, concordant with the PCA and ADMIXTURE analysis (fig. 5).

The present results estimated that *S. niponica* and *S. watanabei* sp. nov. are distributed at lake coasts. The specimens from both coastal Onoe Port and Chikubu-shima Island belonged to *S. nakanoi* sp. nov. and *S. salebrosa* sp. nov. consisted of the two insular populations. The populations of both Lake Biwa and Lake Yogo belonged to *S. fuscata*. The snails from a single population were estimated to originate from a single species in most sites, whereas two phylogenies were found sympatrically in Oura, Nihonmatsu, and Kitakomatsu

Port. The Neighbor-net estimated a close relationship among the snails within populations of *S. niponica* from Oura Port and Kitakomatsu Port, *S. fuscata* from Lake Yogo and three other species from all sites. In contrast, this was not the case with the other populations of *S. niponica* and *S. fuscata*. The results of the three analyses estimated that an individual from Nihonmatsu had intermediate genetic components between *S. fuscata* and *S. watanabei* sp. nov. (figs. 3A, 4, 5)

### Morphological analyses

Measurements and counts obtained from the adult and embryonic shells, radula, and genitalia and the classification of sculpture types are shown in table 2 and supplementary tables S1 and S2. Sculpture types mainly corresponded to the genetic relationships of three species: nodose type, *S. niponica*; ribbed type, *S. watanabei* sp. nov.; smooth type, *S. fuscata* (table 2). However, the *biwae* type was divided into *S. nakanoi* sp. nov. and *S. salebrosa* sp. nov. Several sculpture types were present sympatrically at eight localities (fig. 6). The dominant types were consistent with the lineages

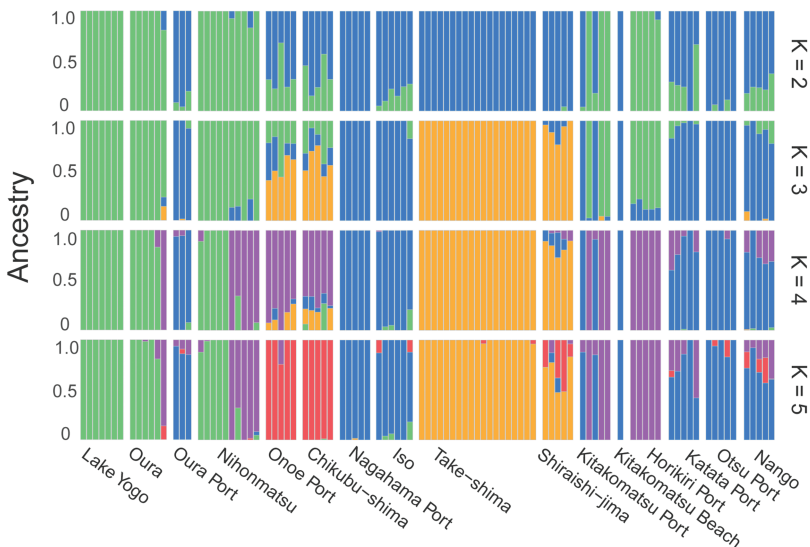


FIGURE 4 Results of ADMIXTURE analysis based on 738 SNPs conducted for five *Semisulcospira* species. Bar colours of the species in  $K=5$  correspond to the colour coding in fig. 3

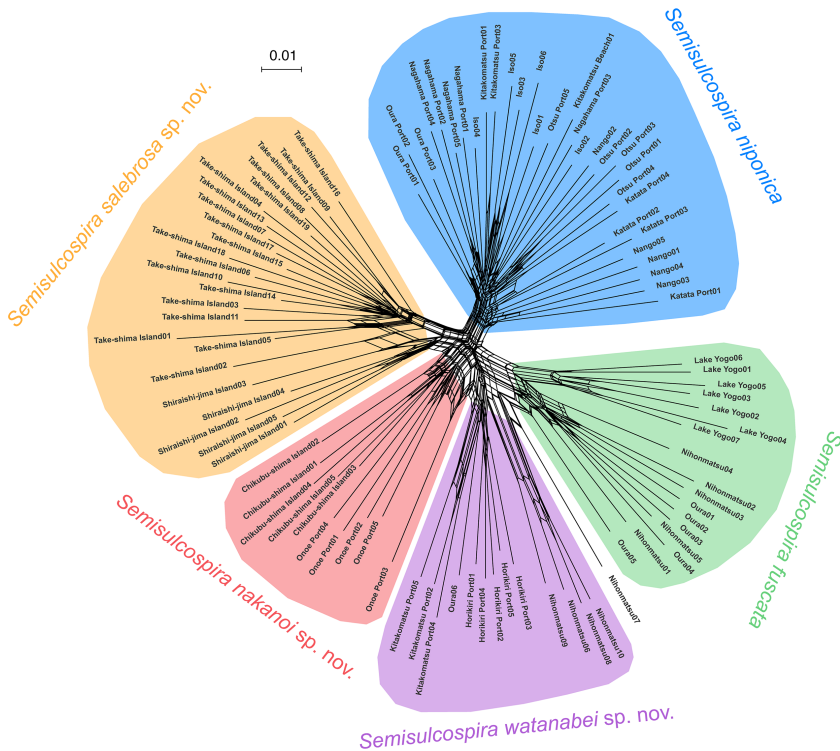


FIGURE 5 Phylogenetic relationships of five *Semisulcospira* species estimated by the Neighbor-Net reconstructed based on uncorrected *p*-distances of 738 SNPs

of the populations except in Nihonmatsu and Kitakomatsu Port. At the latter two sites, where two species coexisted, sculpture type differences were largely congruent with the genetic differences.

Although the number of dental cusps and the proportion of denticle lengths of radulae varied among the snails, no significant inter-specific variation was detected. The length of the protrusions in the seminal receptacle of the female reproductive organs represented intraspecific variation. They were discordant with the genetic relationships of the snails.

The RF analyses correctly classified 82.4% of females into five species discriminated by the present genetic analyses. Bootstrap samplings identified 90.4% of *S. niponica*, 73.0% of *S. fuscata*, 61.1% of *S. watanabei* sp. nov., 83.9% of *S. nakanoi* sp. nov., and 93.3% of *S. salebrosa* sp. nov. Ten characters accurately

distinguished 84.5% of the males of the five species: 96.4% of *S. niponica*, 60% of *S. fuscata*, 86.7% of *S. watanabei* sp. nov., 53.8% of *S. nakanoi* sp. nov., and 71.4% of *S. salebrosa* sp. nov.

The contributions of each variable for the classification of each species are shown in table 3. In the females, the Gini coefficients of ASL, BWL, SA, and sculpture type were larger, significantly contributing to the classification. These four characters were significant for the morphological discrimination of each species: ASL, sculpture type, and SA for *S. niponica*; sculpture type and ASL for *S. fuscata*; sculpture type, SA, and BWL for *S. watanabei* sp. nov.; ASL, sculpture type, and SA for *S. nakanoi* sp. nov.; ASL and BWL for *S. salebrosa* sp. nov. The measurements of ASL were smaller in *S. fuscata*, larger in *S. nakanoi* sp. nov. and *S. salebrosa* sp. nov., and intermediate in

TABLE 2 Morphometric characters of five *Semisulcospira* species examined in the present study. Measurements and counts: minimum–maximum value (mean  $\pm$  SD)

Characters	<i>S. niponica</i>	<i>S. fuscata</i>	<i>S. watanabei</i> sp. nov.	<i>S. nakanoi</i> sp. nov.	<i>S. salebrosa</i> sp. nov.
Female					
Aperture height (AH)	7.7–12.6 (10.2 $\pm$ 0.9)	8.4–11.7 (10.3 $\pm$ 1.0)	6.9–11.8 (9.5 $\pm$ 0.9)	9.6–12.3 (11.1 $\pm$ 0.8)	11.4–14.8 (13.2 $\pm$ 1.0)
Aperture length (AL)	8.3–12.9 (10.8 $\pm$ 1.0)	8.5–12.1 (10.6 $\pm$ 0.9)	7.7–11.9 (10.0 $\pm$ 0.8)	10.1–13.5 (11.8 $\pm$ 0.9)	11.7–15.6 (13.7 $\pm$ 1.0)
Aperture swell length (ASL)	0–0.14 (0.06 $\pm$ 0.04)	0–0.12 (0.04 $\pm$ 0.04)	0–0.23 (0.06 $\pm$ 0.05)	0.11–0.31 (0.20 $\pm$ 0.05)	0.27–0.82 (0.44 $\pm$ 0.12)
Aperture slenderness ratio (ASR)	1.34–1.84 (1.6 $\pm$ 0.1)	1.63–1.91 (1.8 $\pm$ 0.1)	1.52–1.95 (1.7 $\pm$ 0.1)	1.32–1.74 (1.6 $\pm$ 0.1)	1.47–1.90 (1.6 $\pm$ 0.1)
Aperture width (AW)	5.0–8.2 (6.6 $\pm$ 0.7)	5.0–6.9 (6.0 $\pm$ 0.5)	4.7–7.1 (5.9 $\pm$ 0.5)	6.0–8.6 (7.4 $\pm$ 0.7)	6.7–10.2 (8.5 $\pm$ 0.9)
Basal cord number (BCN)	2–5 (3.3 $\pm$ 1.0)	2–6 (3.9 $\pm$ 1.0)	2–4 (2.8 $\pm$ 0.8)	2–3 (2.5 $\pm$ 0.5)	2–5 (2.5 $\pm$ 0.7)
Body whorl length (BWL)	12.9–21.2 (17.3 $\pm$ 1.6)	13.9–19.4 (17.1 $\pm$ 1.7)	11.9–19.3 (15.9 $\pm$ 1.3)	15.9–21.6 (18.9 $\pm$ 1.3)	19.3–24.4 (21.9 $\pm$ 1.5)
Fourth whorl length (FWL)	2.4–5.2 (3.5 $\pm$ 0.5)	2.4–4.8 (3.6 $\pm$ 0.6)	2.3–4.6 (3.5 $\pm$ 0.5)	2.7–4.6 (3.9 $\pm$ 0.4)	3.8–5.3 (4.4 $\pm$ 0.4)
Penultimate whorl length (PWL)	4.4–8.2 (6.3 $\pm$ 0.8)	4.9–7.5 (6.2 $\pm$ 0.8)	4.4–7.4 (5.7 $\pm$ 0.6)	5.5–8 (7.0 $\pm$ 0.5)	7.1–9.0 (7.9 $\pm$ 0.5)
Longitudinal rib number of penultimate whorl (RN)	9–20 (13.1 $\pm$ 2.1)	9–18 (13.8 $\pm$ 3.0)	7–16 (11.1 $\pm$ 1.8)	8–14 (11.3 $\pm$ 1.3)	8–14 (11.1 $\pm$ 1.6)
Spire angle (SA)	16.5–28.9 (23.2 $\pm$ 2.7)	15.6–24.7 (19.2 $\pm$ 2.1)	16.7–28.4 (21.1 $\pm$ 2.7)	17.2–26.7 (21.5 $\pm$ 2.6)	17.0–27.7 (21.2 $\pm$ 2.5)
Spiral cord number of penultimate whorl (SCN)	3–6 (4.2 $\pm$ 0.8)	4–7 (5.0 $\pm$ 0.8)	4–6 (4.6 $\pm$ 0.6)	3–5 (3.7 $\pm$ 0.6)	3–6 (3.4 $\pm$ 0.7)
Shell height (SH)	21.6–36.4 (27.7 $\pm$ 2.9)	21.1–35.0 (29.3 $\pm$ 3.9)	20.1–37.0 (26.8 $\pm$ 3.0)	24.0–35.7 (30.6 $\pm$ 2.7)	32.2–39.8 (35.7 $\pm$ 2.0)
Shell width (SW)	9.1–14.7 (12.0 $\pm$ 1.2)	9.1–12.1 (10.7 $\pm$ 0.8)	8.2–12.8 (10.6 $\pm$ 0.9)	11.1–15.7 (13.5 $\pm$ 1.2)	12.8–18.2 (15.3 $\pm$ 1.2)
Third whorl length (TWL)	3.7–6.4 (4.8 $\pm$ 0.5)	3.7–5.8 (4.8 $\pm$ 0.5)	3.5–6.0 (4.5 $\pm$ 0.5)	4.6–6.5 (5.5 $\pm$ 0.4)	5.3–7.3 (6.2 $\pm$ 0.5)
Whorl elongation ratio (WER)	2.15–3.56 (2.9 $\pm$ 0.3)	2.31–3.64 (2.9 $\pm$ 0.3)	2.30–3.41 (2.8 $\pm$ 0.2)	2.47–3.55 (2.9 $\pm$ 0.2)	2.52–3.55 (3.0 $\pm$ 0.3)
Whorl number (WN)	3.00–6.25 (4.3 $\pm$ 0.6)	4.00–6.00 (4.9 $\pm$ 0.6)	3.25–6.00 (4.7 $\pm$ 0.7)	3.50–5.25 (4.2 $\pm$ 0.5)	3.25–6.25 (4.4 $\pm$ 0.6)
Sculpture type (node / rib / smooth)	88% / 12% / 0%	8% / 49% / 43%	19% / 81% / 0%	100% / 0% / 0%	100% / 0% / 0%
Number of embryos (EN)	2–145 (50.0 $\pm$ 27.4)	17–82 (48.3 $\pm$ 20.4)	21–90 (49.0 $\pm$ 22.2)	10–66 (34.4 $\pm$ 17.2)	15–68 (32.0 $\pm$ 13.0)

TABLE 2 Morphometric characters of five *Semisulcospira* species examined in the present study. Measurements and counts: minimum–maximum value (mean  $\pm$  SD) (cont.)

Characters	<i>S. niponica</i>	<i>S. fuscata</i>	<i>S. watanabei</i> sp. nov.	<i>S. nakanoi</i> sp. nov.	<i>S. salebrosa</i> sp. nov.
Longitudinal rib number on body whorl of the largest embryonic shell (RNE)	9–15 (12.5 $\pm$ 1.3)	8–15 (10.8 $\pm$ 1.6)	9–12 (11.0 $\pm$ 0.9)	8–14 (10.9 $\pm$ 1.6)	10–13 (11.7 $\pm$ 1.2)
Shell height of the largest embryonic shell (SHE)	1.9–4.0 (2.9 $\pm$ 0.5)	2.1–3.6 (2.9 $\pm$ 0.4)	1.5–3.7 (2.4 $\pm$ 0.4)	1.8–3.2 (2.4 $\pm$ 0.4)	2.2–3.9 (2.9 $\pm$ 0.4)
Shell width of the largest embryonic shell (SWE)	1.6–2.7 (2.2 $\pm$ 0.3)	1.7–2.6 (2.1 $\pm$ 0.2)	1.4–2.7 (1.9 $\pm$ 0.3)	1.7–2.6 (2.1 $\pm$ 0.2)	2.0–3.0 (2.5 $\pm$ 0.3)
Whorl number of the largest embryonic shell (WNE)	2.25–3.75 (3.2 $\pm$ 0.3)	2.50–4.00 (3.1 $\pm$ 0.3)	2.25–3.50 (2.9 $\pm$ 0.4)	2.25–3.25 (2.8 $\pm$ 0.3)	2.50–3.75 (2.9 $\pm$ 0.3)
Male					
Aperture height (AH)	6.4–10.9 (8.7 $\pm$ 0.8)	8.1–9.8 (9.0 $\pm$ 0.8)	7.7–11.1 (9.0 $\pm$ 0.8)	6.8–10.7 (8.7 $\pm$ 1.1)	8.2–13.3 (9.9 $\pm$ 1.7)
Aperture length (AL)	6.8–11.9 (9.4 $\pm$ 0.9)	8.7–9.7 (9.2 $\pm$ 0.4)	8.2–11.8 (9.4 $\pm$ 0.9)	7.3–11.5 (9.2 $\pm$ 1.1)	8.5–13.9 (10.4 $\pm$ 1.8)
Aperture swell length (ASL)	0–0.19 (0.09 $\pm$ 0.04)	0–0.09 (0.04 $\pm$ 0.05)	0–0.12 (0.07 $\pm$ 0.04)	0.16–0.35 (0.24 $\pm$ 0.06)	0.20–0.75 (0.33 $\pm$ 0.14)
Aperture slenderness ratio (ASR)	1.36–1.91 (1.7 $\pm$ 0.1)	1.63–1.88 (1.8 $\pm$ 0.1)	1.68–1.83 (1.8 $\pm$ 0.1)	1.47–1.67 (1.6 $\pm$ 0.1)	1.41–1.79 (1.6 $\pm$ 0.1)
Aperture width (AW)	4.1–7.3 (5.7 $\pm$ 0.6)	5.0–5.9 (5.2 $\pm$ 0.4)	4.5–6.6 (5.3 $\pm$ 0.5)	4.7–6.9 (5.8 $\pm$ 0.6)	5.2–9.9 (6.5 $\pm$ 1.4)
Basal cord number (BCN)	2–5 (3.2 $\pm$ 0.9)	3	2–5 (2.9 $\pm$ 0.9)	2–4 (2.3 $\pm$ 0.6)	2–3 (2.3 $\pm$ 0.5)
Body whorl length (BWL)	10.6–17.7 (14.8 $\pm$ 1.3)	13.6–16.1 (15.1 $\pm$ 1.2)	13.2–18.5 (14.8 $\pm$ 1.3)	12.0–18.1 (14.8 $\pm$ 1.7)	13.9–22.4 (16.6 $\pm$ 2.8)
Fourth whorl length (FWL)	2.0–3.8 (3.0 $\pm$ 0.4)	2.5–3.9 (3.3 $\pm$ 0.6)	2.6–3.8 (3.0 $\pm$ 0.3)	1.8–3.8 (2.7 $\pm$ 0.5)	2.0–4.6 (3.2 $\pm$ 0.9)
Penultimate whorl length (PWL)	3.8–7.2 (5.3 $\pm$ 0.5)	5.0–6.1 (5.6 $\pm$ 0.5)	4.7–6.7 (5.2 $\pm$ 0.5)	4.4–6.5 (5.4 $\pm$ 0.6)	5.1–8.3 (6.1 $\pm$ 1.1)
Longitudinal rib number of penultimate whorl (RN)	9–18 (11.8 $\pm$ 1.9)	9–14 (11.0 $\pm$ 2.0)	7–12 (10.1 $\pm$ 1.3)	8–12 (9.4 $\pm$ 1.2)	9–15 (10.6 $\pm$ 1.4)
Spire angle (SA)	18.2–29.7 (22.7 $\pm$ 2.6)	14.3–18.4 (16.9 $\pm$ 1.6)	13.3–22.8 (20.5 $\pm$ 2.5)	16.7–27.7 (21.4 $\pm$ 3.5)	16.7–33.3 (21.5 $\pm$ 4.6)
Spiral cord number of penultimate whorl (SCN)	3–5 (3.9 $\pm$ 0.8)	5–6 (5.3 $\pm$ 0.6)	4–5 (4.4 $\pm$ 0.5)	3–4 (3.4 $\pm$ 0.5)	3–4 (3.2 $\pm$ 0.4)
Shell height (SH)	17.5–28.4 (23.8 $\pm$ 2.1)	21.8–29.2 (26.1 $\pm$ 3.3)	22.0–30.9 (25.0 $\pm$ 2.2)	18.0–27.3 (23.1 $\pm$ 3.0)	22.1–34.9 (27.6 $\pm$ 4.9)

TABLE 2 Morphometric characters of five *Semisulcospira* species examined in the present study. Measurements and counts: minimum–maximum value (mean ± SD) (cont.)

Characters	<i>S. niponica</i>	<i>S. fuscata</i>	<i>S. watanabei</i> sp. nov.	<i>S. nakanoi</i> sp. nov.	<i>S. salebrosa</i> sp. nov.
Shell width (sw)	7.3–12.6 (10.2 ± 0.9)	8.4–9.9 (9.1 ± 0.5)	8.2–11.9 (9.5 ± 0.9)	8.8–13.0 (10.5 ± 1.2)	9.0–16.7 (11.4 ± 2.2)
Third whorl length (TWL)	3.1–5.0 (4.1 ± 0.4)	3.8–4.9 (4.4 ± 0.5)	3.5–4.8 (4.1 ± 0.4)	2.9–5.2 (4.2 ± 0.7)	3.5–6.4 (4.6 ± 0.9)
Whorl elongation ratio (WER)	2.40–3.76 (3.0 ± 0.3)	2.39–3.19 (2.8 ± 0.4)	2.65–3.29 (3.0 ± 0.2)	2.58–3.84 (3.2 ± 0.3)	2.35–4.54 (3.2 ± 0.6)
Whorl number (WN)	3.25–5.75 (4.4 ± 0.6)	4.25–5.00 (4.7 ± 0.3)	4.00–6.00 (5.0 ± 0.6)	3.50–5.25 (4.2 ± 0.4)	4.00–6.00 (4.9 ± 0.5)
Sculpture type (node / rib / smooth)	86% / 14% / 0%	0% / 60% / 40%	7% / 93% / 0%	77% / 23% / 0%	100% / 0% / 0%

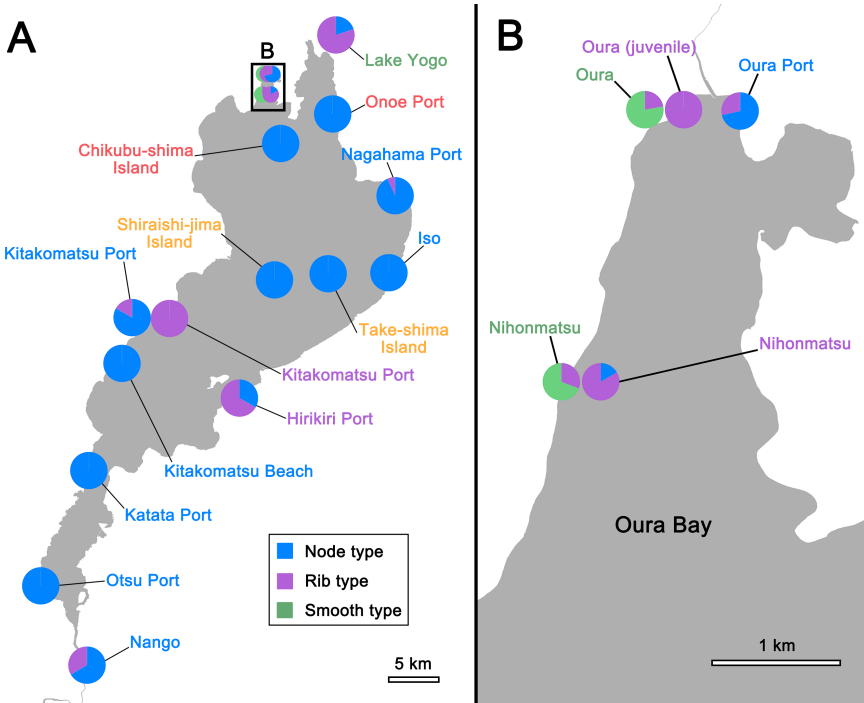


FIGURE 6 Map of Lake Biwa indicating geographical variation in the frequency of sculpture types in the adult females of five *Semisulcospira* species. Colours of locality names correspond to the colour coding in fig. 1

*S. niponica* and *S. watanabei* sp. nov. (table 2). BWL was prominently large in *S. salebrosa* sp. nov., while *S. fuscata* possessed the smallest SA values. The dominant type of sculpture was nodose type in *S. niponica*, *S. nakanoi*

sp. nov., and *S. salebrosa* sp. nov., ribbed type in *S. watanabei* sp. nov., and smooth type in *S. fuscata*.

The following four characters significantly contributed to the male's classification: ASL

TABLE 3 Results of the Random Forest analyses conducted for five *Semisulcospira* species with contributions of each character and mean Gini coefficients to each species

Characters	<i>S. niponica</i>	<i>S. fuscata</i>	<i>S. watanabei</i> sp. nov.	<i>S. nakanoi</i> sp. nov.	<i>S. salebrosa</i> sp. nov.	Mean Gini coefficient
Female						
Aperture swell length (ASL)	0.3336	0.1129	0.0291	0.3336	0.4716	38.9714
Aperture slenderness ratio (ASR)	0.0286	0.0832	-0.0138	0.0286	0.0011	10.5154
Basal cord number (BCN)	0.0280	0.0297	0.0099	0.0280	0.0141	5.4140
Body whorl length (BWL)	0.0321	0.0467	0.0663	0.0321	0.2978	22.9041
Longitudinal rib num- ber of penultimate whorl (RN)	0.0113	0.0052	0.0165	0.0113	0.0350	8.8127
Spire angle (SA)	0.0861	0.0362	0.0831	0.0861	0.0239	16.3837
Spiral cord number of penultimate whorl (SCN)	0.0140	0.0716	0.0171	0.0140	0.0412	7.9527
Whorl elongation ratio (WER)	0.0005	0.0040	0.0174	0.0005	0.0061	7.9218
Whorl number (WN)	0.0115	0.0189	0.0106	0.0115	0.0001	5.6933
Sculpture type	0.0923	0.2130	0.1121	0.0923	0.0686	20.4833
Number of embryos (EN)	0.0177	0.0603	0.0291	0.0177	0.0063	10.1515
Shell height of the largest embryo (SHE)	-0.0027	0.0899	0.0049	-0.0027	0.0003	12.9411
Male						
Aperture swell length (ASL)	0.1503	0.0948	0.0810	0.2563	0.3442	19.4303
Aperture slenderness ratio (ASR)	0.0038	0.0223	0.1714	0.0555	-0.0178	7.1020
Basal cord number (BCN)	0.0035	0.0289	-0.0026	0.0038	0.0176	1.7985
Body whorl length (BWL)	0.0041	0.0270	-0.0051	0.0047	0.0154	4.4192
Longitudinal rib num- ber of penultimate whorl (RN)	0.0202	-0.0105	0.0512	0.0702	0.0343	5.4080
Spire angle (SA)	0.0447	0.1879	0.0154	-0.0118	-0.0200	7.1994
Spiral cord number of penultimate whorl (SCN)	-0.0009	0.1092	0.0231	-0.0044	0.0156	3.0544

TABLE 3 Results of the Random Forest analyses conducted for five *Semisulcospira* species with contributions of each character and mean Gini coefficients to each species (cont.)

Characters	<i>S. niponica</i>	<i>S. fuscata</i>	<i>S. watanabei</i> sp. nov.	<i>S. nakanoi</i> sp. nov.	<i>S. salebrosa</i> sp. nov.	Mean Gini coefficient
Whorl elongation ratio (WER)	0.0014	-0.0187	0.0045	0.0119	-0.0048	4.3564
Whorl number (WN)	0.0068	0.0163	0.0603	0.0584	0.0648	5.6811
Sculpture type	0.0380	0.1412	0.2962	-0.0133	0.0582	7.3610

for *S. niponica*, *S. nakanoi* sp. nov., and *S. salebrosa* sp. nov.; SA and sculpture type for *S. fuscata*; sculpture type and ASR for *S. watanabei* sp. nov. The males showed a similar correlation with females in ASL, SA, and sculpture type (table 2). ASR values were higher in *S. watanabei* sp. nov. and *S. fuscata*.

The Euclidean distances generated from proximities among individuals showed that *S. nakanoi* sp. nov. and *S. salebrosa* sp. nov. are similar in shell morphology (fig. 7A, B). The morphology of *S. watanabei* sp. nov. is intermediate between *S. niponica* and *S. fuscata*, with a significant overlap of interspecific variation among the three species in the females. The Euclidean distances of *S. watanabei* sp. nov. overlapped with that of *S. fuscata* in the males, while the males of *S. niponica* were distinguished from the other two species. A putative hybrid was predicted as *S. niponica* with a 45.1% probability and *S. watanabei* sp. nov. with a 34.9% likelihood. The lectotype of *S. niponica* and the holotype of *S. biwae* were predicted to be *S. niponica* with a high probability by the RF trained with all the females and males (table 4).

## Discussion

### *Phylogeny and morphology*

The present genetic and morphological study revealed the overlooked species diversity of

the *S. niponica*-group. The results of PCA independently separated the nodose, ribbed types of *S. niponica* and smooth types from lakesides. In addition, the insular *biwae* type and one coastal population of the nodose type were divided into two groups. Although the optimal number of clusters was estimated to be four by the ADMIXTURE analysis, the results for  $K = 5$  showed a similar low cross-validation error value and well corresponded with the PCA results. The five groups detected by the genetic analyses were also highly distinct in their morphological traits.

The present investigation discovered sympatric occurrences of the coastal *S. niponica*-group species at three sites: *S. niponica* and *S. watanabei* sp. nov. at Kitakomatsu Port, and *S. fuscata* and *S. watanabei* sp. nov. at Oura and Nihonmatsu. The maintenance of their genetic identity suggests reproductive isolation among the species. In contrast, the evidence of the isolation between the two insular groups and other coastal species could not be obtained in this study due to their allopatric distributions. However, the former group has adapted to the insular reefs, which are different from the coastal rocky bottoms occupied by the latter group, suggesting niche differentiation among them. In addition, it has been revealed that their insular habitats are separated from lake coasts by deep-water, muddy habitats unsuitable for the *S. niponica*-group (Geospatial

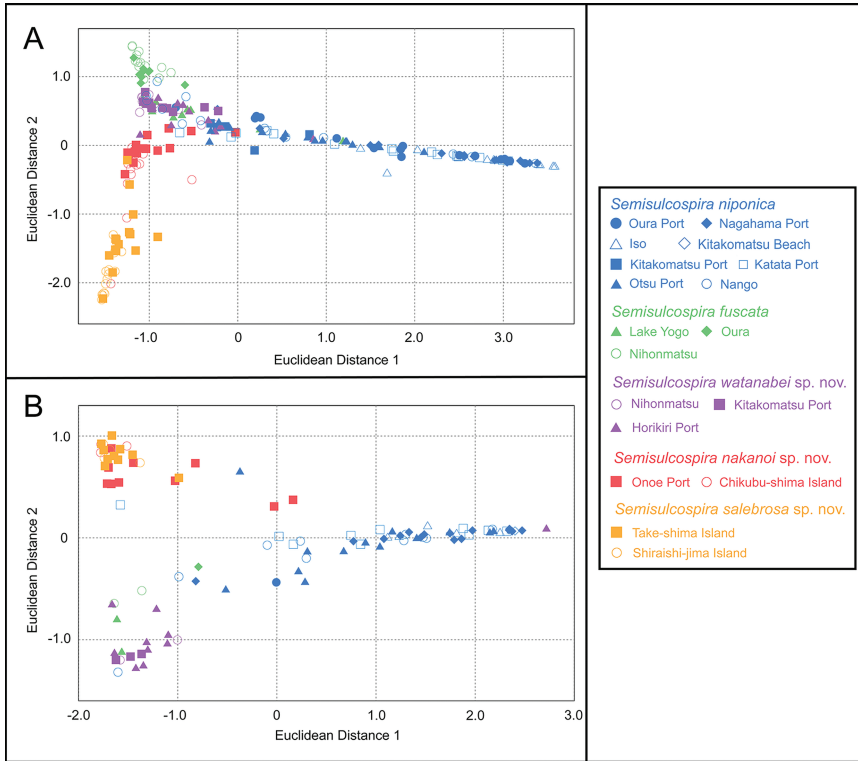


FIGURE 7 Results of Random Forest analyses conducted for five *Semisulcospira* species. Euclidean distances generated from proximities among individuals are plotted. A, Female. B, Male

TABLE 4 Result of the prediction of shell morphological similarity of a lectotype of *Semisulcospira niponica* and a holotype of *S. bivae* for newly collected specimens of five *Semisulcospira* species

Species	<i>S. niponica</i>		<i>S. fuscata</i>		<i>S. watanabei</i> sp. nov.		<i>S. nakanoi</i> sp. nov.		<i>S. salebrosa</i> sp. nov.	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Lectotype of <i>S. niponica</i>	44.94%	45.05%	1.09%	0.23%	5.89%	1.07%	0.84%	0.37%	0.33%	0.19%
Holotype of <i>S. bivae</i>	17.40%	47.53%	0.97%	0%	6.15%	0.10%	19.18%	6.07%	0.49%	2.11%

Information Authority of Japan, 2022). The coastal distribution of *S. nakanoi* sp. nov. also suggests that migration of the snails among coasts and islands does not necessarily lead to their hybridization. According to such potential ecological isolation and the genetic isolation of insular groups supported by the

well-separated clades in the reconstructed Neighbor-net, we consider the present five genetic groups to be independent species.

The results of the genetic analyses were partially consistent with those of the previous studies. A previous genome-wide SNP analysis based on the RAD-seq analysis (Miura et al., 2019)

showed that *S. niponica* comprised two clades; one included snails from Nihonmatsu and the other consisted of individuals from Otsu and the three islands. In this study, the former was discriminated as *S. watanabei* sp. nov., and the latter included *S. niponica*, *S. nakanoi* sp. nov. and *S. salebrosa* sp. nov. However, the two independent lineages and their hybrids discovered from Take-shima Island, using partial sequences of few nuclear loci (Nomoto, 2001), were not detected in our study, despite a relatively large sample number from the island.

A single putative hybrid between *S. fuscata* and *S. watanabei* sp. nov. (fig. 8AY–BA) was detected from the 96 examined snails. This specimen was classified as *S. niponica* by the RF analyses. These results suggests that hybridization events may infrequently occur in the *S. niponica*-group, and hybrids may not necessarily resemble their parents.

The diversification of the *S. habei*-group, including the *S. niponica*-group species, was estimated to have occurred approximately 0.35 million years ago based on genome-wide data (Miura et al., 2019). It has also been known that Lake Yogo was separated from Lake Biwa around 28.5–3.2 thousand years ago (Horie, 1967). The occurrence of *S. fuscata* in Lake Yogo suggests that speciation of the common ancestor of the *S. niponica*-group likely took place before the lake was formed.

The adult shell morphology of the present specimen showed significant divergence, concordant with their genetic relationships. The three characters, ASL, SA, and sculpture type, contributed to the RF classification in both sexes, suggesting that both sexes represent similar interspecific variations in their adult shells. However, the morphological relationships among *S. niponica*, *S. fuscata*, and *S. watanabei* sp. nov. and the contribution of BWL and ASR differed between the

sexes. It has been known in *S. niponica* and other freshwater snails of the Viviparidae and Ampullariidae that a mature female has a larger shell than a male (Minton & Wang, 2011; Cabuga et al., 2017; Sawada & Nakano, 2022). Different shell growth patterns between the sexes have caused the observed sex differences in those taxa. In addition, pronounced female-biased sex ratios have been observed in Lake Biwa semisulcospirids (Köhler, 2016). Therefore, interspecific morphological variation obtained from fully mature females rather than males and juveniles would be more reliable and practical for discriminating species.

The concordance between adult shell morphology and genetic relationships reinforces the possibility of applying this characteristic on species delimitation of other lacustrine species. Substantial interspecific overlap of adult shell morphological traits has been revealed in the riverine congeners in Japan and Korea (Urabe, 1992; Köhler, 2017). The Lake Biwa species likely possess unique evolutionary histories in which species diversification is manifested in their adult shell morphology. In contrast to adult shells, significant interspecific differences in embryonic shell, radula, and reproductive organ morphology were not observed. Differences in the embryonic shell characters have been detected among the *S. niponica*-group species and several lacustrine species such as *S. habei* and *S. decipiens* (Watanabe, 1970; Watanabe & Nishino, 1995). It has been also revealed that radula morphology is stable in Korean *Semisulcospira* (Ko et al., 2001). Hence, the characters of embryonic shell and radula appear to be preserved within the closely related species and/or the genus.

In freshwater snails, it has been estimated that interspecific diversity of radula morphology is associated with different habitat preferences and that rupicolous species have more

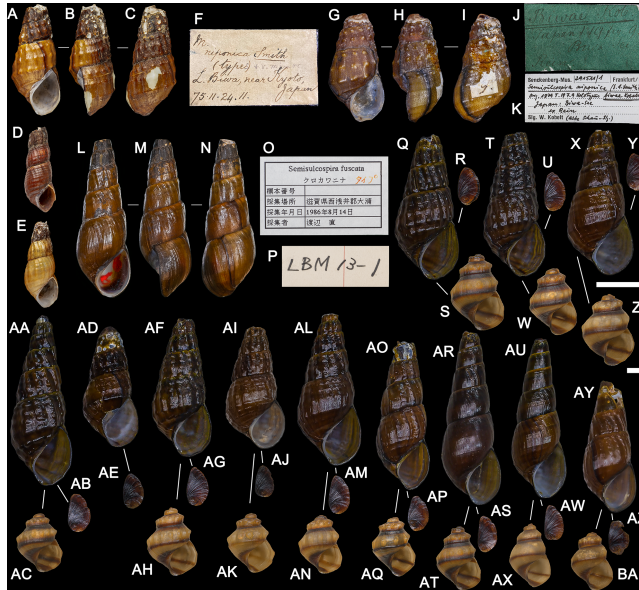


FIGURE 8 Shells of *Semisulcospira niponica* and *S. fuscata*. A–F, Lectotype (A–C), NHM 1875.11.24.11/1, paralectotypes (D, E), NHM 1875.11.24.11/2, 1875.11.24.11/3 and the label of *S. niponica* (F). G–K, Holotype of *S. biwae*, SMF 291511. L–P, Holotype of *S. fuscata*, LBM 13-1. Q–S, *S. niponica* from Oura Port, KUZ Z4093. T–W, *S. niponica* from Nagahama Port, KUZ Z4093. X–Z, *S. niponica* from Iso, KUZ Z4095. AA–AC, *S. niponica* from Kitakomatsu Port, KUZ Z4097. AD–AE, *S. niponica* from Kitakomatsu Beach, KUZ Z4098. AF–AH, *S. niponica* from Katata Port, KUZ Z4099. AI–AK, *S. niponica* from Otsu Port, KUZ Z3766. AL–AN, *S. niponica* from Nango, KUZ Z4101. AO–AQ, *S. fuscata* from Lake Yogo, KUZ Z4103. AR–AT, *S. fuscata* from Oura, KUZ Z4105. AU–AX, *S. fuscata* from Nihonmatsu, KUZ Z4107. AY–BA, Putative hybrid between *S. fuscata* and *S. watanabei* sp. nov. from Nihonmatsu, KUZ Z4140. A–E, G–I, L–N, Q, T, X, AA, AD, AF, AI, AL, AO, AR, AU, AY, Adult shell. R, U, Y, AB, AE, AG, AJ, AM, AP, AS, AW, AZ, Operculum. S, W, Z, AC, AH, AK, AN, AQ, AT, AX, BA, Embryonic shell. F, J, K, O, P, Label. Scale bars: 10 mm (A–E, G–I, L–N, Q, R, T, U, X, Y, AA, AB, AD–AG, AI, AJ, AL, AM, AO, AP, AR, AS, AU, AW, AY, AZ), 1 mm (S, W, Z, AC, AH, AK, AN, AQ, AT, AX, BA). Newly collected specimens (Q–BA) were treated with 3% sodium hypochlorite

rounded denticles (Rintelen et al., 2004). As in *Semisulcospira*, the flat cusps were observed in most *S. niponica*-group snails (fig. 12), whereas *S. davisii* Sawada & Nakano, 2021 from deep muddy areas possesses pointed cusps (Sawada & Nakano, 2021). The interspecific shape variation of radula may be related to the differences in habitat preferences.

Geographical variation in the frequency of sculpture types was observed in several *S. niponica*-group species. More rib types were present in *S. watanabei* sp. nov. in the localities where the species coexists with *S. niponica* or *S. fuscata* (fig. 6). The smooth type of *S. fuscata* was found only in Lake Biwa,

where the species coexists with *S. watanabei* sp. nov. The nodose type was collected from an isolated population in Lake Yogo. It has been revealed in freshwater snails that the coexistence of closely related species can variegated their shell morphology (Fenchel, 1975). The present observations suggest that species composition can affect the specific shell characters as well in *Semisulcospira*. The relationship between the composition and the morphology of sculpture types may lead us to a better understanding of shell morphological variation and characteristics useful for species identification of the genus.

### *Biogeographical implications*

The species of the *S. niponica*-group seem to exhibit habitat specificity, as single species were collected from nearly all the study sites (except for three sites). As aforementioned, the dispersal limitations due to unsuitable environments may have caused insular distributions of the group despite the possibility of niche differentiation among coasts and islands. Although *S. niponica* and *S. watanabei* sp. nov. are widely dispersed in coastal habitats, the sympatric occurrence of four coastal species is relatively infrequent. Accordingly, their species-specific microhabitat differences and/or competitive exclusion may play a role in their exclusive distributions. Feeding habits have been clarified using stable isotopes in lacustrine *Semisulcospira* (Karube et al., 2010). However, knowledge on feed resources and microhabitats at the species level is still lacking. Further studies investigating these factors may explain the distribution pattern of *S. niponica*-group species.

*Semisulcospira niponica* and *S. watanabei* sp. nov. are widespread in Lake Biwa, while the known distribution of the three other species is restricted. These patterns were similar to those of the *S. decipiens*-group species: *S. decipiens* (Westerlund, 1883) widely distributed in the lake, *S. ourense* Watanabe & Nishino, 1995 limited in Oura, *S. morii* in Chikubu-shima Island and Take-shima Island, *S. takeshimensis* Watanabe & Nishino, 1995 in Take-shima Island, and *S. shiraishiensis* in Shiraishi-jima Island (Watanabe & Nishino, 1995). These commonalities in distribution patterns suggest that the common ancestors of the *S. niponica*- and *S. decipiens*-groups experienced similar radiation patterns.

*Semisulcospira* snails are the most diversified molluscs in Lake Biwa, and their species richness is significantly greater than that of other molluscs in the lake (Nishino & Watanabe, 2000). It has been revealed that the

past ecological niche differentiation caused by lake expansion accelerated the diversification of lacustrine species and that radiated species rapidly colonized various environments (Miura et al., 2019). The present investigation highlighted the differences in distribution rather than the substrate preferences of the *S. niponica*-group species. *Semisulcospira* species have low dispersal capability due to their viviparous reproductive mode lacking a planktonic phase (Miura et al., 2020), as well as other viviparous snails (Hendricks, 2012). The restricted distribution of several species in the *S. niponica*-group and their geographical isolation suggest speciation from a common ancestor after the initial increase of the species richness accompanied by lake expansion. Although it remains unclear how *Semisulcospira* snails migrated into the lake islands, the high endemism of the genus in islands suggests that geographical isolation accelerated species diversification, as revealed in other freshwater viviparous snails (Rintelen et al., 2014). Further biogeographic investigations focusing on the divergence time estimation and migration rates of the insular species may lead us to a deeper understanding of the roles of lake islands in allopatric speciation of viviparous snails.

The species diversity of the lacustrine congeners such as *S. habeii* and *S. decipiens* requires re-evaluation. Several divergent clades have been detected in their phylogeny, as with the case of the *S. niponica*-group (Miura et al., 2019).

The reconstructed Neighbor-net estimated the close relationships of the snails within the populations, suggesting their geographical isolation. In contrast, results of other populations indicated the presence of gene flow or migration of snails among sites. The *S. niponica*-group have been collected mainly from rocky areas and harbours, and thus their known habitats are discrete in the lake

(Watanabe & Nishino, 1995; Nishino & Tanida, 2018). However, the present study and a previous one discovered *S. niponica* on sandy beaches distant from their main distribution areas (Miura et al., 2019). These minor snails may connect populations believed to be segmentalized by unsuitable environments, resulting in gene flow among these populations. It has been known that lake molluscs are transferred to fishery ports from other lake sites associated with the fishing industry (Kihira et al., 2009). This transportation may have also caused gene flow among snails originating from distant localities of the lake.

### Systematic status

The shell morphology of the type specimen of *S. niponica* and *S. biwae*, whose exact type locality in Lake Biwa has been unknown, were included in the newly collected *S. niponica* by the RF analyses. Although the taxonomic account of *S. niponica* has included those of the nodose, ribbed and *biwae* types, the present genetic and morphometric analyses showed that the species was represented by its nodose sculpture. Accordingly, the systematic status of the nodose type of *S. niponica* established by Watanabe and Nishino (1995) corresponds to those of *S. niponica* sensu stricto and *S. biwae*. The present results also

highlighted the discordance in the taxonomic accounts between *S. biwae* sensu stricto and *S. biwae* described by Annandale (1916).

It has been believed that *S. biwae* has been distributed in the lake islands, including Chikubu-shima Island since Annandale (1916). Although the type specimen was not examined, the study concluded that the characteristics of 2–3 nodes on a robust shell provided by the original description is consistent with the specimen from the island. However, the present RF analyses classified the holotype of the species into the newly collected *S. niponica*. Moreover, Johannes Justus Rein, the collector of the type specimen, passed Otsu Port and Nagahama Port in his expedition, where the sites are occupied by *S. niponica* (Yamada & Yajima, 2018). Therefore, *S. biwae* sensu stricto was assigned as a junior synonym of *S. niponica* sensu stricto, and *S. biwae* described by Annandale (1916), was described as a new species *S. nakanoi* sp. nov. in this study.

### Systematic descriptions

#### Family Semisulcospiridae Morrison, 1952

#### Genus *Semisulcospira* Boettger, 1886

#### *Semisulcospira niponica* (Smith, 1876)

(table 2, supplementary table S1; figs. 8A–K, Q–AN, 12A–G)

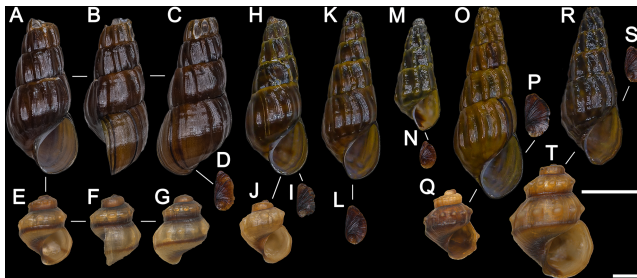


FIGURE 9 Shells of *Semisulcospira watanabei* sp. nov. A–G, Holotype, KUZ Z4109. H–J, Paratype, KUZ Z4110. K–L, Paratype, KUZ Z4114. M–N, Specimen from Oura, KUZ Z4117. O–Q, Specimen from Nihonmatsu, KUZ Z4118. R–T, Specimen from Horikiri Port, KUZ Z4120. A–C, H, K, M, O, R, Adult shell. A–C, H, O, R, Female. K, Male. M, Juvenile. D, I, L, N, P, S, Operculum. E–G, J, Q, T, Embryonic shell. Scale bars: 10 mm (A–D, H–I, K–L, M–N, O–P, R–S), 1 mm (E–G, J, Q, T). All specimens were treated with 3% sodium hypochlorite

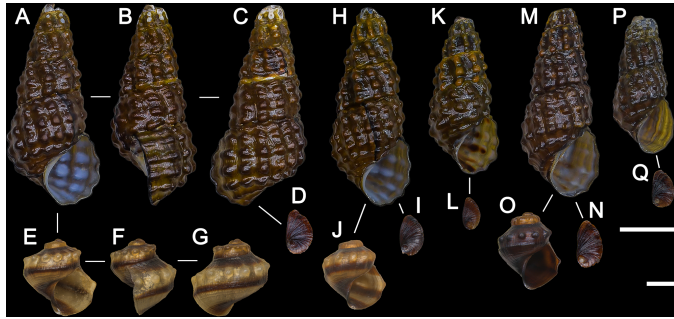


FIGURE 10 Shells of *Semisulcospira nakanoi* sp. nov. A–G, Holotype, KUZ Z4122. H–J, Paratype, KUZ Z4125. K–L, Paratype, KUZ Z4126. M–Q, Specimens from Onoe Port, KUZ Z4129, Z4130. A–C, H, K, M, P, Adult shell. A–C, H, M, Female. K, P, Male. D, I, L, N, Q, Operculum. E–G, J, O, Embryonic shell. Scale bars: 10 mm (A–D, H–I, K–L, M–N, P–Q), 1 mm (E–G, J, O). All specimens were treated with 3% sodium hypochlorite

*Melania niponica* Smith, 1876: 123–124 (part); Martens, 1877: 116 (exact taxonomic account uncertain; TAU); Brot, 1877: 338–339, pl. 34, figs 10 (part); Kobelt, 1879: 131, pl. 19, figs 5–7, 10–14 (part); Pilsbry, 1902: 119; Iwakawa, 1919: 82 (part; TAU); Annandale, 1916: 45, pl. 3, fig. 3A (part).

*Melania biwae* Kobelt, 1879: 132–133, pl. 19, fig. 9; Pilsbry, 1902: 120.

*Melania (Melanoides) niponica* – Nevill, 1884: 264 (TAU).

*Melania (Semisulcospira) niponica* – Boettger, 1886: 8.

*Melania niponica* – De Elera, 1896: 335.

*Semisulcospira niponica* – Kuroda, 1929: 186, 189, pl. 5, figs 32–33 (part); Fukuoka, 1933: 114, 117, fig. 5; Kajiyama & Habe, 1961: 167; Kuroda, 1962: 86 (part; TAU); Kuroda & Habe, 1965: 57 (part); Habe & Kosuge, 1967: 28, pl. 11, fig. 22 (part); Davis, 1969: 243–246, pl. 4, figs 1–3; Watanabe, 1984: fig. 3b–d, f–h (part); Watanabe, 1989: 11–13, 47, figs 10, 27, 28 (part); Köhler, 2016: fig. 4O, P.

*Melanoides (Semisulcospira) niponicus* – Mori, 1936: 229–230; Mori, 1937: 37–38, fig. 1.

*Hua? niponica* – Kira, 1954: 127, pl. 63, fig. 1.

*Biwamelania niponica* – Habe, 1978: 94; Nishino & Tanida, 2018: 56, 251 (part).

*Biwakomelania niponica* – Habe, 1984: 305.

*Semisulcospira (Biwamelania) niponica* – Matsuoka, 1985: 190; Nishino, 1991: 9–10, unnumbered figures (part); Urabe, 2007: 80, fig. 1A; Kihira, et al., 2009: 24–25, unnumbered figure (part); Miura et al., 2019: fig. S1f; Nishino, 2021: 621.

*Semisulcospira niponica* – Higo & Goto, 1993: 97; Goto & Poppe, 1996: 204.

*Semisulcospira (Biwamelania) niponica* (nodose type) – Watanabe & Nishino, 1995: 17–18, fig. 5b (part).

*Material examined:* Voucher specimens: Lectotype: NHM 1875.11.24.11/1, adult shell, sex undetermined, collected from Lake Biwa in central Honshu Island, Japan in 1875 by Charles Wycliffe Goodwin. Paralectotypes: NHM 1875.11.24.11/2, 1875.11.24.11/3, 2 adult shells, sex undetermined, collected with lectotype. SMF 29151, 1 adult shell, sex undetermined, collected from Lake Biwa in 1874 by Johannes Justus Rein. KUZ Z3959 14 males, KUZ Z3766, 15 females, collected from Otsu Port on 23 June 2020 by Naoto Sawada.

Additional materials: KUZ Z4091, 15 females, KUZ Z4092, 1 male, collected from Oura Port on 28 November 2021; KUZ Z4093, 15 females, KUZ Z4094, 15 males, collected from Nagahama Port on 28 November 2021; KUZ Z4095, 12 females,

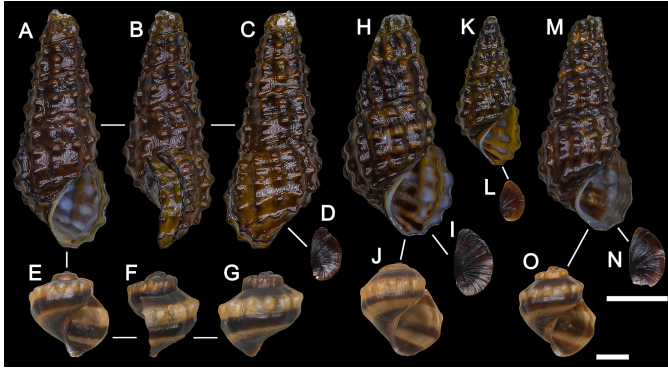


FIGURE 11 Shells of *Semisulcospira salebrosa* sp. nov. A–G, Holotype, KUZ Z4131. H–J, Paratype, KUZ Z4133. K–L, Paratype, KUZ Z4135. M–O, Specimen from Take-shima Island, KUZ Z4138. A–C, H, K, M, Adult shell. A–C, H, M, Female. K, Male. D, I, L, N, Operculum. E–G, J, O, Embryonic shell. Scale bars: 10 mm (A–D, H–I, K–L, M–N), 1 mm (E–G, J, O). All specimens were treated with 3% sodium hypochlorite

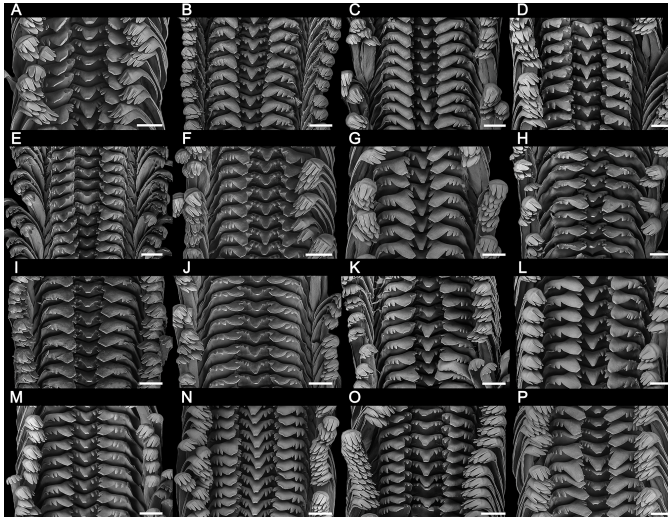


FIGURE 12 Radulae of *Semisulcospira* species. A–G, *Semisulcospira niponica*: A, Oura Port, KUZ Z4091; B, Nagahama Port, KUZ Z4093; C, Iso, KUZ Z4095; D, Kitakomatsu Port, KUZ Z4097; E, Katata Port, KUZ Z4099; F, Otsu Port, KUZ Z4101; G, Nango, KUZ Z4103. H–J, *Semisulcospira fuscata*: H, Lake Yogo, KUZ Z4103; I, Oura, KUZ Z4105; J, Nihonmatsu, KUZ Z4107. K–L, *Semisulcospira watanabei* sp. nov.: K, Kitakomatsu Port, KUZ Z4115; L, Horikiri Port, KUZ Z4120. M–N, *Semisulcospira nakanoi* sp. nov.: M, Onoe Port, KUZ Z4129; N, Chikubu-shima Island, KUZ Z4127. O–P, *Semisulcospira salebrosa* sp. nov.: O, Take-shima Island, KUZ Z4138; P, Shiraiishi-jima Island, KUZ Z4136. Scale bars: 100  $\mu$ m

KUZ Z4096, 5 males, collected from Iso on 24 January 2021; KUZ Z4097, 8 females, collected from Kitakomatsu Port on 28 November 2021; KUZ Z4098, 1 male, collected from Kitakomatsu Beach on 9 January 2022; KUZ Z4099, 15 females, KUZ Z4100, 10 males, collected from Katata Port on 28 November 2021; KUZ Z4101,

15 females, KUZ Z4102, 10 males, collected from Nango on 28 November 2021.

*Amended diagnosis:* Viviparous semisulcospirid. Adult shell medium sized [SH  $27.7 \pm 2.9$  (mean  $\pm$  SD) (female),  $23.8 \pm 2.1$  (male) mm; BWL  $17.3 \pm 1.6$ ,  $14.8 \pm 1.3$  mm], nearly triangular (SA  $23.2 \pm 2.7$ ,  $22.7 \pm 2.6$  degrees); color in

black or brackish brown background; outer lip of aperture hardly swell (ASL  $0.06 \pm 0.04$ ,  $0.09 \pm 0.04$  mm);  $3.3 \pm 1.0$ ,  $3.2 \pm 0.9$  BCN;  $13.1 \pm 2.1$ ,  $11.8 \pm 1.9$  distinct, longitudinal ribs slightly to moderately nodulated on penultimate whorl;  $4.2 \pm 0.8$ ,  $3.9 \pm 0.8$  SCN;  $1.6 \pm 0.1$ ,  $1.7 \pm 0.1$  ASR;  $2.9 \pm 0.3$ ,  $3.0 \pm 0.3$  WER. Embryonic shell medium sized, with distinct nodes on surface; color in black background or beige background with 3 brown or black bands.

*Lectotype designation:* One syntype, NHM 1875.11.24.11/1 (fig. 8A–C), was chosen as the lectotype for *S. niponica* in this study because the specimen possesses 15 longitudinal ribs and 3 spiral cords on the body whorl, which correspond to the values in the original description. The other two syntypes, NHM 1875.11.24.11/2 (fig. 8D) and NHM 1875.11.24.11/3 (fig. 8E) have 6 or 7 spiral cords on the body whorls and were described as a variation of the species (Smith, 1876).

*Description:* Lectotype, NHM 1875.11.24.11/1 (fig. 8A–C): AH 9.1 mm, AL 9.3 mm, ASR 1.62, AW 5.7 mm, BCN 2, BWL 15.4 mm, NL 0.04 mm; PWL 5.5 mm, RN 15, SA 22.1 degrees, SCN 3, SH 22.1 mm, SW 9.9 mm, TWL 4.6 mm, WN 3.25. Shell nearly triangular; suture hardly undulating; whorl sides slightly convex; outer lip of aperture hardly swell; longitudinal ribs distinct, almost straight, oblique, prominently nodulated in penultimate to body whorl; apex of shell eroded; shell color partly faded, colored brown in body to penultimate whorl, blackish brown in remaining whorls, without color band; without operculum.

*Paralectotypes:* NHM 1875.11.24.11/2 (fig. 8D): AH 5.9 mm, AL 5.2 mm, BCN 3, BWL 10.3 mm, FWL 3.0 mm, NL 0 mm, PWL 4.1 mm, SA 11.3 degrees, SCN 7, SH 17.0 mm, SW 6.1 mm, TWL 3.6 mm, WER 1.97; WN 3.25; shell greatly elongated; suture moderately undulating; whorl sides moderately convex; swell of outer lip of aperture absent; longitudinal ribs indistinct, moderately curved, oblique, crossed by

prominent spiral cords in penultimate to body whorl; apex of shell eroded; shell color partly faded, colored blackish brown background; aperture chipped; without operculum. NHM 1875.11.24.11/3 (fig. 8E): AH 6.3 mm, AL 6.1 mm, ASR 1.62, AW 3.8 mm, BCN 3, BWL 10.9 mm, FWL 2.5 mm; PWL 4.2 mm, SA 17.7 degrees, SCN 6, SH 17.2 mm, SW 6.8 mm, TWL 3.2 mm, WER 2.49; WN 3.25; shell elongated; suture moderately undulating; whorl sides slightly convex; swell of outer lip of aperture absent; sculpture distinct, slightly curved, vertical, ribbed longitudinally in penultimate whorl, ribs partly nodulated in body whorl; apex of shell eroded; shell color partly faded, colored beige background; aperture slightly chipped; without operculum.

*Variation:* Adult shells (fig. 8G–I, Q, T, X, AA, AD, AF, AI, AL): Holotype of *S. biwae*, SMF 291511: AH 9.6 mm, AL 9.9 mm, ASR 1.53, AW 6.4 mm, BCN 3, BWL 15.7 mm, PWL 5.3 mm, RN 11, SA 25.6 degrees, SCN 3, SH 21.6 mm, SW 10.9 mm, TWL 4.1 mm, WN 3.25; shell nearly triangular; suture hardly undulating; whorl sides moderately convex; outer lip of aperture slightly swell; longitudinal ribs distinct, almost straight to slightly curved, prominently nodulated in penultimate to body whorl; apex of shell largely eroded; shell colored beige in lower half of body whorl, brown in remaining whorls, without color band; without operculum.

Newly collected specimens: Measurements and counts shown in table 2 and supplementary table S1. Shell near thick to slightly elongated triangular; suture hardly to slightly undulating; whorl sides slightly convex; outer lip of aperture hardly to slightly swell; longitudinal ribs on penultimate whorl distinct, almost straight to slightly curved, oblique, slightly to prominently nodulated by spiral cords, rarely spiral cords alone on penultimate whorl; sculptures often ribbed on upper whorls, granulated in lower whorls; shell colored black or brackish brown in

background, sometimes several indistinct brown band on lower part of each whorl.

Operculum (fig. 8R, U, Y, AB, AE, AG, AJ, AM): 5.5–8.5 mm in long diameter; nearly egg-shaped subcircular, paucispiral, comprising around 3 whorls; nucleus subcentral.

Embryonic shells (fig. 8S, W, Z, AC, AH, AK, AN): Measurements and counts shown in table 2 and supplementary table S1. Shell globose; suture prominently depressed by discrepancy in adjacent whorls; nodes remarkable, on central part of whorls; keels weak, on lower part of whorls; shell colored black background or beige background with 3 brown or black bands on upper and lower part of each whorl and on basal part of shell.

Radulae (fig. 12A–G): Taenioglossa consisting of rachidian in single, lateral teeth, interior and exterior marginal teeth in double row. Rachidian roughly triangular, with large central denticle and 2–3 minor pointed triangular cusps on each side. Lateral teeth with large central denticle, 2–3 inner and outer pointed cusps. Central denticle tip of rachidian and lateral teeth mostly flat, rarely pointed; central denticle of rachidian approximately regular triangular, about 2.5 to 6.0 times longer than other triangular cusps; central denticle of lateral teeth irregular triangular, about 1.5 to 4.0 times longer than other triangular cusps. Interior and exterior marginal teeth spoon-shaped, with 4–6 rounded denticles.

Reproductive organs: Female: Long narrow oviduct, entering near seminal receptacle with long protrusions. Ventral edge of spermatophore bursa with curved sperm gutter, extending toward mantle cavity. Brood pouch on dorsal side of spermatophore bursa, inflated dorsally, separated into many cells, including eggs and embryos; eggs and embryos radially developing from base of brood pouch near seminal receptacle and embryos in anterior or dorsal cells more developed.

Male: Gonad consisting of testes, vas deferens, and prostate without penis. Posterior ventral part of inflated prostate with deep groove, forming U-shape in transverse section. Anterior prostate narrowly opening to mantle cavity.

*Distribution and ecology:* *Semisulcospira niponica* is one of the most widespread species in Lake Biwa (Watanabe & Nishino, 1995; fig. 5b, *S. (B.) niponica* nodose). The species was discovered in eight localities in this study and is most widely distributed in the *S. niponica*-group (fig. 1). *Semisulcospira niponica* mainly inhabits coastal shallow rocky areas and on concrete blocks around harbours at 0–1 m, while it is rarely found on small rocks in shallow sandy areas (e.g., Kitakomatsu Beach and Iso). This species was found with *S. watanabei* at Kitakomatsu Port and occurred with other Lake Biwa species: *S. habei* at Oura Port, Nagahama Port, Kitakomatsu Beach, Otsu Port; *S. dilatata* Watanabe & Nishino, 1995 at Iso; *S. decipiens* at Otsu Port.

*Remarks:* One of paralectotypes of *S. niponica* (NHM 1875.11.24.11/2) was identified as *S. decipiens* by the small SA and shell surface with longitudinal ribs and spiral cords following Davis (1969) and Watanabe and Nishino (1995). The other paralectotype (NHM 1875.11.24.11/3) was similar to *S. habei* in the middle SA and had a moderately convex shell with shell surface sculptures. A note “+ v. minor” was added to the label of the type lot (fig. 8F). As Urabe (2007) discussed the name-bearing types of *S. niponica* cannot be a type specimen of *S. niponica* var. minor (Brot, 1883). The taxonomic account of the latter species was established based on specimens collected from Taiwan. The three species included in the type lot of *S. niponica* are indigenous to Lake Biwa.

*Semisulcospira niponica* resembles *S. watanabei* sp. nov. in shell outline shape and outer lip of the aperture, which hardly

undulates. However, the former species tends to possess granulated longitudinal ribs on the shell surface, and this tendency is stronger where both species coexist (fig. 7). *Semisulcospira niponica* also represents a greater value of BWL, RN, SA, and SHE than *S. watanabei* sp. nov. *Semisulcospira niponica* can be discriminated from other congeners with its blackish triangular adult shell and few longitudinal ribs and spiral cords, and globose embryonic shells with several brown bands (supplementary fig. S1).

*Semisulcospira fuscata* Watanabe & Nishino, 1995

(table 2, supplementary table S1; figs 8L–P, AO–AX, 12H–J)

*Semisulcospira* type B – Watanabe, 1989: 23–25, 53, figs 17, 32.

*Semisulcospira (Biwamelania)* sp. 1 – Nishino, 1991: 16, unnumbered figures.

*Semisulcospira (Biwamelania) fuscata* Watanabe & Nishino, 1995: 5–6, pl. 1, figs A–C, pl. 3, figs a, 5h; Miura et al., 2019: fig. Sig–i; Nishino, 2021: 606.

*Biwamelania fuscata* – Nishino & Tanida, 2018: 48, 246, unnumbered figure.

*Material examined:* Voucher specimens: Holotype: LBM 13-1, 1 adult shell, sex undetermined, collected from Oura, Nagahama City, Shiga Prefecture, Japan on 14 August 1986 by Naoshi Watanabe.

*Additional materials:* KUZ Z4103, 15 females, KUZ Z4104, 2 males, collected from Lake Yogo on 28 November 2021; KUZ Z4105, 9 females, KUZ Z4106, 1 male, collected from Oura on 28 November 2021; KUZ Z4107, 14 females, KUZ Z4108, 2 males, collected from Nihonmatsu on 24 January 2021.

*Amended diagnosis:* Viviparous semi-sulcospirid. Adult shell medium sized (SH  $29.3 \pm 3.9$ ,  $26.1 \pm 3.3$  mm, BWL  $17.1 \pm 1.7$ ,  $15.1 \pm 1.2$  mm), greatly elongated (SA  $19.2 \pm 2.1$ ,

$16.9 \pm 1.6$  degrees); color in black or brackish brown background; outer lip of aperture hardly swell (ASL  $0.04 \pm 0.04$ ,  $0.04 \pm 0.05$  mm);  $3.9 \pm 1.0$ , 3 BCN; sculpture absent on lower whorls in Lake Biwa, sculpture distinct, mostly ribbed on penultimate whorl in Lake Yogo (RN  $13.8 \pm 3.0$ ,  $10.1 \pm 1.3$ );  $5.0 \pm 0.8$ ,  $5.3 \pm 0.6$  SCN;  $1.8 \pm 0.1$ ,  $1.8 \pm 0.1$  ASR;  $2.9 \pm 0.3$ ,  $2.8 \pm 0.4$  WER. Embryonic shell medium sized; color in beige background with 3 brown or black bands.

*Description:* Holotype, LBM 13-1 (fig. 8L–P): AH 10.4 mm, AL 10.0 mm, ASR 1.58, AW 6.3 mm, BCN 3, BWL 17.9 mm, PWL 7.2 mm, SA 15.6 degrees, SH 31.4 mm, SW 10.6 mm, TWL 4.9 mm, WN 5.00; Shell greatly elongated; suture hardly undulating; sides of whorls slightly convex; outer lip of aperture hardly swell; shell surface smooth, or with fine growth lines and sparse nodes on penultimate whorl, longitudinal ribs indistinct, present on upper whorls; shell colored brown in background, band on lower part of body whorl, without color band; without operculum.

*Variation:* Adult shells (fig. 8AO, AR, AU): Measurements and counts shown in table 2 and supplementary table S1. Shell greatly elongated, sometimes nearly triangular in Lake Yogo; suture hardly to slightly undulating; sides of whorls slightly convex; outer lip of aperture hardly swell; shell surface smooth, or with fine growth lines and spiral striae on penultimate whorl, longitudinal ribs indistinct, present on upper whorls in Lake Biwa; longitudinal ribs and spiral cords distinct on penultimate whorl in Lake Yogo; shell colored black or brackish brown in background, rarely several indistinct brown band on lower part of body whorl.

Operculums (fig. 8AP, AS, AW): 5.8–7.8 mm in long diameter; nearly egg-shaped subcircular, paucispiral, comprising around 3 whorls; nucleus subcentral.

Embryonic shells (fig. 8AQ, AT, AX): Measurements and counts shown in table 2 and supplementary table S1. Shell globose to

slightly elongated; suture moderately undulating, or prominently depressed by discrepancy in adjacent whorls; nodes remarkable, on central part of whorls, keels distinct, on lower part of whorls, nodes sometimes absent on lower whorls in Lake Biwa; shell colored beige background with 3 brown or black bands on upper and lower part of each whorl and on basal part of shell.

*Radulae* (fig. 12H–J): Taenioglossa consisting of rachidian in single, lateral teeth, interior and exterior marginal teeth in double row. Rachidian roughly triangular with central denticle and 2–4 small pointed triangular cusps on each side. Lateral teeth with large central denticle, 1–2 inner and outer pointed cusps. Central denticle tip of rachidian and lateral teeth mostly flat, rarely pointed; central denticle of rachidian approximately regular triangular, about 3.5 to 5.5 times longer than other triangular cusps; central denticle of lateral teeth irregular triangular, about 2.5 to 5.0 times longer than other triangular cusps. Interior and exterior marginal teeth spoon-shaped with 4–5 rounded denticles.

Reproductive organs: Female: Long narrow oviduct, entering near seminal receptacle with mostly long, rarely short protrusions. Ventral edge of spermatophore bursa with curved sperm gutter, extending toward mantle cavity. Brood pouch on dorsal side of spermatophore bursa, inflated dorsally, separated into many cells, including eggs and embryos; eggs and embryos radially developing from base of brood pouch near seminal receptacle and embryos in anterior or dorsal cells more developed.

Male: Gonad consisting of testes, vas deferens, and prostate without penis. Posterior ventral part of inflated prostate with deep groove, forming U-shape in transverse section. Anterior prostate narrowly opening to mantle cavity.

*Distribution and ecology*: Distribution of *S. fuscata* is restricted to the west side of Oura Bay, northern shore of Lake Biwa and Lake Yogo (fig. 1; Watanabe & Nishino, 1995; fig. 5h).

The species was found on the piled rock bottom at a depth of 0–3 m. *Semisulcospira fuscata* was collected with *S. watanabei*, *S. habeii* and *S. decipiens* at Oura and Nihonmatsu.

*Remarks*: *Semisulcospira fuscata* can be discriminated from other congeners with a blackish, greatly elongated adult shell without sculptures on the shell surface and embryonic shells with several brown bands in Lake Biwa (supplementary fig. S1). *Semisulcospira fuscata* from Lake Yogo is also distinguishable from other species except for *S. watanabei* sp. nov. and *S. niponica* with the same traits. Although these three species are similar in shell outline shape, the outer lip of the aperture without undulation, and granulated or ribbed sculptures, *S. fuscata* tends to exhibit a lower SA, greater RN, and indistinct longitudinal ribs than the other two species.

***Semisulcospira watanabei* Sawada sp. nov.**

[New Japanese name: Tokitama-kawanina]

(table 2, supplementary table S2; figs 9, 12K, L)

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*Melania niponica* – Martens, 1877: 116 (TAU).

*Melania (Melanoides) niponica* – Nevill, 1884: 264 (TAU).

*Semisulcospira niponica* – Kuroda, 1962: 86 (part; TAU).

*Semisulcospira (Biwamelania) niponica* – Nishino, 1991: 9–10, unnumbered figures (part); Kihira et al., 2009: 24–25 (part).

*Semisulcospira (Biwamelania) niponica* (ribbed type) – Watanabe & Nishino, 1995: 17–18, pl. 3, figs n–p (part).

*Biwamelania niponica* – Nishino & Tanida, 2018: 56, 251 (part).

*Material examined*: Holotype: KUZ Z4109, adult female with embryonic shells treated with sodium hypochlorite, collected from depth of 0.3 m of Lake Biwa at Kitakomatsu Port, Otsu City, Shiga Prefecture, Japan, on 28 November 2021 by first author. Paratypes: KUZ

Z4110–Z4114, 4 adult females, 1 adult male, collected with holotype.

*Additional materials:* KUZ Z4115, 4 females, KUZ Z4116, 2 males, collected with type specimens; KUZ Z4117, 1 juvenile, collected from Oura on 28 November 2021; KUZ Z4118, 12 females, KUZ Z4119, 2 males, collected from Nihonmatsu on 24 January 2021; KUZ Z4120, 15 females, KUZ Z4121, 10 males, collected from Horikiri Port on 28 November 2021.

*Etymology:* The specific name is dedicated to Dr Naoshi Watanabe, who significantly contributed to the systematics of *Semisulcospira* in Lake Biwa.

*Diagnosis:* Viviparous semisulcospirid. Adult shell small (SH  $26.8 \pm 3.0$ ,  $25.0 \pm 2.2$  mm, BWL  $15.9 \pm 1.3$ ,  $14.8 \pm 1.3$  mm), nearly triangular to elongated (SA  $21.1 \pm 2.7$ ,  $20.5 \pm 2.5$  degrees); color in black or brown background; outer lip of aperture hardly swell (ASL  $0.06 \pm 0.05$ ,  $0.07 \pm 0.04$  mm);  $2.8 \pm 0.8$ ,  $2.9 \pm 0.9$  BCN; sculptures prominent, relatively few, ribbed on penultimate whorl (RN  $11.1 \pm 1.8$ ,  $10.1 \pm 1.3$ );  $4.6 \pm 0.6$ ,  $4.4 \pm 0.5$  SCN;  $1.7 \pm 0.1$ ,  $1.8 \pm 0.1$  ASR;  $2.8 \pm 0.2$ ,  $3.0 \pm 0.2$  WER. Embryonic shell small, with distinct nodes on surface; color in black background or beige background with 1–3 brown or black bands.

*Description of holotype:* Adult shell (fig. 9A–C): AH 9.1 mm, AL 9.4 mm, ASR 1.57, AW 6.0 mm, BCN 2, BWL 16.1 mm, FWL 3.4 mm; NL 0 mm; PWL 6.3 mm, RN 11, SA 23.4 degrees, SH 25.0 mm, SW 11.0 mm, TWL 4.5 mm, WER 2.67; WN 3.50; shell nearly thick triangular; suture hardly undulating; whorl sides slightly convex; swell of outer lip of aperture absent; sculpture prominent, almost straight, vertical to oblique, fully ribbed in shell apex to body whorl, faded in body whorl near aperture, spiral striae absent; apex of shell eroded; shell colored black background without color band.

Operculum (fig. 9D): 6.5 mm in long diameter; nearly egg-shaped subcircular,

paucispiral, comprising around 3 whorls; nucleus subcentral.

Embryonic shells (fig. 9E–G): EN 81, RNE 11, SHE 2.3 mm, SWE 1.8 mm, WNE 3; shell globose; suture moderately depressed by discrepancy in adjacent whorls; nodes remarkable, on central part of whorls, keels weak, on lower part of whorls; shell colored blackish beige background with 3 brown or black bands on upper and/or lower part of each whorl and/or on basal part of shell.

Radula: Taenioglossa consisting of rachidian in single, lateral teeth, interior and exterior marginal teeth in double row. Rachidian roughly triangular with large central denticle and 2–3 minor pointed triangular cusps on each side. Lateral teeth with large central denticle, 2–3 inner and outer pointed cusps. Central denticle tip of rachidian and lateral teeth mostly flat, rarely pointed; central denticle of rachidian approximately regular triangular, about 3.5 times longer than other triangular cusps; central denticle of lateral irregular triangular, teeth about 2.5 times longer than other triangular cusps. Interior and exterior marginal teeth spoon-shaped with 4–5 rounded denticles.

Reproductive organ: Female: Long narrow oviduct, entering near seminal receptacle with long protrusions. Ventral edge of spermatophore bursa with curved sperm gutter, extending toward mantle cavity. Brood pouch on dorsal side of spermatophore bursa, inflated dorsally, separated into many cells, including eggs and embryos; eggs and embryos radially developing from base of brood pouch near seminal receptacle and embryos in anterior or dorsal cells more developed.

*Variation:* Adult shells (fig. 9M, O, R): Measurements and counts shown in table 2 and supplementary table S2. Shell nearly slightly elongated triangular; suture hardly to slightly undulating; whorl sides hardly to slightly convex; outer lip of aperture hardly

to slightly swell; longitudinal ribs distinct, slightly curved, vertical to oblique, on upper to body whorl; spiral cords indistinct, sometimes absent; shell colored black to brackish brown or beige in background, rarely indistinct brown band on lower part of each whorl and/or basal part of shell; surface of shells covered with thin algae layer before treatment.

Operculum (fig. 9I, L, N, P, S): 5.5–8.2 mm in long diameter.

Embryonic shells (fig. 9J, Q, T): Measurements and counts shown in table 2 and supplementary table S2. Shell colored blackish brown background or beige background with 1–3 brown or black bands on upper and/or lower part of each whorl and/or on basal part of shell.

Radulae (fig. 12K, L): Lateral teeth with 2–4 inner and outer pointed cusps; central denticle of rachidian about 3.5 to 5.5 times longer than other triangular cusps; central denticle of lateral teeth about 2.5 to 3.0 times longer than other triangular cusps; 4–6 rounded denticles in interior and exterior marginal teeth.

Reproductive organs: Male: Gonad consisting of testes, vas deferens, and prostate without penis. Posterior ventral part of inflated prostate with deep groove, forming U-shape in transverse section. Anterior prostate narrowly opening to mantle cavity.

*Distribution and ecology*: The known distribution of the new species is discrete and limited to four sites in the coast of Lake Biwa (fig. 1). However, the species may be widespread on the Northern shore of the lake because the ribbed type of *S. niponica*, whose sculpture type is consistent with *S. watanabei* sp. nov. has been recorded there (Watanabe & Nishino, 1995, fig. 5b, *S. (B.) niponica* ribbed). *Semisulcospira watanabei* inhabits shallow rocky areas and concrete blocks around harbours. The new species was found with *S. niponica* at Kitakomatsu Port and with *S. fuscata* at Oura and Nihonmatsu.

*Semisulcospira watanabei* sp. nov. was collected with other Lake Biwa species: *S. habei* at Oura, Nihonmatsu, and Horikiri Port; *S. decipiens* at Oura and Nihonmatsu.

*Remarks*: The new species was first described as an intraspecific variation of *S. niponica* by Watanabe and Nishino (1995). *Semisulcospira watanabei* sp. nov. can be discriminated from *S. niponica* in the smaller size of the adult and embryonic shell and fewer prominent longitudinal ribs on the shell surface. Traits of a blackish, elongated triangular adult shell, prominent longitudinal ribs, and globose embryonic shells with several brown bands distinguish *S. watanabei* sp. nov. from other *Semisulcospira* species (supplementary fig. S1).

***Semisulcospira nakanoi* Sawada sp. nov.**

[New Japanese name: Chikubu-kawanina]

(table 2, supplementary table S2; figs 10, 12M, N)  
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*Melania niponica* – Annandale, 1916: 45, pl. 3, fig. 3B (part).

*Melania biwae* – Annandale, 1916: 45, pl. 3, fig. 4.

*Semisulcospira niponica* – Kuroda, 1929: 186, 189, pl. 5, fig. 31 (part); Hirase & Taki, 1951: pl. 82, fig. 15; Kuroda, 1962: 86 (part; TAU); Kuroda & Habe, 1965: 57 (part); Habe & Kosuge, 1967: 28, pl. 11, fig. 21 (part); Watanabe, 1984: fig. 3b, f; Watanabe, 1989: 11–13, 47, figs 10, 27, 28 (part).

*Semisulcospira niponica* var. *biwae* – Fukuoka, 1933: 114, 117, fig. 6.

*Semisulcospira (Biwamelania) niponica* – Nishino, 1991: 9–10, unnumbered figures (part); Kihira et al., 2009: 24–25 (part).

*Semisulcospira nipponica biwae* – Higo & Goto, 1993: 97; Goto & Poppe, 1996: 204.

*Semisulcospira (Biwamelania) niponica (biwae type)* – Watanabe & Nishino, 1995: 17–18, fig. 5b (part).

*Biwamelania niponica* – Nishino & Tanida, 2018: 56, 251 (part).

*Material examined:* Holotype: KUZ Z4122, adult female with embryonic shells treated with sodium hypochlorite, collected from depth of 3.0 m of Lake Biwa at Chikubushima, Nagahama City, Shiga Prefecture, Japan, on 9 September 2020 by first author. Paratypes: KUZ Z4123–Z4126, 3 adult females, 1 adult male, collected with holotype.

Additional materials: KUZ Z4127, 11 females, KUZ Z4128, 2 males, collected with type specimens; KUZ Z4129, 16 females, KUZ Z4130, 5 males, collected from Onoe Port on 25 July 2021.

*Etymology:* The specific name is dedicated to Dr Takafumi Nakano, who greatly supported the first author's study of Japanese *Semisulcospira*.

*Diagnosis:* Viviparous semisulcospirid. Adult shell medium sized (SH  $30.6 \pm 2.7$ ,  $23.1 \pm 3.0$  mm; BWL  $18.9 \pm 1.3$ ,  $14.8 \pm 1.7$  mm), nearly triangular (SA  $21.5 \pm 2.6$ ,  $21.4 \pm 3.5$  degrees); color in black background; outer lip of aperture moderately swell (ASL  $0.20 \pm 0.05$ ,  $0.24 \pm 0.06$  mm);  $2.5 \pm 0.5$ ,  $2.3 \pm 0.6$  BCN; longitudinal ribs relatively few, prominently nodulated on penultimate whorl (RN  $11.3 \pm 1.3$ ,  $9.4 \pm 1.2$ );  $3.7 \pm 0.6$ ,  $3.4 \pm 0.5$  SCN;  $1.6 \pm 0.1$ ,  $1.6 \pm 0.1$  ASR;  $2.9 \pm 0.2$ ,  $3.2 \pm 0.6$  WER. Embryonic shell small, with distinct nodes on surface; color in black background or beige background with 3 brown or black bands.

*Description of holotype:* Adult shell (fig. 10A–C): AH 11.5 mm, AL 12.5 mm, ASR 1.48, AW 8.5 mm, BCN 2, BWL 19.7 mm, FWL 4.4 mm; NL 0.18 mm; PWL 7.3 mm, RN 9, SA 23.1 degrees, SCN 4, SH 34.1 mm, SW 15.7 mm, TWL 5.9 mm, WER 2.61; WN 4.75; shell nearly thick triangular; suture hardly undulating; whorl sides slightly convex; outer lip of aperture moderately swell; longitudinal ribs slightly curved, oblique, prominently nodulated in upper to body whorl; apex of shell eroded; shell colored black background without color band.

Operculum (fig. 10D): 8.9 mm in long diameter; nearly egg-shaped subcircular, paucispiral, comprising around 3 whorls. Nucleus subcentral.

Embryonic shells (fig. 10E–G): EN 62, RNE 12, SHE 2.3 mm, SWE 2.2 mm, WNE 2.5; shell globose; suture prominently undulating; remarkable nodes on central part of whorls and weak keels on lower part of whorls; shell colored beige background with 3 brown or black bands on upper and/or lower part of each whorl and/or on basal part of shell.

Radula: Taenioglossa consisting of rachidian in single, lateral teeth, interior and exterior marginal teeth in double row. Rachidian roughly triangular with large central denticle and 3 minor pointed triangular cusps on each side. Lateral teeth with large central denticle, 3–4 inner and outer pointed cusps. Central denticle tip of rachidian and lateral teeth mostly flat; central denticle of rachidian approximately regular triangular, about 2.5 times longer than other triangular cusps; central denticle of lateral teeth irregular triangular, about 1.5 times longer than other triangular cusps. Interior and exterior marginal teeth spoon-shaped with 5–6 rounded denticles.

Reproductive organ: Female: Long narrow oviduct, entering near seminal receptacle with long protrusions. Ventral edge of spermatophore bursa with curved sperm gutter, extending toward mantle cavity. Brood pouch on dorsal side of spermatophore bursa, inflated dorsally, separated into many cells, including eggs and embryos; eggs and embryos radially developing from base of brood pouch near seminal receptacle and embryos in anterior or dorsal cells more developed.

*Variation:* Adult shells (fig. 10M, P): Measurements and counts shown in table 2 and supplementary table S2. Shell nearly thick to elongated triangular; surface sculptures almost straight to slightly curved, oblique,

rarely ribbed on upper whorls; shell colored black to brackish olive background with or without several color bands on upper and lower part of each whorl and basal part of shell; surface of shells covered with thin algae layer before treatment.

Operculums (fig. 10I, L, N, Q): 7.1–8.9 mm in long diameter.

Embryonic shells (fig. 10J, Q): Measurements and counts shown in table 2 and supplementary table S2. Shell with or without keels on lower part of whorls; shell colored blackish brown background or beige background with 3 brown or black bands on upper and/or lower part of each whorl and/or on basal part of shell.

Radulae (fig. 12M, N): Rachidian with 2–4 minor pointed triangular cusps on each side; lateral teeth with 2–4 inner and outer pointed cusps; central denticle of rachidian about 1.5 to 4 times longer than other triangular cusps; central denticle of lateral teeth about 1.5 to 4.0 times longer than other triangular cusps; 4–6 rounded denticles in interior and exterior marginal teeth.

Reproductive organs: Male: Gonad consisting of testes, vas deferens, and prostate without penis. Posterior ventral part of inflated prostate with deep groove, forming U-shape in transverse section. Anterior prostate narrowly opening to mantle cavity.

*Distribution and ecology:* The new species has been found only in Chikubu-shima Island and the coastal Onoe Port in Lake Biwa (fig. 1). *Semisulcospira nakanoi* sp. nov. inhabits the wharf and rocky bottom of Chikubu-shima Island and concrete blocks around Onoe Port. This species was found with *S. morii* and *S. habeii* at Chikubu-shima Island.

*Remarks:* The new species has been identified as *S. biwae* together with *S. salebroza* sp. nov. and has been treated as a junior synonym of *S. niponica* since Kuroda (1929). *Semisulcospira nakanoi* sp. nov. resembles *S. salebroza* sp. nov. with well-developed

nodes on the shell surface. However, the former species is distinguishable from the latter by the smaller adult and embryonic shell size and less undulating outer lip of the aperture. The new species can be discriminated from other congeners by its large, blackish, triangular adult shell, prominent granulated ribs, the moderately undulating outer lip of the aperture, and globose embryonic shells with several brown bands (supplementary fig. S1).

***Semisulcospira salebroza* Sawada sp. nov.**

[New Japanese name: Konpeito-kawanina]

(table 2, supplementary table S2; figs 11, 12O, P)  
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*Semisulcospira niponica* – Watanabe, 1984: fig. 3c–d, g–h.

*Semisulcospira (Biwamelania) niponica* – Nishino, 1991: 9–10, unnumbered figures (part); Kihira et al., 2009: 24–25, unnumbered figure (part); Miura et al., 2019: fig. S1d, e.

*Biwamelania nipponica* – Minato, 1991: 79, unnumbered figure.

*Semisulcospira (Biwamelania) niponica (biwae type)* – Watanabe & Nishino, 1995: 17–18, fig. 5b (part).

*Biwamelania niponica* – Nishino & Tanida, 2018: 56, 251, unnumbered figure (part).

*Material examined:* Holotype: KUZ Z4131, adult female with embryonic shells treated with sodium hypochlorite, collected from a depth of 3.0 m of Lake Biwa at Shiraiishi-jima Island, Takashima City, Shiga Prefecture, Japan, on 9 September 2020 by first author. Paratypes: KUZ Z4132–Z4135, 3 adult females, 1 adult male, collected with holotype.

Additional materials: KUZ Z4136, 11 females, KUZ Z4137, 3 males, collected with type specimens; KUZ Z4138, 15 females, KUZ Z4139, 10 males, collected from Take-shima Island on 25 July 2021.

*Etymology:* The specific name is after prominent granulated longitudinal ribs on adult shells of the new species.

*Diagnosis:* Viviparous semisulcospirid. Adult shell large (SH  $35.7 \pm 2.0$ ,  $27.6 \pm 4.9$  mm, BWL  $21.9 \pm 1.5$ ,  $16.6 \pm 2.8$  mm), nearly triangular (SA  $21.2 \pm 2.5$ ,  $21.5 \pm 4.6$  degrees); color in black background; outer lip of aperture prominently swell (ASL  $0.44 \pm 0.12$ ,  $0.33 \pm 0.14$  mm);  $2.5 \pm 0.7$ ,  $2.3 \pm 0.5$  BCN; ribs relatively few, prominently nodulated on penultimate whorl (RN  $11.1 \pm 1.6$ ,  $10.6 \pm 1.4$ );  $3.4 \pm 0.7$ ,  $3.2 \pm 0.4$  SCN;  $1.6 \pm 0.1$ ,  $1.6 \pm 0.1$  ASR;  $3.0 \pm 0.3$ ,  $3.2 \pm 0.6$  WER. Embryonic shell medium sized, with distinct nodes on surface; color in beige background with 3–4 brown or black bands.

*Description of holotype:* Adult shell (fig. 11A–C): AH 14.2 mm, AL 14.5 mm, ASR 1.63, AW 8.9 mm, BCN 3, BWL 23.0 mm, FWL 5.3 mm; NL 0.42 mm; PWL 8.2 mm, RN 9, SA 18.5 degrees, SCN 3, SH 39.4 mm, SW 15.7 mm, TWL 6.6 mm, WER 2.71; WN 4.75; shell near slightly elongated triangular; suture hardly undulating; whorl sides slightly convex; outer lip of aperture swell prominently; longitudinal ribs slightly curved, straight, nodulated prominently in upper to body whorl; apex of shell eroded; shell colored black background without color band.

Operculum (fig. 11D): 8.8 mm in long diameter; nearly egg-shaped subcircular, paucispiral, comprising around 3 whorls; nucleus subcentral.

Embryonic shells (fig. 11E–G): EN 30, RNE 12, SHE 2.7 mm, SWE 2.5 mm, WNE 2.5; shell globose; suture prominently undulating; remarkable nodes present on central part of whorls, keels absent on lower part of whorls; shell colored beige background with 3 black bands on upper and/or lower part of each whorl and/or on basal part of shell.

Radula: Taenioglossa consisting of rachidian in single, lateral teeth, interior and exterior marginal teeth in double row. Rachidian roughly triangular with large central denticle

and 2 minor pointed triangular cusps on each side. Lateral teeth with large central denticle, 2–3 inner and outer pointed cusps. Central denticle tip of rachidian and lateral teeth mostly flat, rarely pointed; central denticle of rachidian approximately regular triangular, about 3.0 times longer than other triangular cusps; central denticle of lateral teeth irregular triangular, about 3.5 times longer than other triangular cusps. Interior and exterior marginal teeth spoon-shaped with 4–5 rounded denticles.

Reproductive organ: Female: Long narrow oviduct, entering near seminal receptacle with long protrusions. Ventral edge of spermatophore bursa with curved sperm gutter, extending toward mantle cavity. Brood pouch on dorsal side of spermatophore bursa, inflated dorsally, separated into many cells, including eggs and embryos; eggs and embryos radially developing from base of brood pouch near seminal receptacle and embryos in anterior or dorsal cells more developed.

*Variation:* Adult shells (fig. 11M): Measurements and counts shown in table 2 and supplementary table S2. Shell nearly triangular to slightly elongated; suture slightly to moderately undulating; whorl sides slightly to moderately convex; sculptures prominent, almost straight to slightly curved, vertical to slightly oblique, prominently nodulated, rarely ribbed on upper whorls; shell colored black to brackish brown background with or without several color bands on upper, middle, and lower part of each whorl and basal part of shell; surface of shells covered with thin algae layer before treatment.

Operculums (fig. 11I, L, N): 8.8–11.1 mm in long diameter.

Embryonic shells (fig. 11J, O): Measurements and counts shown in table 2 and supplementary table S2. Suture slightly or prominently undulating; distinct keels present or absent on lower part of whorls; shell colored beige

background with 3 brown or black bands on upper and/or lower part of each whorl and/or on basal part of shell.

Radulae (fig. 12O, P): 2–4 minor pointed triangular cusps on each side of rachidian; lateral teeth with 1–4 inner and outer pointed cusps; central denticle of rachidian about 2.5 to 5.0 times longer than other triangular cusps; central denticle of lateral teeth about 1.5 to 3.0 times longer than other triangular cusps.

Reproductive organs: Male: Gonad consisting of testes, vas deferens, and prostate without penis. Posterior ventral part of inflated prostate with deep groove, forming U-shape in transverse section. Anterior prostate narrowly opening to mantle cavity.

*Distribution and ecology*: The known distribution of *S. salebroso* sp. nov. is restricted to Shiraishi-jima Island and Take-shima Island in Lake Biwa (fig. 1). The new species inhabits the wharf and rocky bottom of the islands. *Semisulcospira salebroso* sp. nov. was found with *S. shiraishiensis* in the Shiraishi-jima Island and *S. takeshimensis* in Take-shima Island.

*Remarks*: The new species has been identified as *S. biwae* together with *S. nakanoi* sp. nov. and assigned as an intraspecific variation of *S. niponica*. The new species can be discriminated from *S. nakanoi* sp. nov. and other semisulcospirids by the large, blackish, triangular adult shell with well-granulated ribs and strongly undulating outer lip of the aperture and globose embryonic shells with several brown bands (supplementary fig. S1).

## Conclusions

The overlooked species diversity of the *Semisulcospira niponica*-group was revealed by integrative taxonomy using genome-wide SNP-based genetic analyses and machine learning classification based on shell

morphological traits. Morphological examination of the type specimens successfully resolved the five genetic clusters and synonymized *S. biwae* under *S. niponica*. The five clusters corresponded to the two described species, *S. niponica* and *S. fuscata*, and three new species, *S. watanabei* sp. nov., *S. nakanoi* sp. nov., and *S. salebroso* sp. nov.

The results also provide new insights into the diversification of freshwater snails in the ancient lake and their morphology and distribution. Similar substrate preferences and adaptation of radula morphology for rocky areas were observed in all the species. Distributions of *S. fuscata*, *S. nakanoi* sp. nov., and *S. salebroso* sp. nov. are restricted in the coasts and islands, while two other species are widespread in the lake. Their distribution patterns suggest that their speciation was accelerated by their low dispersal ability due to their viviparous reproductive mode and geographical isolation after the initial diversification of the genus associated with ecological niche differentiation.

The allopatric distribution among the species was observed in most sites. In addition, intraspecific variation in the frequency of sculpture types was detected among sites dominated by a single species and those inhabited by several species. Accordingly, it has been suggested that competitive exclusion among them may have led to their current distributions and that the presence of closely-related congeners can affect their shell morphological characteristics.

## Acknowledgements

The authors are very grateful to Jonathan Ablett (NHM), Sigrid Hof (SMF), and Shigefumi Kanao (LBM) for their kind support and for providing photographs of the type specimens. We also thank Misako Urabe

(The University of Shiga Prefecture), Hiroshi Fukuda (Okayama University), Kazunori Hasegawa (National Museum of Nature and Science, Tokyo), and Paul Callomon (Drexel University) for providing information. We also appreciate the constructive comments of Takafumi Nakano (Kyoto University; KU), Danwei Huang (Tropical Marine Science Institute, Singapore), and two anonymous reviewers. The first author is also grateful to Toshiaki Nishimura, Yoshiharu Sasaki, Yoshitaka Tamura, Kenichiro Ishii, Jamen Uiriamu Otani, Kentaro Miura, Yugo Miuchi, and Kaoru Sawada for assisting with sample collection. We thank Katsutoshi Watanabe, Tomohisa Makino, Taku Okamoto, Tomoki Kadokawa, and Minoru Tamura in KU for assisting with the genetic and morphological analyses. This study was financially supported, in part, by the Research Institute of Marine Invertebrates Grant Number 1KU 2021-01 and JSPS KAKENHI Grant Number JP21J22917. We thank Editage (www.editage.com) for English language editing.

### Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.20411001>

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