Evolutionary Systematics. 8 2024, 127–144 | DOI 10.3897/evolsyst.8.124491

PENSOFT. EVOLUTIONARY SYSTEMATICS

Redescription of *Semisulcospira reticulata* (Mollusca, Semisulcospiridae) with description of a new species from Lake Biwa, Japan

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Academic editor: Andreas Schmidt-Rhaesa ♦ **Received** 1 April 2024 ♦ **Accepted** 23 May 2024 ♦ **Published** 10 June 2024

Abstract

Semisulcospira Boettger, 1886 is a freshwater snail genus divergent in Lake Biwa, Japan. Although recent taxonomic studies of the genus have arranged taxonomic accounts of many species in the lake, our knowledge of morphological traits has still been insufficient in *S. reticulata* Kajiyama & Habe, 1961, which belongs to the *S. niponica*-group in the genus. Among the various habitats of the lacustrine species, in addition, the species richness of this group in coastal sandy mud areas of the lake has not been completely clarified. In this study, we revisited the taxonomic account of *S. reticulata* and described a new *S. niponica*-group species, *S. nishimurai* **sp. nov.**, from Lake Biwa by evaluating the genetic and morphological relationships within the group. The reconstructed maximum likelihood and singular value decomposition quartets trees, and ADMIXTURE analyses using genome-wide SNP data strongly supported the genetic distinctiveness of most *S. niponica*-group species. The Random Forest classification showed that *S. reticulata* possesses a unique, large protoconch without distinct keels, and that *S. nishimurai* **sp. nov.** is characterized by a small, less elongated teleoconch and tiny protoconch. The large protoconch of *S. reticulata* and the narrow radulae of *S. reticulata* and *S. nishimurai* **sp. nov.** suggest their specialization in life history and feeding habits, respectively. The discovery of the new species from sandy mud substrates highlights niche differentiation among *Semisulcospira* species, and provides new insights into the diversification of the genus in ancient lakes.

Key Words

Ancient lake, freshwater snail, MIG-seq, next-generation sequencing, radula, Random Forest, taxonomy, type specimen

Introduction

The freshwater snail genus *Semisulcospira* Boettger, 1886, a member of the family Semisulcospiridae, is characterized by its synapomorphic trait of viviparity (Strong and Köhler 2009; Köhler 2017). The genus is distributed in Japan, Korea, Taiwan, and China, and it contains unique populations in the Lake Biwa water system in Japan (Davis 1969; Du et al. 2019a). Of the 30 extant species, 18 are endemic to the water system (Watanabe and Nishino 1995; Du et al. 2019b; Sawada and Fuke 2022; Suppl. material 1: table S1), and exhibit interspecific diversity in shell morphology, karyotypes, and habitat preferences (Burch 1968; Watanabe and Nishino 1995; Miura et al. 2019).

Semisulcospira was originally erected as a subgenus of the genus *Melania* Lamarck, 1799, and contained six species (Boettger 1886). Subsequently, 15 extant species indigenous to the Lake Biwa system had been described by 1995 (Watanabe and Nishino 1995). Recent studies employing genome-wide single nucleotide polymorphisms (SNP) analyses have clarified the phylogenetic relationships among the Japanese species and arranged their taxonomic accounts, resulting in the synonymization of three species and the description of five new species (Miura et al. 2019; Sawada and Fuke 2022, 2023). Phylogenetic studies have also identified two clades in the lake endemics (Nomoto 2001; Miura et al. 2019), and subsequently, they were defined with morphological traits as *S. niponica*- and *S. nakasekoae*-groups (Sawada and Fuke 2022).

Recent integrative taxonomy revisited the taxonomic accounts of many lake endemics of *Semisulcospira* using name-bearing types and newly collected specimens (Sawada and Fuke 2022, 2023). The delimitation of the lacustrine species was updated based on their morphological and genetic distinctiveness. The reproductive isolation among the closely related species suggested by their sympatric distribution was also emphasized in the taxonomic revision. However, these studies also indicated that genetic and morphological traits have been insufficiently elucidated to re-examine the validity in several species, such as *S. reticulata* Kajiyama & Habe, 1961. *Semisulcospira reticulata* is one of the endemic species of the Lake Biwa system and was described offshore of the lake. Although this species was examined in several studies (Davis 1969; Watanabe and Nishino 1995; Miura et al. 2019), knowledge of its phylogenetic position, along with intraspecific variation in shell morphology and traits of the radulae and reproductive organs, is still limited.

Habitat preferences, especially for substrates and water depth, have been observed in *Semisulcospira* species (Miura et al. 2019). Different species have been recorded on steep rocky, shore pebble, coastal sand, and offshore muddy lakebeds (Watanabe and Nishino 1995; Sawada and Fuke 2023). Among the various habitats in the lake, coastal sandy mud substrates are one of the environments where investigations on *Semisulcospira* snails are lacking. An overlooked species, *S. elongata* Sawada, 2022, in the *S. nakasekoae*-group, was recently described from such substrates (Sawada and Fuke 2022). However, the species richness of the *S. niponica*-group in sandy mud areas has not been completely clarified. In fact, an unidentified *Semisulcospira* species with shell morphology partially resembling other *S. niponica*-group congeners, has been collected from the habitat by an amateur conchologist (Nishimura 2024).

In this study, the taxonomic accounts of *S. reticulata* and the unidentified sandy mud species were investigated using genetic analysis and morphological comparisons within the *S. niponica*-group. This study improves our knowledge of *S. reticulata* and describes the unidentified species, that has been overlooked in previous taxonomic studies, as a new species.

Materials and methods

Taxon sampling

A total of 221 specimens were used for genetic and/or morphological analyses (Table 1). Among them, specimens of 45 *S. reticulata* and 49 unidentified species were newly obtained. In addition, seven specimens of *S. kurodai* Kajiyama & Habe, 1961 were newly collected for phylogenetic analysis. The remainder of this study included voucher specimens deposited at the Zoological Collection of Kyoto University (**KUZ**) by Sawada and Fuke (2022, 2023) and holotype of *S. reticulata* at the National Museum of Nature and Science, Tokyo (**NSMT**).

The newly collected snails were sampled by hand using a snorkel and aeration device (LIEYU Z270 scuba diving compressor; Dongguan Zuoji Diving Equipment Co., Ltd., Guangdong, China), and using dredge from seven localities in Lake Biwa, central Japan in 2019–2022 with the permission of the government of Shiga Prefecture, Japan (Fig. 1). Water depth was measured using a COSMIQ+ Dive computer (Deepblu, København, Denmark) at the localities where snails were collected by hand. Specimens of teleoconchs, protoconchs, radulae, and reproductive organs were prepared, cleaned, and observed as described by Sawada and Fuke (2023). The newly collected specimens were deposited at KUZ.

Sequencing and SNP detection

Genetic relationships among *S. niponica*-group species were clarified using Multiplexed ISSR Genotyping by sequencing (MIG-seq) analyses (Suyama and Matsuki 2015). Genomic DNA extraction, library preparation, and sequencing were performed on 11 *S. reticulata*, 22 unidentified species, and seven *S. kurodai* specimens. Pooled libraries were prepared following the method of Sawada and Fuke (2023) using the Onuki and Fuke (2022) primer set or the modified Suyama et al. (2022) primer set, and were outsourced to Novogene for 150-bp paired-end sequencing with Illumina NovaSeq 6000. Newly obtained raw MIG-seq data were deposited in the DNA Data Bank of Japan (**DDBJ**) Sequence Read Archive (accession numbers: PRJDB16526 and PRJDB17676). Raw data were demultiplexed using the process_shortreads program in Stacks v2.59 (Rochette et al. 2019).

MIG-seq data of the following *S. niponica*-group species, including their type materials and topotypes, were obtained via DDBJ and used for the analysis (Table 1): *S. niponica* (Smith, 1876), *S. decipiens* (Westerlund, 1883), *S. reticulata*, *S. habei* Davis, 1969, *S. rugosa* Watanabe & Nishino, 1995, *S. fuscata* Watanabe & Nishino, 1995, *S. watanabei* Sawada, 2022, *S. nakanoi* Sawada, 2022, and *S. salebrosa* Sawada, 2022.

Table 1. Specimen list of presently examined *Semisulcospira niponica*-group species with voucher numbers, collection localities, and DDBJ Sequence Read Archive (DRA) accession numbers for the specimens used for the genetic analysis. The locality numbers correspond to Fig. 1. Acronym: KUZ, Zoological Collection of Kyoto University; NSMT, National Museum of Nature and Science, Japan.

Vouncher number	Collection locality (reference)	DRA accession number (reference)
Semisulcospira niponica (Smith, 1876)		
KUZ Z4093	Concrete brock bottom at depths of 0–1 m, Lake Biwa, Nagahama Port, Nagahama City, Shiga Prefecture, Japan (putative type locality; Sawada and Fuke 2023)	DRR360330–DRR360334 (Sawada and Fuke 2023)
KUZ Z3959	Piled rocky bottom at depths of 0–1 m, Lake Biwa, Otsu Port, Otsu City, Shiga Prefecture, Japan (putative type locality; Sawada and Fuke 2023)	DRR360355–DRR360359 (Sawada and Fuke 2023)
Semisulcospira decipiens (Westerlund, 1883)		
KUZ Z4208	Piled rocky bottom at depths of 0–1 m, Lake Biwa, Hannoura, Nagahama City, Shiga Prefecture, Japan (locality #1)	DRR398459–DRR398463 (Sawada and Fuke 2022)
KUZ Z4210	Muddy bottom at depths of 0–1 m, creak flows into Lake Biwa, Ebie, Nagahama City, Shiga Prefecture, Japan	DRR398445–DRR398449 (Sawada and Fuke 2022)
KUZ Z4215–Z4217	Rocky bottom at depths of 0–1 m, Lake Biwa, Iso, Maibara City, Shiga Prefecture, Japan	DRR398477–DRR398480 (Sawada and Fuke 2022)
KUZ Z4220	Muddy bottom at depths of 6–12 m, Lake Biwa, Mano, Otsu City, Shiga Prefecture, Japan (locality #7) (type locality; Takigawa et al. 2020)	DRR398523, DRR398524 (Sawada and Fuke 2022)
Semisulcospira reticulata Kajiyama & Habe, 1961		
NSMT-Mo39770 (holotype)	Muddy bottom at depths of 5–10 m, Lake Biwa, offshore of Oki-shima Island, Okishima-cho, Omi-hachiman City, Shiga Prefecture, Japan (Kajiyama and Habe 1961)	
KUZ Z4925	Muddy bottom at depths of around 10–15 m, Lake Biwa, offshore of Oki-shima Island, Okishima-cho, Omi-hachiman City, Shiga Prefecture, Japan (locality #6)	DRR502630–DRR502631 (this study)
KUZ Z4926–Z4929	Sandy mud to muddy bottom at depths of 6–8 m, Lake Biwa, Hannoura, Nagahama City, Shiga Prefecture, Japan (locality #1)	DRR502547–DRR502549 (this study)
KUZ Z4930–Z4934	Sandy mud to muddy bottom at depths of 5–8 m, Lake Biwa, Kitafunaki, Takashima City, Shiga Prefecture, Japan (locality #4)	DRR502594–DRR502599 (this study)
KUZ Z4229, Z4230, Z4286	Muddy bottom at depths of 6–12 m, Lake Biwa, Mano, Otsu City, Shiga Prefecture, Japan (locality #7)	DRR398530–DRR398532 (Sawada and Fuke 2022)
Semisulcospira habei Davis, 1969		
KUZ Z4224	Piled rocky bottom at depths of 0–0.5 m, Uji River, Oshima, Uji City, Kyoto Prefecture, Japan (type locality; Davis 1969)	DRR398605–DRR398609 (Sawada and Fuke 2022)
KUZ Z4225	Rocky and sandy bottoms at depths of 0–0.5 m, Uji River, Yokoohji-shimomisu-higashinokuchi, Fushimi-ku, Kyoto City, Kyoto Prefecture, Japan	DRR398450–DRR398454 (Sawada and Fuke 2022)
KUZ Z4226	Sandy and concrete brock bottoms at depths of 0–0.5 m, Uji River, Yawata-zaiohji, Yawata City, Kyoto Prefecture, Japan	DRR398620–DRR398624 (Sawada and Fuke 2022)
Semisulcospira rugosa Watanabe & Nishino, 1995		
KUZ Z2493–Z2497	Piled rocky bottom at depths of 0–1 m, Lake Biwa, Kitafunaki, Takashima City, Shiga Prefecture, Japan (locality #1) (type locality; Watanabe and Nishino 1995)	DRR398496–DRR398500 (Sawada and Fuke 2022)
Semisulcospira fuscata Watanabe & Nishino, 1995		
KUZ Z4105	Piled rocky bottom at depths of 0–1 m, Lake Biwa, Oura, Nagahama City, Shiga Prefecture, Japan (type locality; Watanabe and Nishino 1995)	DRR360363–DRR360367 (Sawada and Fuke 2023)
Semisulcospira watanabei Sawada, 2022		
KUZ Z4109, Z4110, Z4115	Concrete brock bottom at depths of 0–1 m, Lake Biwa, Kitakomatsu Port, Otsu City, Shiga Prefecture, Japan (type locality; Sawada and Fuke 2023)	DRR360325, DRR360327, DRR360328 (Sawada and Fuke 2023)
Semisulcospira nakanoi Sawada, 2022		
KUZ Z4122, Z4123, Z4127	Rocky bottom at depths of 0–3 m, Lake Biwa, Chikubu-shima Island, Nagahama City, Shiga Prefecture, Japan (type locality; Sawada and Fuke 2023)	DRR360304–DRR360308 (Sawada and Fuke 2023)
Semisulcospira salebrosa Sawada, 2022		
KUZ Z4131, Z4132, Z4136	Rocky bottom at depths of 0–3 m, Lake Biwa, Okino-shiraishi Island, Takashima City, Shiga Prefecture, Japan (type locality; Sawada and Fuke 2023)	DRR360369–DRR360373 (Sawada and Fuke 2023)
Semisulcospira kurodai K1 sensu Morita et al. (2024)		
KUZ Z5145	Muddy bottom at depths of 0–1 m, creek, Tambasasayama City, Hyogo Prefecture, Japan	DRR537224–DRR537228 (this study)
Semisulcospira kurodai K2 sensu Morita et al. (2024)		
KUZ Z5146, Z5147	Muddy and rocky bottoms at depths of 0–1 m, river, Toyooka City, Hyogo Prefecture, Japan	DRR537229, DRR537230 (this study)
Semisulcospira nishimurai sp. nov.		
KUZ Z4935 (holotype)	Sandy bottom at a depth of 3 m, Lake Biwa, Hannoura, Nagahama City, Shiga Prefecture, Japan (locality #1)	DRR502539 (this study)
KUZ Z4936–Z4939 (paratypes)	Sandy to sandy mud bottom at depths of 3–6 m, Lake Biwa, Hannoura, Nagahama City, Shiga Prefecture, Japan (locality #1)	DRR502540, DRR502542 (this study)
KUZ Z4940–Z4944	Sandy gravel to sandy mud bottom at depths of 2–7 m, Lake Biwa, Hannoura, Nagahama City, Shiga Prefecture, Japan (locality #1)	DRR502541 (this study)
KUZ Z4945	Piled rocky bottom at depths of 0–1 m, Lake Biwa, Oura, Nagahama City, Shiga Prefecture, Japan (locality #2)	DRR502632–DRR502633 (this study)
KUZ Z4946–Z4951	Pebble to sandy mud bottoms at depths of 1–8 m, Lake Biwa, Minamihama-cho, Nagahama City, Shiga Prefecture, Japan (locality #3)	DRR502617–DRR502620 (this study)
KUZ Z4952–Z4956	Pebble to sandy mud bottoms at depths of 1–7 m, Lake Biwa, Kitafunaki, Takashima City, Shiga Prefecture, Japan (locality #4)	DRR502580–DRR502589, DRR502591 (this study)
KUZ Z4957	Sandy mud bottom at depths of 6–8 m, Lake Biwa, Oki-shima Island, Okishima-cho, Omihachiman City, Shiga Prefecture, Japan (locality #5)	DRR502629 (this study)

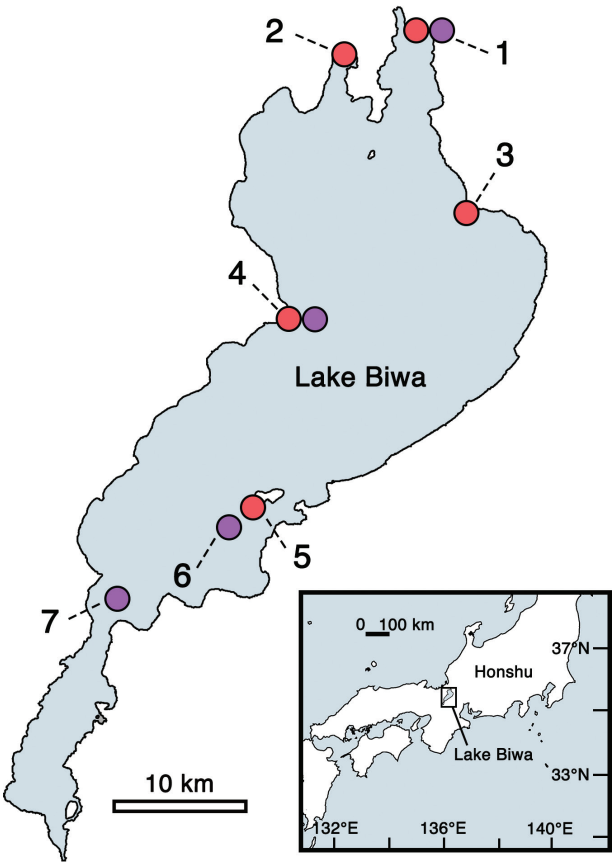

Figure 1. Map representing seven localities where *Semisulcospira reticulata* (purple) and *S. nishimurai* sp. nov. (red) were collected in Lake Biwa, Japan. The detailed localities are shown in Table 1.

Low-quality bases ($Q < 30$) and the adapter sequences were removed with fastp v0.20.1 (Chen et al. 2018) and the read length was trimmed to 109 bp to match the shorter Read 1. Quality-controlled reads were mapped to the whole genome sequence assembly of *S. habei* (GenBank accession number: BTPG01000001–BTPG01000578) (Miura et al. 2023) using Strobealign v0.13.0 (Sahlin 2022) with the default settings. SNP detection was performed using the ref_map.pl pipeline of Stacks. SNP filtering and output were conducted using populations with the following settings: SNPs common to more than 70% of all samples ($R = 0.7$) retained; SNPs with heterozygosity greater than 75% (max-obs-het = 0.75) and minor alleles less than two (min-mac = 2) excluded. All SNPs were used for the reconstruction of a phylogenetic tree, whereas only one SNP from a locus (write-single-snp) was retained for the ADMIXTURE analysis. All other parameters were set to the default settings. Only samples with less than 50% missing were used in the subsequent analyses.

Phylogenetic analyses and population structure

A phylogenetic tree of 107 *S. niponica*-group specimens was reconstructed using maximum-likelihood (ML) with IQ-TREE v1.6.12 (Nguyen et al. 2015). The best-fit models in the ML phylogenies were calculated using automatic model selection with ascertainment bias correction (-m MFP+ASC). The branch support for the ML phylogenetic tree was assessed using 1,000 Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) and 1,000 ultrafast bootstrap replicates (UFBoot).

A tree topology of the *S. niponica*-group populations was estimated based on multi-species coalescence using singular value decomposition quartets (SVDquartets) (Chifman and Kubatko 2014) implemented in PAUP* v4.0a (Swofford 2003). The branch support for the SVDquartets tree was assessed using 200 bootstrap replicates. All other parameters were set to the default settings. Based on the phylogenetic relationships within the *S. niponica*-group estimated by Miura et al. (2019) and Morita et al. (2024), *S. kurodai* K1 sensu Morita et al. (2024) and *S. kurodai* K2 sensu Morita et al. (2024) were used as outgroups for the present analysis.

Individual admixture proportions of *S. reticulata*, *S. decipiens*, *S. habei* and unidentified species were calculated according to the morphological similarities among *S. reticulata*, *S. decipiens*, and the unidentified species implied by Nishimura (2024), and closer genetic relationships of the four species in the *S. niponica*-group (Miura et al. 2019; Sawada and Fuke 2022; see also Results). The analysis was conducted with likelihood-model-based clustering using ADMIXTURE v1.3.0 (Alexander et al. 2009) with the following settings: the number of genetic populations (K) was set to 1–10, and the convergence criterion (C) was set to 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 for each K-value with the ADMIXTURE. The estimated admixture proportions were visualized in the seed value for $K = 2$–4, where the analyses estimated lower mean cross-validation error values (see Results). Summarization of cross-validation error values and visualization of the ADMIXTURE plot were performed in R v3.6.1 (R Core Team 2019).

Morphological examination

The teleoconchs, protoconchs, opercula, radulae, and reproductive organs of the newly collected *S. reticulata* and unidentified species were examined. In addition, teleoconch and protoconch of the holotype of *S. reticulata* preserved at NSMT were examined. Measurements and counts of 24 shell morphological characters were obtained following the methods described by Sawada and Nakano (2021) and Sawada and Fuke (2022). Reproductive organs were observed under a Leica M125C stereoscopic microscope (Leica Microsystems, Wetzlar, Germany) according to the methods by Strong and Köhler (2009). After dissection, the radulae were extracted by soaking the oral tissues in 1 M sodium hydroxide solution for a day. The extracted radulae were photographed using a Hitachi TM1000 scanning electron microscope (Hitachi Ltd., Tokyo, Japan).

The specimens were fixed horizontally and photographed ventrally using a Nikon D7500 camera (Nikon Corporation, Tokyo, Japan) with a Nikon-compatible Tamron SP 90 mm f /2.8 1:1 macro lens (Tamron Co., Ltd., Saitama, Japan). Measurements of morphological characters were obtained from the digital images with ImageJ v1.51 (Schneider et al. 2012). Protoconchs, opercula, radulae, and reproductive organs were obtained from larger specimens in each population, given that the correlations among teleoconch size and the characteristics of these parts are known (Watanabe 1970b; Takami 1994; Sawada and Nakano 2022).

Abbreviations of morphological characters examined are as follows: Teleoconch: **ASR**, aperture slenderness ratio (proportion of aperture length to fourth aperture width); **BCN**, basal cord number; **BWL**, body whorl length; **RN**, longitudinal rib number of penultimate whorl; **SA**, spire angle; **SCN**, spiral cord number of penultimate whorl; **WER**, whorl elongation ratio (proportion of aperture height to fourth whorl length); **WN**, whorl number. Protoconch: **PN**, number of protoconchs; **RNP**, longitudinal rib number on body whorl of the largest protoconch; **SLP**, shell length of the largest protoconch; **WNP**, whorl number of the largest protoconch; **Node Number**, number of nodes on body whorl of the largest protoconch; **Keel Type**, keel type on body whorl of the largest embryonic shell.

Morphological comparison

The shell morphologies of *S. reticulata*, *S. decipiens*, *S. habei* and the unidentified species, whose admixture proportions were calculated by the present genetic analysis, were compared using 179 specimens. Their shell morphological relationships were evaluated by principal component analysis (PCA) using normalized data for 14 morphological characters: ASR, BCN, BWL, RN, SA, SCN, WER, WN, PN, SLP, RNP, WNP, Node Number, and Keel Type.

Discriminant analysis was performed for the same datasets using the Random Forest (RF) algorithm (Breiman 2001). In the discriminant analysis, three variables were randomly sampled for each split as candidates, and 100,000 trees were generated, given that the out-of-bag error rate fully decreased with a large number of trees. Only specimens of mature females were used for morphological analyses because sexual dimorphism has been revealed in the genus (Matsuda and Miura 2022; Sawada and Nakano 2022). In addition, the central cusp morphology of the rachidian and lateral teeth of the radula were compared in the four species, following Watanabe (1970b) and Sawada and Fuke (2022). PCA was performed using R. RF analyses were conducted using the package randomForest v4.6–14 (Andy and Matthew 2002) for R.

Results

Phylogenetic analyses and population structure

After de-multiplexing, 13.4K–62.0K reads were obtained for each individual. The average coverage per individual at each locus was 11.5. The Stacks pipeline detected 19,767 SNPs from 12,417 loci with a total alignment length of 3,334,746 bp and 14.7% missing data on average in 107 snails.

The topology of the reconstructed ML and SVDquartets trees based on 19,767 SNPs were well consistent. ML tree strongly supported the monophyly of all ingroup *S. niponica*-group species (SH-aLRT = 98.3–100; UFBoot = 100) (Figs 2, 3). The monophyly of *S. reticulata*, *S. nishimurai* sp. nov., *S. habei*, and *S. niponica* was also recovered with strong support in the SVDquartets tree (BS = 100). Strong support for the monophyly of the clade, including ten ingroup species in the Lake Biwa system, was obtained from both trees (SH-aLRT = 100; UFBoot = 100; BS = 100). A monophyletic clade consisting of all *S. niponica*-group species other than *S. reticulata* was recovered with moderate support (SH-aLRT = 95.8; UFBoot = 99; BS = 86.5). The monophyly of clades consisting of *S. decipiens* and *S. habei* (SH-aLRT = 100; UFBoot = 100; BS = 100), as well as clades including *S. fuscata*, *S. watanabei*, *S. niponica*, *S. salebrosa*, and *S. nakanoi* (SH-aLRT = 100; UFBoot = 100; BS = 100), were also robustly supported by both trees. Clades consisting of specimens from Kitafunaki (SH-aLRT = 100; UFBoot = 100) and other localities (SH-aLRT = 95.8; UFBoot = 99; BS = 98.5) were strongly supported within *S. reticulata*. The monophyly of *S. decipiens* (SH-aLRT = 98.6; UFBoot = 100), the clade consisting of *S. nishimurai* sp. nov., *S. decipiens*, and *S. habei* (SH-aLRT = 96.7; UFBoot = 97), and the clade including *S. rugosa*, *S. fuscata*, *S. watanabei*, *S. niponica*, *S. salebrosa*, and *S. nakanoi* (SH-aLRT = 100; UFBoot = 99) were supported only by ML tree.

ADMIXTURE analysis based on 4,401 SNPs estimated lower mean cross-validation error values for two to four genetic clusters (Suppl. material 1: table S2). *Semisulcospira reticulata* and *S. nishimurai* sp. nov. were clearly divided into independent clusters with *K* values greater than two (Fig. 4). Common ancestries were detected between *S. decipiens* and *S. habei* at $K = 2$ and 3, whereas the two species were divided into almost independent clusters at *K* values greater than three.

Morphological comparison

PCA conducted for *S. reticulata*, *S. nishimurai* sp. nov., *S. decipiens*, and *S. habei* using 14 morphological characters summarized their morphological relationships. PC1 and PC2 explained 35.7% and 16.1% of total variation, respectively. The first PC separated *S. reticulata*, *S. nishimurai* sp. nov., and *S. decipiens*, with slight overlaps in scores (Fig. 5). A large overlap was observed between the PC1 scores of *S. habei* and *S. decipiens*.

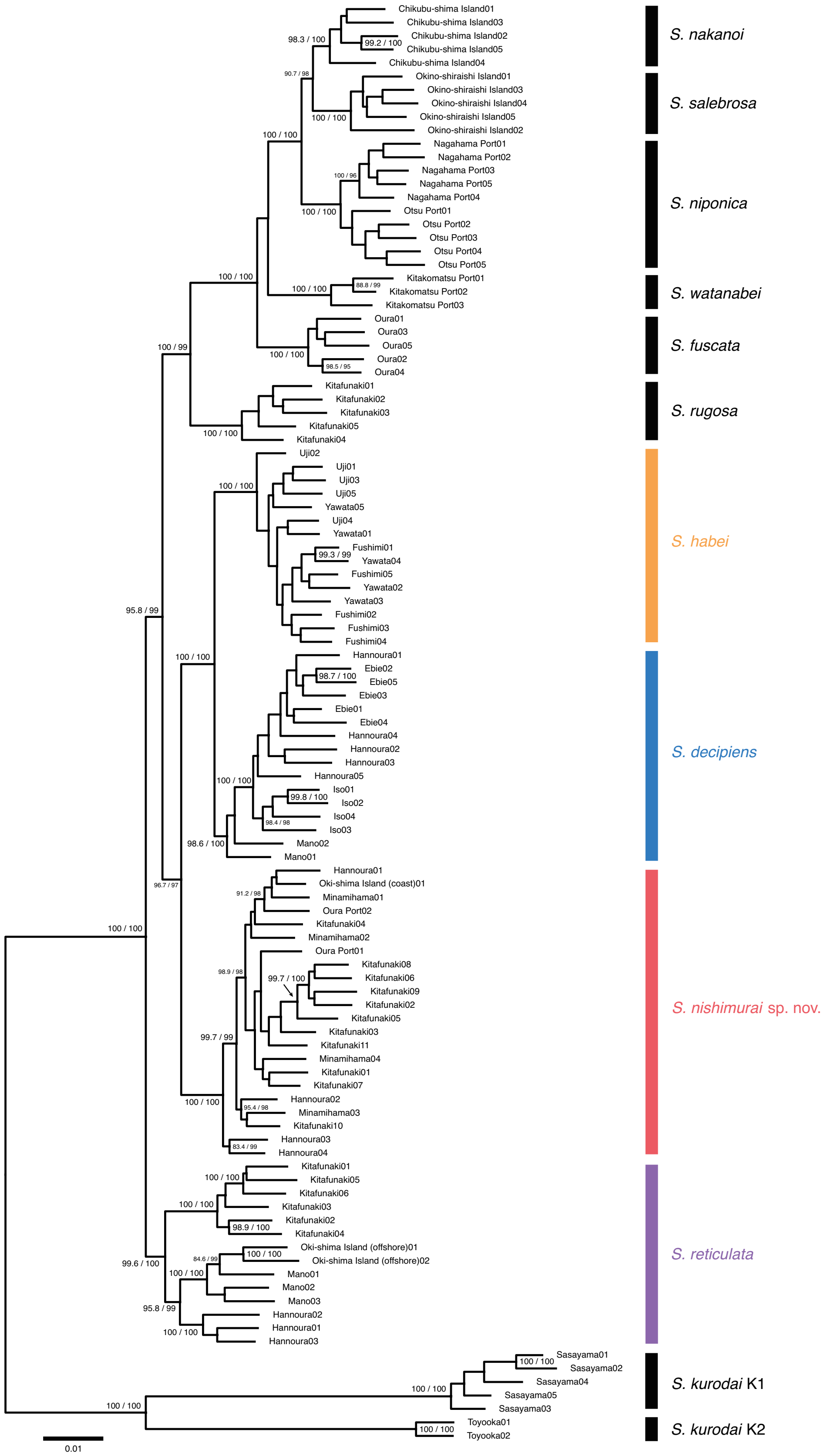

Figure 2. The maximum likelihood tree of 11 *Semisulcospira niponica*-group species calculated based on 19,767 SNPs. The numbers associated with the nodes represent values of SH-aLRT (left) / ultrafast bootstrap (right). Values of SH-aLRT higher than 80.0 and that of ultrafast bootstrap greater than 95 are indicated.

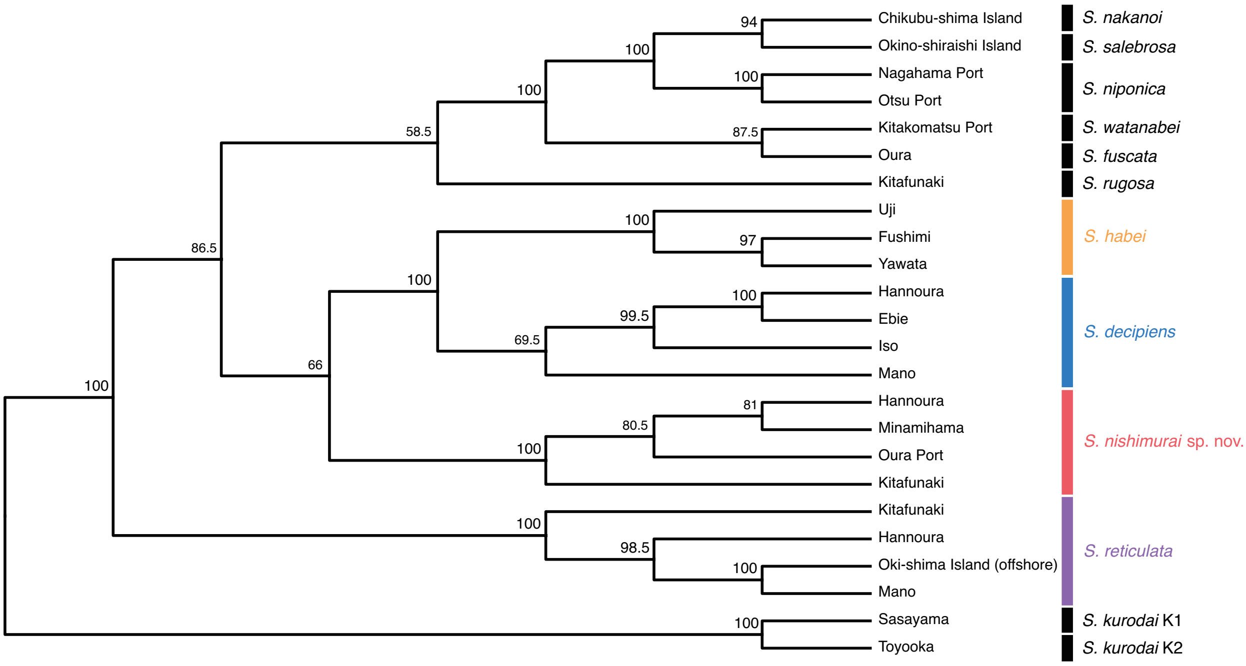

Figure 3. SVDquartets tree of *Semisulcospira niponica*-group populations calculated based on 19,767 SNPs. The numbers associated with the nodes represent bootstrap values.

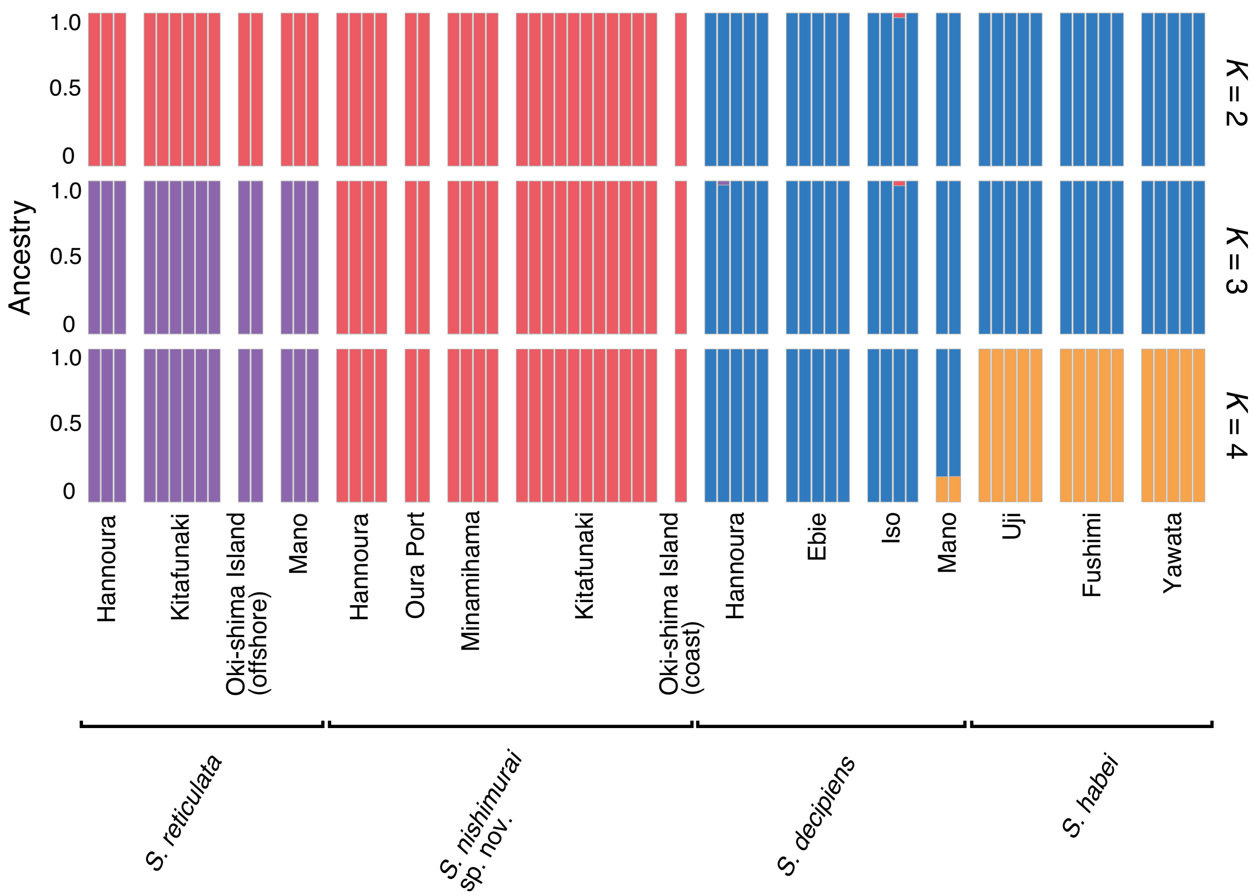

Figure 4. Results of the ADMIXTURE analysis based on 4,401 SNPs conducted for *S. reticulata*, *S. nishimurai* sp. nov., *S. decipiens*, and *S. habei*.

The scores of *S. habei* partially overlapped with those of *S. nishimurai* sp. nov. The PC1 scores of *S. nishimurai* sp. nov. were intermediate between those of the other three species. The values of other PCs largely overlapped among the four species. Among the morphological characters analyzed, Keel Type, SLP, WNP, RN, Node Number, and WER contributed more strongly to explaining the variation in PC1 than the others (Suppl. material 1: table S3).

RF classification using the same dataset as PCA correctly classified 93.3% of the specimens into the four species. Bootstrap tests identified 98% *S. reticulata*, 94% *S. nishimurai* sp. nov., 95% *S. decipiens*, and 85% *S. habei*. The mean decrease in the Gini coefficient was larger for SLP, Node Number, WER, BWL, and PN (Table 2). The mean decrease in accuracy was greater for the following morphological characters: SLP, Keel Type, and PN in *S. reticulata*; SLP, WER, and BWL in *S. nishimurai* sp. nov.; RN, BWL, and Node Number in *S. decipiens*; and Node Number, WER, and ASR in *S. habei*. The specimens of *S. reticulata* possessed protoconchs without distinct keels that were fewer and larger than those of the other three species (Table 3). *Semisulcospira nishimurai* sp. nov. represented smaller teleoconchs and protoconchs than the other three species. Measurements of WER were lower for *S. nishimurai* sp. nov. and *S. reticulata* than for *S. decipiens* and *S. habei*.

Semisulcospira reticulata and *S. nishimurai* sp. nov. possessed narrow, pointed cusps on the rachidian and lateral teeth of the radula (Table 3; Fig. 6). The central cusps of the two species were typically sharper than those of *S. decipiens* and *S. habei*.

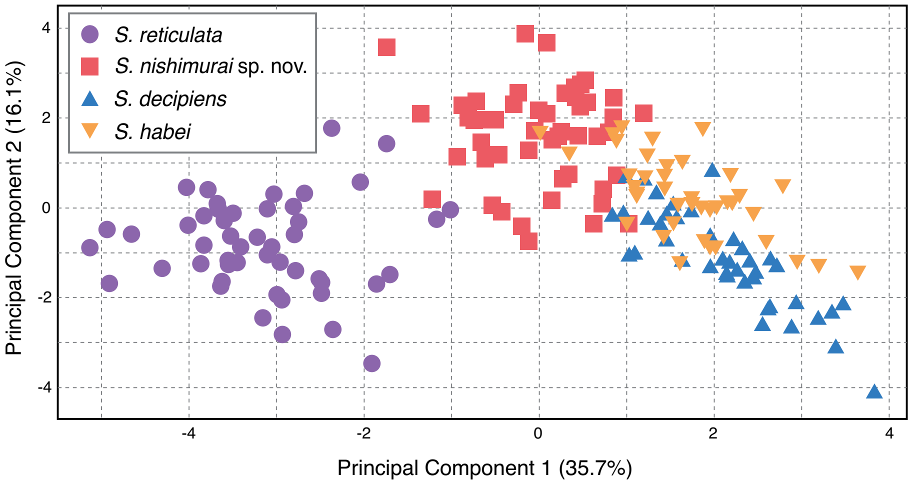

Figure 5. Scatter plot of PC1 and PC2 generated by principal component analysis based on the normalized data of 14 morphological characters conducted for the four *Semisulcospira niponica*-group species.

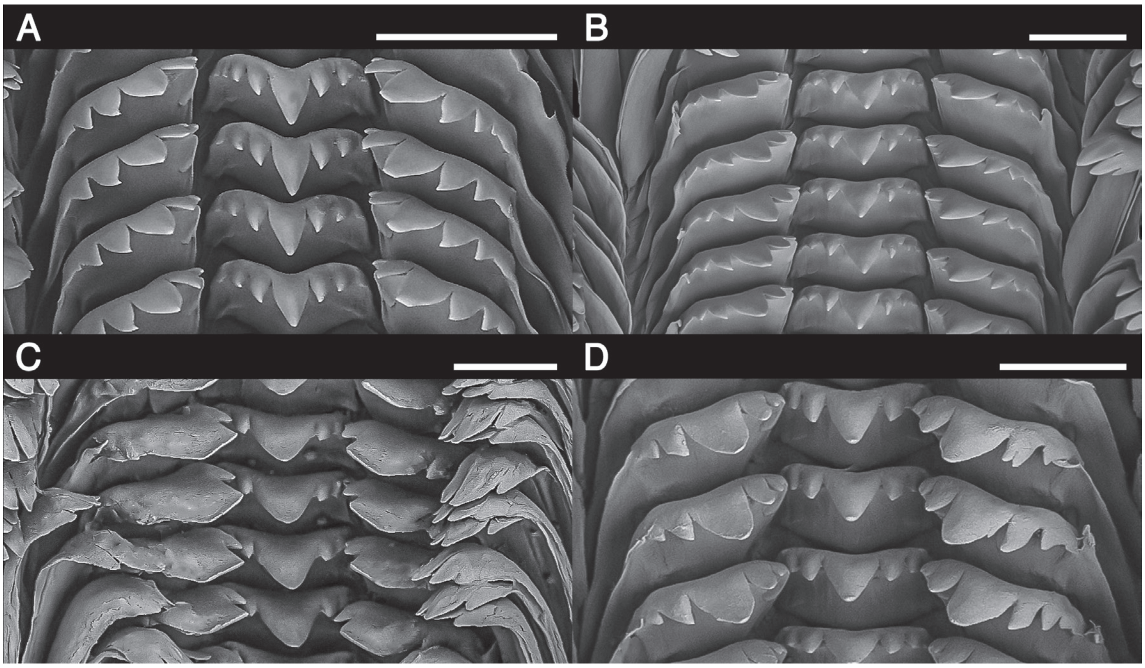

Figure 6. Radula morphology of four *Semisulcospira niponica*-group species. **A.** *S. reticulata*; **B.** *S. nishimurai* sp. nov.; **C.** *S. decipiens*; **D.** *S. habei*. Scale bars: 100 µm.

Table 2. Results of the Random Forest analyses conducted for four *Semisulcospira niponica*-group species with specimen numbers, mean decrease in accuracy in each character, and mean decreases in Gini coefficients.

Character	*S. reticulata*	*S. nishimurai* sp. nov.	*S. decipiens*	*S. habei*	Mean decrease of Gini coefficients
Specimen number	49	49	41	40	
Aperture slenderness ratio (ASR)	0.002	0.049	0.007	0.091	9.049
Basal cord number (BCN)	0	0.041	0.053	0	5.125
Body whorl length (BWL)	0.019	0.085	0.122	0.048	14.856
Longitudinal rib number of penultimate whorl (RN)	0.076	0.008	0.127	0.046	11.396
Spire angle (SA)	0.001	0.011	0.020	0.001	4.409
Spiral cord number of penultimate whorl (SCN)	0	0	0.001	0.001	1.188
Whorl elongation ratio (WER)	0.025	0.095	0.079	0.120	15.024
Whorl number (WN)	0.007	0.001	0.009	0.002	2.927
Number of protoconchs (PN)	0.154	0.029	0.073	0.058	14.250
Longitudinal rib number on body whorl of the largest protoconch (RNP)	0.012	0.001	0.019	0.003	3.005
Shell length of the largest protoconch (SLP)	0.252	0.109	0.068	0.050	19.478
Whorl number of the largest protoconch (WNP)	0.024	0.012	0.028	0.005	5.213
Number of nodes on body whorl of the largest protoconch (Node Number)	0.077	0.007	0.102	0.248	17.454
Keel type on body whorl of the largest protoconch (Keel Type)	0.157	0.017	0.072	0.076	9.691

Table 3. Morphometric characters of the four *Semisulcospira niponica*-group examined in the present study. Measurements and counts: minimum–maximum value (mean ± SD).

Character	*S. reticulata*	*S. nishimurai* sp. nov.	*S. decipiens*	*S. habei*
Aperture slenderness ratio (ASR)	1.30–1.91 (1.6 ± 0.1)	1.42–1.81 (1.6 ± 0.1)	1.54–1.81 (1.7 ± 0.1)	1.58–1.92 (1.7 ± 0.1)
Basal cord number (BCN)	2–5 (3.3 ± 0.7)	2–4 (2.9 ± 0.6)	3–6 (4.4 ± 0.8)	3–6 (3.8 ± 0.8)
Body whorl length (BWL) (mm)	13.7–22.4 (18.4 ± 1.8)	12.8–22.0 (15.9 ± 1.7)	15.9–23.9 (19.7 ± 1.7)	15.1–20.0 (17.2 ± 1.2)
Longitudinal rib number of penultimate whorl (RN)	20–34 (26.4 ± 3.2)	14–31 (20.4 ± 3.7)	14–22 (17.4 ± 1.6)	15–23 (19.7 ± 1.8)
Spire angle (SA) (degrees)	13.7–22.4 (18.0 ± 2.3)	13.0–23.2 (17.3 ± 2.4)	15.0–24.8 (20.3 ± 2.3)	13.7–23.6 (18.4 ± 2.5)
Spiral cord number of penultimate whorl (SCN)	5–8 (6.2 ± 0.8)	5–9 (6.3 ± 0.7)	4–8 (6.3 ± 0.8)	5–8 (6.5 ± 0.8)
Whorl elongation ratio (WER)	1.97–2.78 (2.4 ± 0.2)	2.09–2.98 (2.4 ± 0.2)	2.47–3.48 (2.8 ± 0.2)	2.44–3.58 (2.9 ± 0.3)
Whorl number (WN)	4.50–7.50 (6.0 ± 1.0)	3.50–8.75 (5.6 ± 1.1)	4.00–6.50 (4.8 ± 0.7)	3.5–7.0 (5.00 ± 0.8)
Number of protoconchs (PN)	1–17 (7.4 ± 3.7)	4–102 (34.6 ± 23.2)	9–158 (64.9 ± 39.4)	8–82 (30.7 ± 11.8)
Longitudinal rib number on body whorl of the largest protoconch (RNP)	10–19 (14.0 ± 1.8)	9–16 (12.3 ± 1.4)	10–13 (11.6 ± 0.8)	11–15 (12.3 ± 0.9)
Shell length of the largest protoconch (SLP) (mm)	2.5–7.5 (5.1 ± 1.0)	1.8–3.1 (2.4 ± 0.3)	1.3–3.1 (2.6 ± 0.3)	1.9–3.6 (2.7 ± 0.4)
Whorl number of the largest protoconch (WNP)	2.50–5.00 (3.8 ± 0.4)	2.25–4.00 (3.2 ± 0.3)	2.25–3.50 (3.1 ± 0.3)	2.50–4.00 (3.1 ± 0.4)
Number of nodes on body whorl of the largest protoconch (Node Number) (0 / 1 / 2 or 3) (%)	90 / 4 / 6	12 / 67 / 21	0 / 93 / 7	0 / 8 / 92
Keel type on body whorl of the largest protoconch (Keel Type) (absent / weak / prominent) (%)	73 / 27 / 0	0 / 27 / 73	0 / 0 / 100	0 / 0 / 100
Typical shape of the largest cusp of rachidian tooth	narrow, pointed	narrow, pointed	wide, rounded	narrow, rounded
Typical shape of the largest cusp of lateral tooth	narrow, pointed	narrow, pointed	wide, flat	wide, rounded

Taxonomy

Semisulcospiridae Morrison, 1952

Semisulcospira Boettger, 1886

Type species. *Melania libertina* Gould, 1859 by subsequent designation (Wenz 1939).

Semisulcospira reticulata Kajiyama & Habe, 1961

Table 4, Figs 7A–Y, 8A–D, 9C

Japanes name: Kagome-kawanina (Kajiyama and Habe 1961)

Semisulcospira decipiens reticulata Kajiyama & Habe, 1961: 171–173, 175–176, figs 6, 6a; Habe 1965: 57, unnumbered figure; Kawase et al. 2012: 37, figs 2–11.

Semisulcospira reticulata – Burch and Davis 1967: 36–37; Burch 1968: 6, 26–29, fig. 1D, pl. 4, figs 4, 5, pl. 5, fig. 8; Davis, 1969: 249, 255, pl. 5, figs 1, 2, pl. 6, figs 1, 2, pl. 10, figs 10, 11, pl. 11., figs 1–4; Watanabe 1970a: 17–23, fig. 15-3, 8, 9; Habe 1986: 322, fig. 17-1-17; Köhler 2016: fig4L–N; Sawada and Fuke 2022: fig. 11bq–bu.

Biwamelania decipiens – Habe 1978: 94; Minato 1991: 79, unnumbered fig.

Semisulcospira (*Biwamelania*) *reticulata* – Matsuoka 1985: 190; Watanabe and Nishino 1995: app. pl. 1, fig. 8, app. pl. 2, figs 22, 23; Nishino and Watanabe 2000: fig. 2–12; Matsuda 2000: 8–9, unnumbered fig.; Kihira et al. 2009: 26–27, 33, unnumbered figs; Miura et al. 2019: fig. S1p–r; Nishino 2021: 618.

Biwamelania reticulata – Kihira and Matsuda 1990: 22–23, 25, unnumbered figs; Nishino and Tanida 2018: 60, 253, unnumbered figs.

Semisulcospira decipiens multigranosa – Kawase et al. 2012: 37, figs 2–10.

Material examined. ***Holotype.*** NSMT-Mo39770, adult female, collected from “Off Okinoshima in Lake Biwa” (Lake Biwa, offshore of Oki-shima Island, Okishima-cho, Omi-hachiman City, Shiga Prefecture, Japan).

Additional materials. KUZ Z4926, 15 females, Z4927, 9 males, collected from 6–8 m water depths at Hannoura on 26 June 2022; KUZ ZZ4930, 15 females, Z4931, 5 males, from 5–8 m depths at Kitafunaki on 10 July 2022; KUZ Z4925, 15 females, from 10–15 m depths offshore at Oki-shima Island on 28 April 2019; KUZ Z4229, 4 females, Z4286, 4 males, from 6–12 m depths at Mano on 12 October 2021.

Table 4. Morphometric characters of *Semisulcospira reticulata* and *S. nishimurai* sp. nov. Measurements and counts: minimum–maximum value (mean ± SD).

Species	***Semisulcospira reticulata***				***Semisulcospira nishimurai* sp. nov.**				
Locality	Hannoura	Kitafunaki	Oki–shima Island	Mano	Hannoura	Oura Port	Minamihama	Kitahunaki	Oki–shima Island
specimen number of shells (mature male / mature female / operculum / protoconch)	3 / 15 / 5 / 9	5 / 15 / 8 / 15	0 / 15 / 7 / 10	4 / 4 / 2 / 3	12 / 15 / 11 / 14	0 / 2 / 2 / 2	13 / 15 / 7 / 12	5 / 14 / 5 / 11	0 / 3 / 3 / 2
specimen number of radulae	5	5	4	3	5	2	5	5	2
specimen number of reproductive organs (male / female)	3 / 8	3 / 8	0 / 10	5 / 4	6 / 8	0 / 0	5 / 7	5 / 7	0 / 1
Morphological characters of mature female teleoconchs									
Aperture height (AH) (mm)	10.2–13.1 (11.6 ± 0.8)	9.5–12.0 (10.1 ± 0.6)	8.9–12.0 (10.2 ± 0.9)	8.0–12.0 (10.3 ± 1.7)	8.1–12.5 (9.9 ± 1.0)	10.0–10.1 (10.0 ± 0.1)	7.4–9.7 (8.8 ± 0.5)	8.0–11.9 (9.0 ± 0.9)	8.1–8.2 (8.1 ± 0.1)
Aperture length (AL) (mm)	9.0–13.6 (11.9 ± 1.1)	9.7–12.9 (10.5 ± 0.8)	9.5–12.5 (10.8 ± 0.8)	8.7–12.1 (10.7 ± 1.4)	8.7–13.1 (10.2 ± 1.1)	10.2–10.3 (10.3 ± 0.1)	7.4–9.8 (8.9 ± 0.6)	7.8–12.0 (9.0 ± 1.0)	7.9–8.4 (8.1 ± 0.2)
Aperture slenderness ratio (ASR)	1.31–1.68 (1.5 ± 0.1)	1.52–1.89 (1.7 ± 0.11)	1.47–1.91 (1.6 ± 0.1)	1.30–1.50 (1.4 ± 0.1)	1.42–1.68 (1.6 ± 0.1)	1.62–1.64 (1.6 ± 0.1)	1.46–1.77 (1.6 ± 0.1)	1.42–1.81 (1.6 ± 0.1)	1.49–1.64 (1.6 ± 0.1)
Aperture width (AW) (mm)	6.9–8.5 (7.7 ± 0.5)	5.7–7.2 (6.2 ± 0.4)	5.6–7.8 (6.6 ± 0.5)	6.1–8.4 (7.6 ± 1.0)	5.6–7.8 (6.4 ± 0.5)	6.3	4.5–6.1 (5.6 ± 0.4)	4.6–7.2 (5.6 ± 0.7)	5.1–5.3 (5.2 ± 0.1)
Basal cord number (BCN)	2–5 (3.7 ± 0.7)	3–4 (3.3 ± 0.5)	2–4 (2.9 ± 0.5)	3–4 (3.5 ± 0.6)	2–4 (3.2 ± 0.7)	3–4 (3.5 ± 0.7)	2–4 (2.7 ± 0.6)	2–4 (2.9 ± 0.5)	2–3 (2.7 ± 0.6)
Body whorl length (BWL) (mm)	17.9–22.4 (20.1 ± 1.2)	16.3–20.6 (17.3 ± 1.1)	16.2–20.7 (18.3 ± 1.4)	13.7–19.9 (17.6 ± 2.8)	15.0–22.0 (17.2 ± 1.7)	17.0–17.9 (17.5 ± 0.6)	12.8–16.5 (15.1 ± 0.8)	14.0–19.4 (15.5 ± 1.4)	13.7–14.2 (13.9 ± 0.2)
Fourth whorl length (FWL) (mm)	4.0–5.2 (4.7 ± 0.3)	3.8–4.6 (4.1 ± 0.3)	3.9–5.2 (4.4 ± 0.4)	3.4–4.4 (4.1 ± 0.5)	3.3–5.3 (4.1 ± 0.5)	3.6–4.4 (4.0 ± 0.6)	3.1–4.2 (3.7 ± 0.3)	3.3–4.4 (3.8 ± 0.3)	3.2–3.3 (3.3 ± 0.1)
Penultimate whorl length (PWL) (mm)	6.8–8.3 (7.7 ± 0.5)	5.8–7.7 (6.5 ± 0.5)	6.1–8.7 (7.4 ± 0.8)	5.1–7.4 (6.7 ± 1.1)	6.0–8.5 (6.6 ± 0.7)	6.4–7.4 (6.9 ± 0.7)	4.8–6.3 (5.8 ± 0.4)	4.9–7.4 (6.1 ± 0.7)	5.2–5.5 (5.3 ± 0.2)
Longitudinal rib number of penultimate whorl (RN)	20–31 (26.5 ± 2.9)	22–33 (27.4 ± 3.0)	20–30 (24.7 ± 2.8)	25–34 (29.3 ± 4.4)	16–28 (22.8 ± 3.4)	21–25 (23.0 ± 2.8)	14–21 (18.6 ± 1.8)	17–31 (20.5 ± 4.0)	15–17 (15.7 ± 1.2)
Spire angle (SA) (degrees)	13.7–22.3 (19.0 ± 2.4)	14.1–20.4 (17.4 ± 2.2)	14.4–22.4 (17.4 ± 2.1)	18.5–20.3 (19.5 ± 0.8)	15.0–20.5 (17.9 ± 1.8)	17.7–18.6 (18.1 ± 0.6)	13.8–23.2 (18.0 ± 2.9)	13.0–18.1 (15.3 ± 1.6)	18.2–20.4 (19.3 ± 1.1)
Spiral cord number of penultimate whorl (SCN)	6–8 (6.5 ± 0.7)	5–7 (6.2 ± 0.9)	5–7 (6.0 ± 0.7)	5–6 (5.5 ± 0.6)	5–7 (5.9 ± 0.7)	6–7 (6.5 ± 0.7)	6–7 (6.4 ± 0.5)	5–9 (6.5 ± 1.1)	6–7 (6.5 ± 0.7)
Shell length (SL) (mm)	32.1–41.7 (38.0 ± 2.9)	30.1–39.5 (33.4 ± 2.5)	31.9–43.8 (37.7 ± 3.4)	28.2–41.2 (36.6 ± 5.7)	29.3–40.4 (33.8 ± 2.7)	30.3–31.9 (31.1 ± 1.1)	27.3–34.1 (29.5 ± 1.8)	26.2–33.9 (29.6 ± 2.7)	23.3–28.3 (25.6 ± 2.5)
Shell width (SW) (mm)	12.6–15.9 (14.5 ± 1.0)	11.4–13.9 (12.1 ± 0.7)	11.4–14.3 (13.1 ± 0.7)	10.8–14.6 (13.3 ± 1.7)	10.5–14.2 (11.7 ± 1.0)	11.2–11.3 (11.3 ± 0.1)	8.4–11.1 (10.1 ± 0.6)	8.8–12.2 (10.0 ± 0.9)	9.3–9.7 (9.5 ± 0.2)
Third whorl length (TWL) (mm)	5.1–6.8 (6.0 ± 0.5)	4.4–5.9 (5.1 ± 0.5)	5.0–6.8 (5.8 ± 0.5)	4.2–5.9 (5.4 ± 0.8)	4.5–6.6 (5.2 ± 0.6)	4.8	3.6–5.2 (4.5 ± 0.4)	4.3–6.0 (4.9 ± 0.4)	4.0–4.3 (4.2 ± 0.2)
Whorl elongation ratio (WER)	2.21–2.78 (2.5 ± 0.2)	2.19–2.75 (2.5 ± 0.2)	1.97–2.59 (2.3 ± 0.2)	2.37–2.73 (2.5 ± 0.2)	2.21–2.98 (2.4 ± 0.2)	2.28–2.79 (2.5 ± 0.4)	2.09–2.76 (2.4 ± 0.2)	2.13–2.68 (2.4 ± 0.1)	2.44–2.51 (2.5 ± 0.1)
Whorl number (WN)	4.50–7.25 (5.3 ± 0.8)	4.50–7.00 (5.6 ± 0.7)	5.00–7.50 (6.7 ± 0.9)	6.25–7.00 (6.7 ± 0.4)	4.25–7.00 (5.9 ± 0.7)	5	3.50–8.00 (5.8 ± 1.0)	3.50–8.75 (5.5 ± 1.5)	4.00–5.75 (4.9 ± 0.1)
Sculpture Type (node / granulated rib / smooth rib / spiral cord / smooth) (%)	87 / 0 / 0 / 13 / 0	47 / 53 / 0 / 0 / 0	80 / 20 / 0 / 0 / 0	100 / 0 / 0 / 0 / 0	40 / 60 / 0 / 0 / 0	100 / 0 / 0 / 0 / 0	27 / 47 / 27 / 0 / 0	64 / 29 / 7 / 0 / 0	0 / 67 / 33 / 0 / 0
Morphological characters of mature male teleoconchs									
Aperture height (AH) (mm)	7.6–9.2 (8.4 ± 0.8)	7.7–9.1 (8.2 ± 0.5)	–	8.0–8.6 (8.2 ± 0.3)	7.5–10.6 (9.1 ± 0.9)	–	6.9–9.5 (8.3 ± 0.7)	7.9–9.2 (8.3 ± 0.6)	–
Aperture length (AL) (mm)	8.5–9.8 (9.0 ± 0.6)	8.0–9.3 (8.5 ± 0.5)	–	8.3–8.9 (8.6 ± 0.3)	7.8–10.7 (9.2 ± 0.8)	–	6.8–9.7 (8.4 ± 0.8)	7.7–9.3 (8.5 ± 0.6)	–
Aperture slenderness ratio (ASR)	1.55–1.68 (1.6 ± 0.1)	1.34–1.76 (1.6 ± 0.2)	–	1.47–1.69 (1.6 ± 0.1)	1.45–1.69 (1.6 ± 0.1)	–	1.45–1.91 (1.7 ± 0.1)	1.74–1.81 (1.8 ± 0.1)	–
Aperture width (AW) (mm)	5.3–5.8 (5.5 ± 0.3)	4.6–6.0 (5.2 ± 0.6)	–	5.3–5.7 (5.5 ± 0.2)	4.6–6.6 (5.8 ± 0.6)	–	4.3–5.9 (5.0 ± 0.5)	4.4–5.3 (4.8 ± 0.4)	–
Basal cord number (BCN)	4–5 (4.3 ± 0.6)	2–4 (3.4 ± 0.9)	–	2–3 (2.8 ± 0.5)	2–4 (2.9 ± 0.7)	–	2–6 (3.4 ± 1.0)	3	–
Body whorl length (BWL) (mm)	13.8–16.2 (14.6 ± 1.4)	13.0–15.3 (14.0 ± 0.9)	–	13.9–14.4 (14.1 ± 0.2)	12.9–18.3 (15.5 ± 1.5)	–	11.1–16.1 (13.7 ± 1.2)	13–15.7 (14.1 ± 1.0)	–
Fourth whorl length (FWL) (mm)	2.7–3.0 (2.9 ± 0.2)	2.0–3.7 (3.0 ± 0.6)	–	3.6–3.8 (3.7 ± 0.1)	3.3–4.2 (3.7 ± 0.3)	–	2.5–3.8 (3.2 ± 0.4)	3.1–3.9 (3.4 ± 0.3)	–
Penultimate whorl length (PWL) (mm)	4.6–6.2 (5.4 ± 0.8)	4.7–5.9 (5.3 ± 0.6)	–	5.0–5.8 (5.5 ± 0.4)	4.9–7.1 (6.0 ± 0.7)	–	4.0–6.1 (4.9 ± 0.6)	4.7–5.9 (5.2 ± 0.4)	–
Longitudinal rib number of penultimate whorl (RN)	20–25 (23.0 ± 2.6)	19–24 (21.4 ± 2.5)	–	20–21 (20.7 ± 0.6)	16–23 (18.8 ± 2.3)	–	15–23 (18.3 ± 2.3)	14–21 (16.6 ± 2.7)	–
Spire angle (SA) (degrees)	21.5–24.8 (23.5 ± 1.7)	16.2–19.3 (17.7 ± 1.5)	–	14.8–19.5 (16.7 ± 2.0)	12.1–18.4 (16.1 ± 1.9)	–	14.6–23.1 (19.1 ± 2.2)	15.4–20.3 (17.4 ± 2.1)	–
Spiral cord number of penultimate whorl (SCN)	5–6 (5.7 ± 0.6)	5–6 (5.4 ± 0.5)	–	4–6 (4.8 ± 1.0)	5–6 (5.8 ± 0.4)	–	5–7 (5.9 ± 0.6)	5–6 (5.8 ± 0.5)	–
Shell length (SL) (mm)	23.2–27.2 (24.8 ± 2.1)	22.7–31.6 (26.6 ± 3.4)	–	28.7–31.1 (29.8 ± 1.1)	25.3–34.0 (29.7 ± 3.2)	–	20.5–30.2 (25.9 ± 2.7)	24.1–28.6 (25.8 ± 1.8)	–
Shell width (SW) (mm)	10.1–11.2 (10.6 ± 0.5)	8.6–10.2 (9.5 ± 0.7)	–	10.0–11.0 (10.6 ± 0.5)	8.5–11.3 (10.2 ± 0.9)	–	7.8–10.2 (8.8 ± 0.7)	8.2–10.2 (9.2 ± 0.8)	–

Species	*Semisulcospira reticulata*				*Semisulcospira nishimurai* sp. nov.				
Third whorl length (TWL) (mm)	3.2–4.3 (3.7 ± 0.5)	3.6–4.1 (3.9 ± 0.2)	–	4.3–5.1 (4.5 ± 0.4)	4.1–5.4 (4.7 ± 0.4)	–	3.4–4.5 (4.0 ± 0.4)	3.6–4.5 (4.0 ± 0.3)	–
Whorl elongation ratio (WER)	2.50–3.15 (2.9 ± 0.4)	2.18–4.05 (2.8 ± 0.7)	–	2.11–2.41 (2.2 ± 0.1)	2.28–2.65 (2.5 ± 0.1)	–	2.18–3.05 (2.6 ± 0.3)	2.19–2.66 (2.5 ± 0.2)	–
Whorl number (WN)	5.00–5.25 (5.1 ± 0.1)	4.50–8.00 (6.0 ± 1.4)	–	6.00–8.00 (6.8 ± 0.9)	4.50–6.25 (5.2 ± 0.6)	–	4.25–8.00 (5.8 ± 1.2)	4.25–5.25 (4.8 ± 0.4)	–
Sculpture Type (node / granulated rib / smooth rib / spiral cord / smooth) (%)	33 / 67 / 0 / 0 / 0	60 / 40 / 0 / 0 / 0	–	100 / 0 / 0 / 0 / 0	50 / 42 / 8 / 0 / 0	–	23 / 62 / 15 / 0 / 0	60 / 20 / 20 / 0 / 0	–
Morphological characters of protoconchs									
Number of protoconchs (PN)	4–17 (9.3 ± 4.8)	1–14 (7.3 ± 4.2)	3–14 (7.7 ± 3.8)	2–4 (3.0 ± 1.0)	26–102 (57.4 ± 18.9)	56–97 (76.5 ± 29.0)	5–52 (23.3 ± 13.8)	5–43 (21.3 ± 9.4)	4–24 (14.0 ± 14.1)
Longitudinal rib number on body whorl of the largest protoconch (RNP)	11–15 (13.3 ± 1.6)	12–19 (15.1 ± 2.4)	12–15 (13.6 ± 1.0)	10–15 (12.7 ± 2.5)	11–16 (12.9 ± 1.4)	11–13 (12.0 ± 1.4)	11–16 (12.6 ± 1.4)	9–15 (11.7 ± 1.7)	11–12 (11.5 ± 0.7)
Shell length of the largest protoconch (SLP) (mm)	4.0–5.9 (5.2 ± 0.5)	2.5–6.6 (4.6 ± 1.1)	4.7–7.5 (5.9 ± 1.0)	2.7–4.5 (3.9 ± 1.0)	2.1–2.9 (2.5 ± 0.2)	2.6–3.1 (2.8 ± 0.4)	1.8–2.5 (2.3 ± 0.2)	2.0–2.7 (2.3 ± 0.2)	2.3–2.9 (2.6 ± 0.4)
Shell width of the largest protoconch (SWP) (mm)	3.4–4.2 (3.8 ± 0.2)	2.1–3.9 (3.2 ± 0.5)	3.4–4.3 (3.8 ± 0.3)	2.8–3.7 (3.3 ± 0.5)	1.5–2.1 (1.8 ± 0.2)	1.8–1.9 (1.9 ± 0.1)	1.6–1.9 (1.8 ± 0.1)	1.5–1.9 (1.7 ± 0.1)	1.8
Whorl number of the largest protoconch (WNP)	3.00–4.15 (3.8 ± 0.4)	2.50–4.50 (3.8 ± 0.5)	3.50–5.00 (4.1 ± 0.5)	2.50–3.50 (3.1 ± 0.5)	2.75–4.00 (3.3 ± 0.3)	3.50–3.75 (3.6 ± 0.2)	2.25–3.50 (3.1 ± 0.3)	2.75–3.50 (3.1 ± 0.2)	2.75–3.50 (3.1 ± 0.5)
Number of nodes on body whorl of the largest protoconch (Node Number) (0 / 1 / 2 or 3) (%)	100 / 0 / 0	100 / 0 / 0	93 / 7 / 0	0 / 33 / 67	7 / 93 / 0	50 / 50 / 0	20 / 73 / 7	0 / 36 / 64	50 / 50 / 0
Keel type on body whorl of the largest protoconch (Keel Type) (absent / weak / prominent) (%)	73 / 27 / 0	80 / 20 / 0	50 / 50 / 0	100 / 0 / 0	0 / 20 / 80	0 / 50 / 50	0 / 27 / 73	0 / 29 / 71	0 / 50 / 50
Morphological characters of radulae									
Cusp number of rachidian	5–7	5–8	5–6	5–8	5–8	5–7	5–7	5–8	5–6
Cusp number of laterial teeth	4–8	5–7	4–6	6–7	5–7	5–6	5–7	4–6	4–6
Cusp number of interior marginal teeth	4–5	5–6	4–5	3–6	4–5	4–5	4–5	4–6	5–7
Cusp number of exterior marginal teeth	3–4	4–5	4	3–5	3–4	3–4	3–4	3–5	4–5
Shape of the largest cusp of rachidian tooth	narrow, pointed	very narrow, pointed	very narrow, pointed	narrow, pointed	narrow, pointed	narrow, pointed	narrow, pointed	narrow, pointed	slightly narrow, pointed
Shape of the largest cusp of lateral tooth	narrow, pointed	narrow, pointed	narrow, pointed	narrow, pointed	narrow, pointed	slightly narrow, pointed	slightly narrow, pointed	slightly narrow, pointed	wide, rounded

Amended diagnosis. Viviparous. Teleoconch elongated, large in the genus [SH 36.4 ± 3.7 (mean ± SD) (female), 27.2 ± 3.1 (male) mm; BWL 18.4 ± 1.8, 14.2 ± 0.8 mm]; spires laterally broadened (SA 18.0 ± 2.3, 18.8 ± 3.3 degrees), axially compressed (WER 2.4 ± 0.2, 2.7 ± 0.6); outer lip of aperture simple, smooth; aperture rounded (ASR 1.6 ± 0.1, 1.6 ± 0.1); basal cords few (BCN 3.3 ± 0.7, 3.4 ± 0.9); longitudinal ribs distinct, granulated, in large number on penultimate whorl, (RN 26.4 ± 3.2, 21.6 ± 2.2); spiral cords in medium number (SCN 6.2 ± 0.8, 5.3 ± 0.8); color in light brown background. Protoconch large in the genus (SLP 5.0 ± 1.1 mm, WNP 3.8 ± 0.4); longitudinal ribs distinct; spiral cord weak or absent; color in yellowish-brown, with or without 1–3 dark brown bands.

Description of holotype (NSMT-Mo39770; Fig. 7A–F). ***Teleoconch.*** AH 9.9 mm, AL 10.4 mm, ASR 1.54, AW 6.8 mm, BCN 4, BWL 17.9 mm, FWL 3.8 mm, PWL 7.1 mm, RN 24, SA 19.5 degrees, SCN 6, SH 35.7 mm, SW 12.8 mm, TWL 4.6 mm, WER 2.60, WN 7.00; shell elongated; spires moderately broadened laterally, slightly convex; suture almost straight; outer lip of aperture simple, smooth; aperture rounded; apex of shell eroded; longitudinal ribs distinct, oblique, opisthocyrt, prominently granulated at intersection with spiral cords; shell surface brown to blackish-brown with deposits.

Protoconchs. RNP 14, SLP 5.8 mm, SWP 4.1 mm, WNP 4.00; shell globose; suture slightly undulating; longitudinal ribs remarkable, with rounded nodes in 2 rows; spiral cord weak on lower part of whorls; shell colored light beige in background, without color band.

Variation. ***Teleoconchs*** (Fig. 7G, J, K, N, P, S, U, X). Measurements and counts shown in Table 4. Spires slightly to moderately broadened, slightly to strongly convex; suture typically slightly undulating, sometimes almost straight; longitudinal ribs distinct, oblique, typically orthocline to opisthocyrt on upper whorls, opisthocyrt on lower whorls, sometimes opisthocline on upper and lower whorls, rarely fade in body whorl; spiral cords rarely distinct; shell colored yellowish-brown to light brown, with dark brown longitudinal stripes; color bands dark brown or dark olive, sometimes present on central part of upper whorls.

Opercula (Fig. 7H, L, O, Q, T, V, Y). 5.8–8.9 mm in long diameter; nearly egg-shaped subcircular, paucispiral, comprising around 3 whorls; nucleus subcentral.

Protoconchs (Fig. 7I, M, R, W). Measurements and counts shown in Table 4. Shell globose or slightly elongated; suture slightly to moderately undulating; longitudinal ribs distinct, smooth, rarely granulated on central part of whorls in 1–2 rows; spiral cords weak or absent on lower part of whorls; shell colored beige to light beige in background; color bands olive or dark brown, thick, sometimes present on upper and lower parts of whorls and basal part.

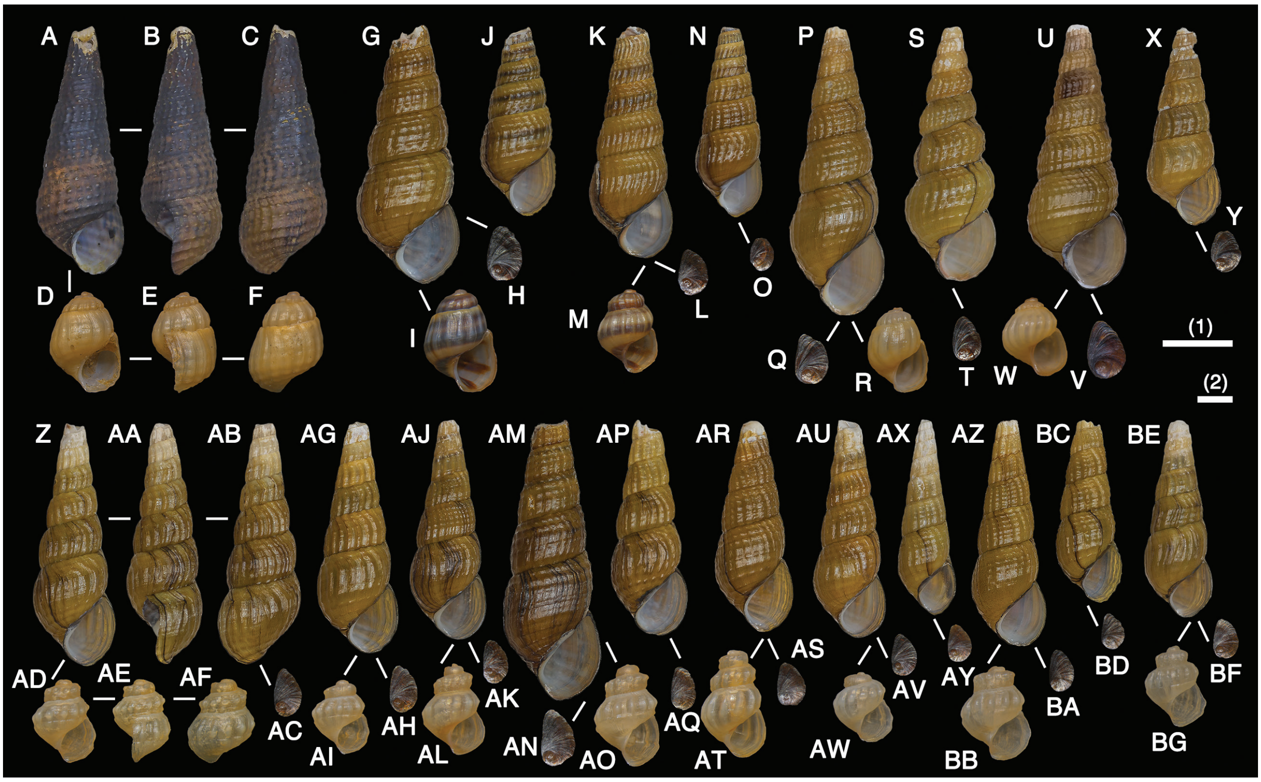

Figure 7. Shells and opercula of *Semisulcospira reticulata* (**A–Y**) and *S. nishimurai* sp. nov. (**Z–BF**). **A–F.** Holotype of *S. reticulata* from Oki-shima Island (offshore), NSMT-Mo39770; **G–J.** Hannoura, KUZ Z4926, Z4927; **K–O.** Kitafunaki, KUZ Z4930, Z4931; **P–T.** Oki-shima Island (offshore), KUZ Z4925; **U–Y.** Mano, KUZ Z4229, Z4286; **Z–AF.** Holotype of *S. nishimurai* sp. nov. from Hannoura, KUZ Z4935; **AG–AQ.** Paratypes of *S. nishimurai* sp. nov. from Hannoura, KUZ Z4936–Z4939; **AR–AT.** Oura Port, KUZ Z4945; **AU–AY.** Minamihama, KUZ Z4946, Z4947; **AZ–BD.** Kitafunaki, KUZ Z4952, Z4953; **BE–BG.** Oki-shima Island (coast), KUZ Z4957. Scale bars: 10 mm (**1**), female teleoconch (**A–C, G, K, P, S, U, Z–AB, AG, AJ, AM, AR, AU, AZ, BE**), male teleoconch (**J, N, AP, AX, BC**), operculum (**H, L, O, Q, T, V, Y, AC, AH, AK, AN, AQ, AS, AV, AY, BA, BD, BF**); 2 mm (**2**), protoconch (**D–F, I, M, R, W**); 1 mm (**2**), protoconch (**AD–AF, AI, AL, AO, AT, AW, BB, BG**).

Radulae (Fig. 8A–D). Taenioglossa. Rachidian teeth consisting of central denticle and 2–4 small pointed triangular cusps on each side; tip of rachidian central denticle narrow, pointed, triangular, 2.0–3.5 times longer than other triangular cusps. Lateral teeth consisting of large central denticle and 2–4 inner and outer pointed cusps; central cusp of lateral teeth narrow, pointed, triangular, 2.0–3.0 times longer than other triangular cusps. Interior and exterior marginal teeth spoon-shaped, with 4–6 rounded denticles.

Reproductive organs: Female (Fig. 9C). Oviduct long, narrow, emerging from ovary entering near seminal receptacle on ventral side of soft body; protrusions of seminal receptacle long or short. Sperm gutter extending from spermatophore bursa towards mantle cavity, curved inward along whorls. Brood pouch extending along dorsal side of spermatophore bursa and sperm gutter, dorsally inflated, separated into many chambers, including eggs and embryos; eggs colored beige to orange, radially developing from base of brood pouch near seminal receptacle.

Male. Vas deferens long, narrow, emerging from testes, entering posterior end of prostate. Prostate without penis elongated, inflated in posterior ventral side, with deep groove, forming U-shape in transverse section, anterior narrowly opening to mantle cavity.

Distribution and ecology. *Semisulcospira reticulata* has been recorded from the northern and southern basins of Lake Biwa (Watanabe and Nishino 1995: fig. 5e; Fig. 1). However, this species has not been collected from the southern basin of the lake in recent years (Nishino and Tanida 2018). *Semisulcospira reticulata* was found on sandy mud to mud bottoms at depths of 4–30 m (Watanabe 1970, 1980; Table 1). The species was collected with other congeners: *S. niponica*-group, *S. decipiens* at Hannoura, Kitafunaki, and Mano, *S. nishimurai* sp. nov. at Hannoura and Kitafunaki; *S. nakasekoae*-group, *S. cryptica* and *S. ourensis* at Hannoura, *S. arenicola* at Kitafunaki, *S. elongata* at Kitafunaki, Oki-shima Island, and Mano, *S. davisi* at Hannoura, Kitafunaki, and Oki-shima Island. *Semisulcospira reticulata* was found with *S. reiniana* at Kitafunaki.

Remarks. Genetic differentiation among specimens from Kitafunaki and the other localities were clarified in this species (Figs 2, 3) whereas morphological distinctiveness were not observed in the Kitafunaki specimens (Table 4). The teleoconch of *S. reticulata* resembles that of *S. nishimurai* sp. nov. However, *S. reticulata* represents a greater number of axial ribs on the teleoconch, and the protoconch size of *S. reticulata* is prominently larger than

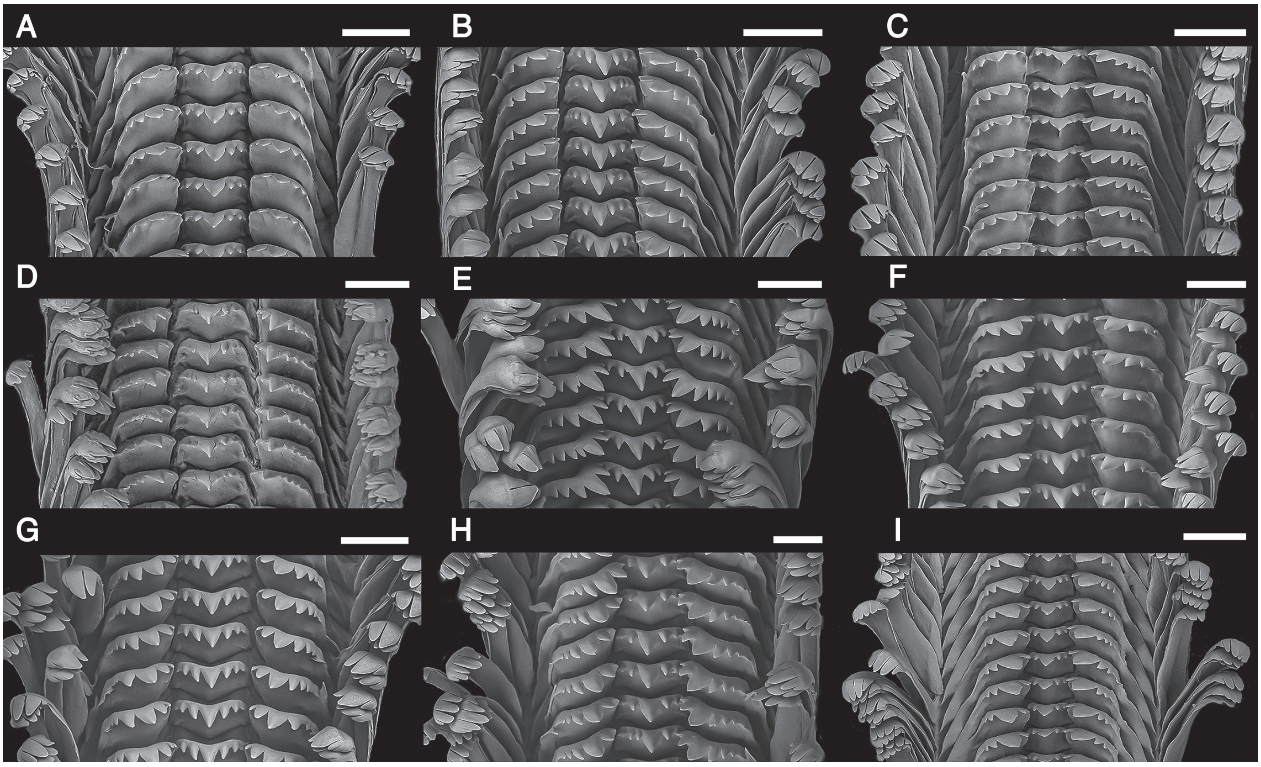

Figure 8. Radulae of *Semisulcospira reticulata* (**A–D**) and *S. nishimurai* sp. nov. (**E–I**). **A.** Hannoura, KUZ Z4926; **B.** Kitafunaki, KUZ Z4930; **C.** Oki-shima Island (offshore), KUZ Z4925; **D.** Mano, KUZ Z4229; **E.** Holotype of *S. nishimurai* sp. nov. from Hannoura, KUZ Z4935; **F.** Oura Port, KUZ Z4945; **G.** Minamihama, KUZ Z4946; **H.** Kitafunaki, KUZ Z4952; **I.** Oki-shima Island (coast), KUZ Z4957. Scale bars: 100 µm.

that of *S. nishimurai* sp. nov. Although the protoconch size and shape of *S. reticulata* are similar to those of *S. davisi*, larger SH, BWL, and SA values of *S. reticulata* discriminate the two species.

Semisulcospira nishimurai Sawada, sp. nov.

https://zoobank.org/9D84A764-FDBA-4AC1-934B-C7B4ACEC0CF6

Table 4, Figs 7Z–BG, 8E–I, 9A, B, D–F

New Japanese name: Azai-kawanina

Melania niponica – Kobelt 1879: 131, pl. 19, fig. 11 (part).

Melania multigranosa – Annandale 1916: 44–45, pl. 3, fig. 2B (part).

Melanoides (*Semisulcospira*) *multigranosa* – Kuroda 1929: 186, 189, pl. 5, fig. 36 (part).

Semisulcospira (*Biwamelania*) sp. – Nishimura 2024: unnumbered figures (part).

Material examined. ***Holotype.*** KUZ Z4935, adult female, collected from Lake Biwa at a water depth of 3 m, Hannoura, Kinomoto-cho, Nagahama City, Shiga Prefecture, Japan on 26 June 2022 by Naoto Sawada.

Paratypes. KUZ Z4936–Z4938, 3 adult females, Z4939, 1 adult male collected from 3–6m depths with holotype.

Additional materials. KUZ Z4940, 11 females, Z4941, 11 males, collected from 2–7 m depths with holotype; KUZ Z4946, 15 females, Z4947, 13 males, collected from 1–8 m depths at Minamihama on 2 July 2022; KUZ Z4952, 14 females, Z4953, 5 males, from 1–7 m depths at Kitafunaki on 10 July 2022; KUZ Z4945, 2 females, from 0–1 m depths from Oura Port on 28 November 2021; KUZ Z4957, 3 females from 6–8 m depths at the coast of Oki-shima Island on 10 August 2019.

Diagnosis. Viviparous. Teleoconch elongated, medium sized in the genus [SH 30.7 ± 3.3 (mean ± SD) (female), 27.4 ± 3.3 (male) mm; BWL 15.9 ± 1.7, 14.5 ± 1.6 mm], spires laterally broadened (SA 17.3 ± 2.4, 17.6 ± 2.4 degrees), axially compressed (WER 2.4 ± 0.2, 2.5 ± 0.2); outer lip of aperture simple, smooth; aperture rounded (ASR 1.6 ± 0.1, 1.7 ± 0.1); basal cords few (BCN 2.9 ± 0.6, 3.1 ± 0.8); longitudinal ribs, distinct, granulated on penultimate whorl, in medium number (RN 20.4 ± 3.7, 18.2 ± 2.4); spiral cords in medium number (SCN 6.3 ± 0.7, 5.8 ± 0.5); color in light brown background. Protoconch small to medium sized in the genus (SLP 2.4 ± 0.3 mm, WNP 3.2 ± 0.3); longitudinal ribs prominently granulated; color in light beige, with or without 1–2 brown bands.

Description of holotype (KUZ Z4935; Fig. 7Z–AF). ***Teleoconch.*** AH 10.2 mm, AL 10.4 mm, ASR 1.60, AW 6.5 mm, BCN 3, BWL 17.1 mm, FWL 4.2 mm, PWL 6.5 mm, RN 26, SA 16.3 degrees, SH 35.0 mm, SW 11.9 mm, TWL 5.0 mm, WER 2.40, WN 6.50; shell elongated; spires moderately broadened laterally, moderately convex; suture strongly undulating; outer lip of aperture simple, smooth; aperture rounded; apex of shell

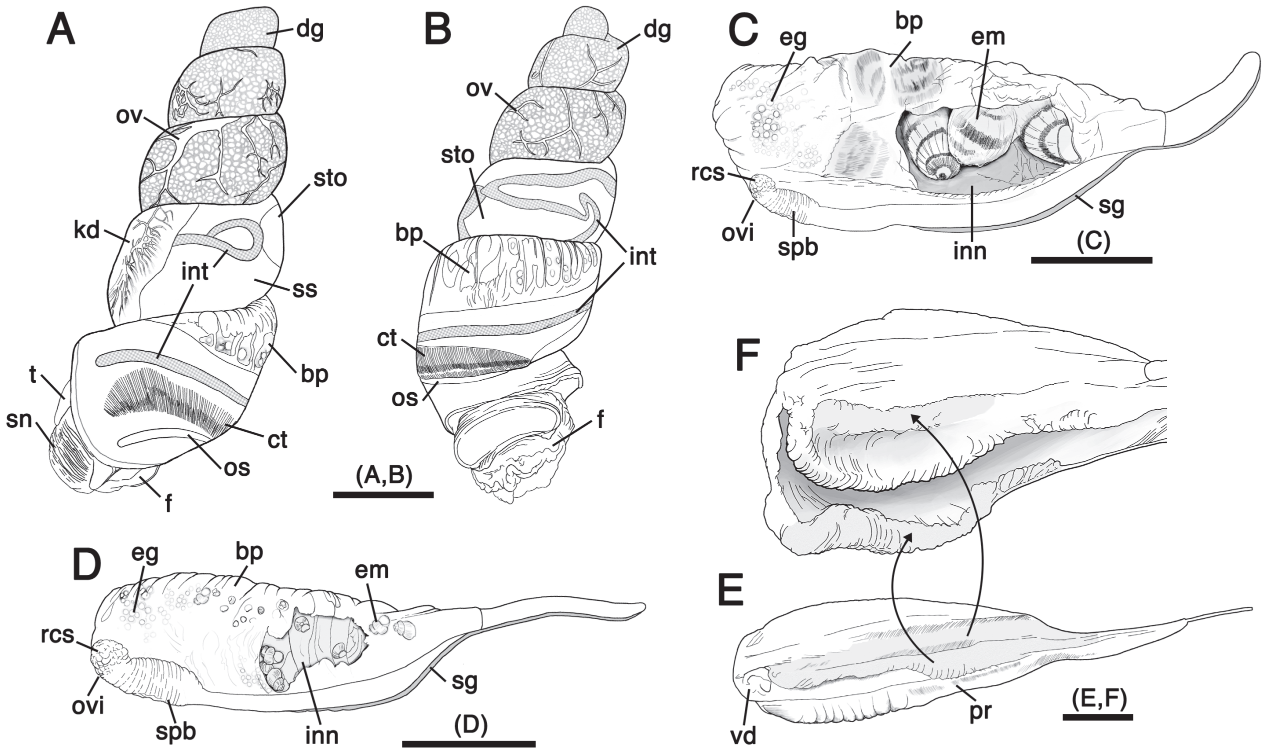

Figure 9. Schematic drawings indicating generalised external features of the soft body and reproductive organs of *Semisulcospira reticulata* and *S. nishimurai* sp. nov.: **A, B.** Dorsal and ventral views of organs in visceral mass of mature female of *S. nishimurai* sp. nov. without operculum; **C.** External, right lateral view of pallial oviduct of *S. reticulata*; **D.** External, view of pallial oviduct of *S. nishimurai* sp. nov.; **E.** External, right upper lateral view of the prostate of *S. nishimurai* sp. nov.; **F** Internal folding structures of the prostate of *S. nishimurai* sp. nov. Abbreviations: bp, brood pouch; ct, ctenidium; dg, digestive gland; eg, egg; em, embryo; f, foot; inn, inner wall of brood pouch; int, intestine; kd, main kidney chamber; os, osphradium; ov, ovary; ovi, renal oviduct; pr, prostate; rcs, seminal receptacle; sg, sperm gutter; sn, snout; spb, spermatophore bursa; ss, style sac; sto, stomach; t, cephalic tentacle; vd, vas deferens. Scale bars: 5 mm.

eroded; longitudinal ribs distinct, oblique, opisthocyrt, almost smooth; spiral cord absent on penultimate whorl; shell colored light yellowish-brown background in upper, yellowish-brown background in lower whorls with dark brown longitudinal stripes; color bands absent.

Operculum. 7.0 mm in long diameter; nearly egg-shaped subcircular, paucispiral, comprising around 3 whorls; nucleus subcentral.

Protoconchs. PN 61, RNP 13, SLP 2.4 mm, SWP 1.9 mm, WNP 3.25; shell globose; suture prominently depressed by discrepancy between adjacent whorls; longitudinal ribs remarkable, with pointed nodes in 1 row on central part of whorls; spiral cord distinct on upper and lower parts of whorls; shell colored beige in background, without color band.

***Radulae* (Fig. 8E).** Taenioglossa. Rachidian teeth consisting of central denticle and 2–3 small pointed triangular cusps on each side; tip of rachidian central denticle narrow, pointed, triangular, 2.5 times longer than other triangular cusps. Lateral teeth consisting of large central denticle and 2–3 inner and outer pointed cusps; central cusp of lateral teeth narrow, pointed, triangular, 2.0 times longer. Interior and exterior marginal teeth spoon-shaped with 4–5, 4 rounded denticles, respectively.

Reproductive organ. Female. Oviduct long, narrow, emerging from ovary entering near seminal receptacle on ventral side of soft body; protrusions of seminal receptacle long. Sperm gutter extending from spermatophore bursa towards mantle cavity, curved inward along whorls. Brood pouch extending along dorsal side of spermatophore bursa and sperm gutter, dorsally inflated, separated into many chambers, including eggs and embryos; eggs colored beige, radially developing from base of brood pouch near seminal receptacle.

Variation. ***Teleoconchs*** (Fig. 7AG, AJ, AM, AP, AR, AU, AX, AZ, BC, BE). Measurements and counts shown in Table 4. Spires broadened slightly to moderately; slightly to strongly convex; suture slightly to moderately undulating; longitudinal ribs distinct, oblique, typically orthocline to opisthocyrt on upper whorls, opisthocyrt on lower whorls, mildly to prominently granulated at intersection with spiral cords, rarely opisthocline on upper and lower whorls, fade on body whorl; spiral cords sometimes indistinct; shell colored yellowish-brown to light brown with dark brown longitudinal stripes; color bands dark brown or dark olive, rarely present on central part of upper whorls.

Opercula (Fig. 7AH, AK, AN, AQ, AS, AV, AX, BA, BD, BF). 5.1–8.2 mm in long diameter.

Protoconchs (Fig. 7AI, AL, AO, AT, AW, BB, BG). Measurements and counts shown in Table 4. Shell globose or slightly elongated; suture slightly to moderately undulating; longitudinal ribs remarkable, usually granulated pointedly on central part of whorls in 1–2 rows; spiral cord distinct or weak on upper and lower part of whorls; shell colored beige to light beige in background; color bands, brown, thick, sometimes present on upper and lower parts of whorls and on basal part.

Radulae (Fig. 8F–I). Rachidian teeth consisting of 5–8 triangular cusps; tip of rachidian central denticle typically narrow, pointed, rarely rounded, 2.0–3.0 times longer than other triangular cusps. Central cusp of lateral teeth typically narrow, pointed, rarely wide, rounded, 1.0–3.5 times longer than other triangular cusps than other triangular cusps. Interior and exterior marginal teeth spoon-shaped with 4–6, 4–5 rounded denticles, respectively.

Reproductive organs. Female (Fig. 9A, B, D). Protrusions of seminal receptacle rarely short.

Male (Fig. 9E, F). Vas deferens long, narrow, emerging from testes, entering posterior end of prostate. Prostate without penis elongated, inflated in posterior ventral part, with deep groove, forming U-shape in transverse section, anterior narrowly opening to mantle cavity.

Etymology. The specific name is dedicated to Toshiaki Nishimura, who first discovered the new species through his exhaustive survey of the distribution of *Semisulcospira* in Lake Biwa (Nishimura 2024). The new Japanese name refers to the Azai clan, a Japanese daimyo (feudal lord) of the Sengoku period, whose largest territory roughly corresponds to the distribution of the new species (Ota 2011).

Distribution and ecology. *Semisulcospira nishimurai* sp. nov. is widely distributed in the northern basin of Lake Biwa (Fig. 1). The new species was found on sandy mud to mud bottoms at depths of 1–8 m and was common at depths greater than 3 m. The new species was collected with other congeners: *S. niponica*-group, *S. decipiens* at Hannoura and Kitafunaki, *S. watanabei* at Hannoura and Oura Port, *S. niponica* at Oura Port and Oki-shima Island, *S. reticulata* at Hannoura and Kitafunaki; *S. nakasekoae*-group, *S. cryptica*, *S. ourensis*, and *S. morii* at Hannoura, *S. arenicola* and *S. elongata* at Minamihama and Kitafunaki, *S. davisi* at Hannoura, Minamihama, and Kitafunaki. This new species was found with *S. reiniana* at Kitafunaki.

Remarks. The new species has been confused with *S. niponica* and *S. multigranosa*. However, the new species can be discriminated from other congeners, including *S. niponica* and *S. multigranosa*, by the combination of an elongated teleoconch with a medium to large number of weakly granulated longitudinal ribs and a medium-sized, granulated, beige-coloured protoconch. Although *S. nishimurai* sp. nov., *S. decipiens*, and *S. reticulata* possess partially similar teleoconch morphologies, the larger number of longitudinal ribs and lower spire angle of teleoconchs discriminate the new species from *S. decipiens*. The new species can be distinguished from *S. reticulata* by its smaller numbers of longitudinal ribs on teleoconchs and smaller protoconchs.

Discussion

The reconstructed ML and SVDquartets trees and the results of the ADMIXTURE analysis supported the genetic distinctiveness of *S. reticulata* and *S. nishimurai* sp. nov. in the *S. niponica*-group. The genetic relationships among the described *S. niponica*-group species estimated by the present analyses were consistent with those reported by previous studies (Miura et al. 2019; Sawada and Fuke 2022, 2023). The sister relationships between *S. reticulata* and the other lacustrine *S. niponica*-group species and between *S. nishimurai* sp. nov., *S. decipiens*, and *S. habei* were also recovered in this study.

Morphological analyses revealed that the morphology of the teleoconch, protoconch, and radula was useful for distinguishing between *S. reticulata*, *S. nishimurai* sp. nov., *S. decipiens*, and *S. habei*. The analyses showed a partial morphological overlap between *S. reticulata*, *S. nishimurai* sp. nov., and *S. decipiens*, as implied by Nishimura (2024). Nonetheless, the first axis generated by PCA correctly identified most specimens of *S. reticulata* and *S. nishimurai* sp. nov., showing an intermediate shell morphology of *S. nishimurai* sp. nov. among the four species. Discriminant analysis also revealed that *S. reticulata* is characterized by its unique protoconch morphology and that *S. nishimurai* sp. nov. possesses a small, less elongated teleoconch and tiny protoconch.

The distribution of *S. reticulata* overlapped with that of other *S. niponica*-group species, including *S. nishimurai* sp. nov. and *S. decipiens*, in several localities (Table 1; see also Taxonomy). Although the genetic diversity among the *S. niponica*-group species was much lower than that of their riverine congeners (Miura et al. 2019, 2020), the suggested reproductive isolation among *S. reticulata* and other sympatric *S. niponica*-group species highlights that *S. reticulata* is an independent species. Based on the genetic and morphological distinctiveness of the new species in coexistence with closely related species, we also considered *S. nishimurai* sp. nov. to be reproductively isolated from other *Semisulcospira* species in the Lake Biwa system.

Previous and present studies have revealed that in the *S. niponica*-group, *S. reticulata* prefers the deepest areas of the lake and possesses the largest protoconches (Kajiyama and Habe 1961; Watanabe and Nishino 1995; Miura et al. 2019). The differences in protoconch size observed among lacustrine *Semisulcospira* species suggest interspecific diversification of their life histories (Reynolds 2003; Jeschke and Kokko 2009). The distinctively large protoconch of *S. reticulata* may be explained by the slow life history in the deep and stable areas of the lake.

The present taxonomic revision detected interspecific differences in radula morphology among lacustrine *Semisulcospira* species. Narrower, more pointed radula cusps were observed in *S. reticulata* and *S. nishimurai* sp. nov. than in the other large-grained *S. niponica*-group members. Knowledge of the relationships between radula morphology and habitats has been fragmented in *Semisulcospira* snails (Watanabe 1970b). In the other lacustrine freshwater snails, however, interspecific differences have been estimated to be associated with their trophic specialization in various substrates (Rintelen et al. 2004). Therefore, further comprehensive investigations into the relationship between radula morphology, substrates, and feeding habits will contribute to elucidating the ecological specialization of *Semisulcospira*.

The discovery of new species in sandy mud substrates may be the key to understanding the diversification history of *Semisulcospira*. Moderate differences in habitat preferences, especially for water depth and substrate, have been known among species in Lake Biwa (Watanabe and Nishino 1995; Miura et al. 2019; Table 1). This interspecific difference has been estimated to be associated with niche differentiation among *Semisulcospira* species during the early stages of their diversification (Miura et al. 2019). The recovery of a well-branched clade of *S. nishimurai* sp. nov. in the present phylogeny supports this hypothesis, and also fits the sandy mud species in the *S. niponica*-group, suggesting that the *S. niponica*- and *S. nakasekoae*-groups exhibit similar species richness in the non-rupicolous areas of Lake Biwa.

Acknowledgements

The authors are grateful to Kazunori Hasegawa (NSMT) for kind support during the examination of the type specimens. We are also grateful to Shiga Prefecture for providing permission for sample collection using aeration devices. We also thank Katsutoshi Watanabe (Kyoto University; KU), Taku Okamoto (KU), Tomoki Kadokawa (KU), Minoru Tamura (KU), and Hiroko Okada for supporting genetic and morphological analyses. We are grateful to Andreas Schmidt-Rhaesa, Thomas von Rintelen, and an anonymous reviewer for their constructive comments. The first author thanks Toshiaki Nishimura, Kiichiro Maida, Jamen Uiriamu Otani, Yoshiharu Sasaki (Shiga Prefectural Fisheries Experiment Station), Yukiko Goda (KU), Tetsuji Akatsuka (KU), Kenichiro Ishii, Yoshitaka Tamura, Kentaro Miura, Yugo Miuchi, Katata fishermen's union in Shiha Prefecture, and Kaoru Sawada for their assistance with field surveys and sample collection. This study was supported by the Center for Ecological Research, Kyoto University, a Joint Usage–Research Center. This study was partly supported by JSPS KAKENHI Grant Number JP21J22917 and the Research Institute of Marine Invertebrates Grant Number IKU 2021□01. We thank Editage (www.editage.jp) for English language editing.

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Supplementary material 1

Result of the ADMIXTURE analysis and principal component analysis

Authors: Naoto Sawada, Yusuke Fuke, Osamu Miura, Haruhiko Toyohara, Takafumi Nakano

Data type: docx

Link: https://doi.org/10.3897/evolsyst.8.124491.suppl1