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**Integrative taxonomy of *Semisulcospira kurodai* (Mollusca, Semisulcospiridae)
with insights into its geographical variation and description of three new species
from Japan**

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22 **Running title:** Sawada et al. Integrative taxonomy of *Semisulcospira kurodai*

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24 *Semisulcospira* Boettger, 1886 is a freshwater snail genus widely distributed in East
25 Asia. The Japanese *Semisulcospira* consists of widespread riverine species and two
26 species groups that have diversified in Lake Biwa (*S. niponica* and *S. nakasekoe*
27 groups). *Semisulcospira kurodai* Kajiya & Habe, 1961 is a member of the *S.*
28 *niponica* group and has been known as the only species that has expanded outside the
29 lake drainage. Although recent studies have suggested the presence of undescribed
30 species in the snails previously identified as *S. kurodai*, their taxonomic accounts have
31 not yet been examined. In this study, the taxonomy of the species was arranged by a
32 comprehensive taxon sampling and an integrative approach. Analyses based on
33 genome-wide SNPs and teleoconch, protoconch, and radula morphologies clearly
34 discriminated *S. kurodai* and the three new species. These species are described here as
35 *S. masudai* Sawada & Miura sp. nov. and *S. praecursa* Sawada & Miura sp. nov. in the
36 *S. niponica* group and *S. miurai* Sawada sp. nov. in the *S. nakasekoe* group.
37 Morphological analysis revealed sexual size differences in each species. The analysis of
38 geographical variation in *S. kurodai* detected four genetic subgroups in the species, and
39 indicated that morphological variation in the shell and radula of this species was
40 increased by local environmental factors. Phylogenetic relationships within *S. kurodai*
41 suggest that this species originated not directly around Lake Biwa but in the Chugoku

42 District, where its common ancestor with *S. masudai* sp. nov. was isolated by the
43 mountainous areas. The similar teleoconch shapes of the *S. niponica* and *S. nakasekoe*
44 group species in riverine habitats suggest parallel morphological evolution between
45 groups, as observed in Lake Biwa endemics. The present investigation also clarified that
46 habitat shrinkage of *S. kurodai* may have caused the current allopatric distribution
47 between *S. kurodai* and *S. praecursa* sp. nov.

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49 **Keywords:** East Asia, endangered species, freshwater snail, MIG-seq, next-generation
50 sequencing, radula, Random Forest, type specimen

Introduction

Semisulcospira Boettger, 1886 is a freshwater snail genus characterised by the synapomorphic trait of viviparity in the family Semisulcospiridae (Köhler, 2017; Strong & Köhler, 2009). Members of this genus are widely distributed in Japan, Korea, Taiwan, and China, and have diversified in a Japanese ancient lake and southern China (Davis, 1969; Du et al., 2019; Watanabe & Nishino, 1995). Taxonomic revision and demonstration of the reproductive isolation of *Semisulcospira* species have been pursued in these areas by recent integrative studies using morphological and genetic data (He et al., 2024; Sawada et al., 2024; Sawada & Fuke, 2022; Ueno et al., 2024).

Kuroda (1929) clarified that Japanese *Semisulcospira* consists of widely distributed riverine species and members endemic to the Lake Biwa system, discriminating them by basal cord numbers and states of teleoconch sculptures. Subsequently, *S. kurodai* Kajimaya & Habe, 1961 was described from a creek in Hyogo Prefecture, central Japan (near localities #24 and #25 in Fig. 1) (Kajiyama & Habe, 1961). This species was collected from the western Shizuoka Prefecture (near locality #1) to southern Hyogo Prefecture (near locality #28), far from the Lake Biwa system in its original description. On the other hand, the study also inferred that *S. kurodai* is closely related to Lake Biwa endemics, based on its few basal cords on teleoconch and large protoconchs. Later, in

his comprehensive revision of the Japanese *Semisulcospira*, Davis (1969) confirmed that *S. kurodai* possesses shell morphological traits similar to those of Lake Biwa species. Further surveys have revealed that this species is widely distributed in central Honshu and eastern Shikoku (from western Shizuoka Prefecture to Okayama and Tokushima Prefectures), as well as in the northern Hyogo Prefecture (Kimura, 1994; Masuda, 2014; Takami, 1997; Fig. 1).

Phylogenetic and taxonomic studies in the 2000s have revealed that *Semisulcospira* species in the Lake Biwa system belong to two species groups, i.e., *S. niponica* and *S. nakasekoe* groups (Kamiya et al., 2011; Miura et al., 2019; Sawada & Fuke, 2022). The *S. niponica* group includes *S. kurodai* distributed outside the lake, whereas all members of the *S. nakasekoe* group are indigenous to the Lake Biwa water system. Based on fossil records of the genus from the old strata of Lake Biwa, it has been estimated that the species richness of the two groups originated in the Lake Biwa system, and that *S. kurodai* expanded from the lake system into rivers (Miura et al., 2019; Morita et al., 2024).

A recent phylogenetic analysis of *S. kurodai* supported four well-differentiated clades within the species (K1–K4) which represent partially different morphological features (Morita et al., 2024). Furthermore, one of these clades was found to be closely

related to the *S. nakasekoe* group species, demonstrating that both *S. niponica* and *S. nakasekoe* groups had advanced into riverine habitats independently. According to the unclarified species richness implied for Japanese *Semisulcospira*, the accounts of snails, that have been previously identified as *S. kurodai*, should be revisited. The taxonomic revision of these snails may also contribute to elucidating their diversification processes outside the lake and their convergent evolution in riverine habitats. However, our knowledge of the genetic, morphological, and ecological traits of *S. kurodai* remains limited. Moreover, the two sympatric clades of *S. kurodai*, obtained around the type locality of the species, have hampered the elucidation of their taxonomic accounts (Morita et al., 2024).

This study investigated the taxonomic accounts of four clades of *S. kurodai* based on an integrative analysis of specimens obtained from its whole distribution, along with a revision of its type specimen. Here, we describe each of the four clades defined by Morita et al. (2024) as *S. kurodai* (K1), *S. masudai* sp. nov. (K2), *S. praecursa* sp. nov. (K3), and *S. miurai* sp. nov. (K4), respectively.

Materials and methods

Taxon sampling

A total of 459 specimens were used for genetic and morphological analyses (Table 1). Among them, 420 specimens were newly obtained. The remainder of this study are voucher specimens deposited at the Zoological Collection of Kyoto University (KUZ) and the holotype of *S. kurodai* at the National Museum of Nature and Science, Tokyo (NSMT).

The habitats of *S. kurodai* and the new species were investigated at 37 localities in central Japan from 2020 to 2024 (Fig. 1). Among these, living specimens were collected from 26 localities, whereas snails were not found at the 11 previously recorded sites (Table S1). The substrates and semisulcospirid fauna were visually recorded following Miura et al. (2019). Specimens of teleoconchs, protoconchs, radulae, and reproductive organs were prepared and observed as described by Sawada et al. (2020). However, the teleoconch specimens were cleaned following Sawada et al. (2021). Newly collected specimens were deposited at the KUZ.

Sequencing and SNP detection

Genetic relationships among Japanese *Semisulcospira* species were elucidated using Multiplexed ISSR Genotyping by sequencing (MIG-seq) analyses (Suyama & Matsuki, 2015). Genomic DNA extraction, library preparation, and sequencing were performed

on 73 *S. kurodai*, eight *S. masudai* sp. nov., 15 *S. praecursa* sp. nov., 22 *S. miurai* sp. nov., 11 *S. libertina* (Gould, 1859), and a putative hybrid (Table 1). Specimens of the *S. libertina* L1, L2, and L4 clades were included in the analyses according to the phylogenetic relationships of Japanese *Semisulcospira* (Miura et al. 2020; Morita et al. 2024). Pooled libraries were prepared using the Onuki & 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 number: PRJDB17676). The raw data were demultiplexed using the process_shortreads program in Stacks v2.66 (Rochette et al., 2019).

MIG-seq data of the following Lake Biwa endemics obtained by Sawada & Fuke (2022, 2023) were used for analysis (Table 1): *S. niponica* group, *S. niponica* (Smith, 1876), *S. reticulata* Kajiyama & Habe, 1961, *S. habei* Davis, 1969, *S. rugosa* Watanabe & Nishino, 1995, *S. fuscata* Watanabe & Nishino, 1995; *S. nakasekoe* group, *S. nakasekoe* (Kuroda, 1929), *S. morii* Watanabe, 1984, *S. arenicola* Watanabe & Nishino, 1995, *S. takeshimensis* Watanabe & Nishino, 1995, *S. davisi* Sawada & Nakano, 2021.

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. Based on the results of the analyses described below, SNP detection was performed independently for the following subsets: 1) all specimens; 2) *S. niponica* group species; 3) *S. nakasekoe* group species, *S. libertina*, and a putative hybrid; 4) *S. kurodai*, *S. masudai* sp. nov., and Lake Biwa endemics of the *S. niponica*-group; and 5) *S. kurodai* specimens. SNP detection was conducted using the ref_map.pl pipeline of Stacks and VCFtools v0.1.13 (Danecek et al., 2011).

SNP filtering and output were conducted using populations with the following settings: SNPs common to more than 70% of all samples ($R = 0.7$) were retained; SNPs with heterozygosity greater than 75% ($\text{max-obs-het} = 0.75$) and minor alleles less than two ($\text{min-mac} = 2$) were excluded; and the minimum depth of coverage was set to five. All SNPs were used for phylogenetic analyses, whereas only one SNP from a locus (write-single-snp) was retained for population structure analysis. All other parameters

were set to default settings. Only samples with fewer than 50% missing sites were used in subsequent analyses.

Phylogenetic network and population structure analyses

Genetic relationships among all specimens were initially estimated by principal component analysis (PCA). PCA was performed using PLINK v1.90b6.25 (Purcell et al., 2007). Subsequently, sequences were separated into two subsets, the *S. niponica* group and the remainder, according to the PCA results.

Neighbour-net was reconstructed for each subset to estimate the phylogenetic relationships via Splitstree v4.15.1 (Huson & Bryant, 2006). The genetic distance was calculated using the Uncorrected P algorithm, and the handling of ambiguous sites was set as MatchState.

Individual admixture proportions of *Semisulcospira* specimens were calculated for each subset with likelihood-model-based clustering using ADMIXTURE v1.3.0 (Alexander et al., 2009). The analyses were conducted 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 visualised for *K*-values with lower mean cross-validation error values. Summarisation of cross-validation error values and visualisation of the ADMIXTURE plot were performed in R v3.6.1 (R Core Team, 2019).

Morphological examination

The measurements and counts of 24 shell morphological characters were obtained with the methods described by Sawada & Nakano (2021). However, states of protoconch sculptures were identified following Sawada & Fuke (2022). Because protoconchs were not preserved in the holotype, however, only shell length, whorl number, and node number, which were available from the original description, were obtained. The reproductive organs were observed under a YS05T stereoscopic microscope (Micronet Inc., Kawaguchi, Japan). After dissection, the radulae were extracted and cleaned with 1 M sodium hydroxide and 1% sodium hypochlorite solutions. The extracted radulae were photographed using a JSM-6510LV scanning electron microscope (JEOL Ltd., Tokyo, Japan) after platinum coating using a JEC-3000FC (JEOL Ltd.).

The specimens were fixed horizontally and photographed ventrally using a Nikon D7500 camera (Nikon Corporation, Tokyo, Japan) with a Nikon-compatible Tamron SP

193 90 mm f/2.8 1:1 macro lens (Tamron Co., Ltd., Saitama, Japan). Morphological
194 characters were measured from the digital images using ImageJ v1.51 (Schneider et al.,
195 2012). Protoconchs, opercula, radulae, and reproductive organs were examined in the
196 larger specimens of each population.

197 Abbreviations of morphological characters examined are as follows: Teleoconch:
198 AH, aperture height; AL, aperture length; ASR, aperture slenderness ratio (the
199 proportion of aperture length to aperture width); AW, aperture width; BCN, basal cord
200 number; BWL, body whorl length; FWL, fourth whorl length; PWL, penultimate whorl
201 length; RN, longitudinal rib number of penultimate whorl; SA, spire angle; SCN, spiral
202 cord number of penultimate whorl; SL, shell length; SW, shell width; TWL, third whorl
203 length; WER, whorl elongation ratio (the proportion of aperture height to fourth whorl
204 length); and WN, whorl number; Sculpture Type, sculpture state on penultimate whorl.
205 Protoconch: PN, number of protoconchs; RNP, longitudinal rib number on the body
206 whorl of the largest protoconch; SLP, shell length of the largest protoconch; WNP,
207 whorl number of the largest protoconch; Node Number, number of nodes at intersection
208 with spiral cords on the central part of the body whorl of the largest protoconch; and
209 Keel Type, strength of the sutural spiral cord on the lower part of the body whorl of the
210 largest embryonic shell.

211

212 **Test of sexual dimorphism**

213 Sexual dimorphisms in shell size and shape were tested in *S. kurodai*, *S. masudai* sp.

214 nov., *S. praecursa* sp. nov., and *S. miurai* sp. nov. using 278 female and 135 male

215 specimens. Based on sexual dimorphisms revealed in the genus (Matsuda & Miura,

216 2022; Sawada & Nakano, 2022), sexual differences in shell size were initially tested

217 within each species using the Wilcoxon rank-sum test for BWL. In addition, differences

218 in PC1 scores generated by PCA conducted for the following nonlinear teleoconch

219 characters were also examined: ASR, BCN, SCN, SA, WER, WN, Sculpture Type, and

220 presence/absence of longitudinal ribs. As the ribs were frequently missing in the

221 specimens, the presence or absence of longitudinal ribs was used instead of RN.

222 Specimens of *S. kurodai* from Sanda (locality #27), from which sufficiently mature

223 specimens could not be obtained, were excluded from the morphological analysis. The

224 Wilcoxon rank sum test and PCA were performed using R. Differences were considered

225 statistically significant at $p < 0.05$.

226

227 **Interspecific morphological comparison**

228 Shell morphologies of *S. kurodai*, *S. masudai* sp. nov., *S. praecursa* sp. nov., and *S.*
229 *miurai* sp. nov. were compared using 278 female specimens. The Random Forest (RF)
230 classification (Breiman, 2001) was used to evaluate the morphological relationships and
231 differences among the four species. The analysis was conducted for normalised data for
232 14 morphological characters: ASR, BCN, BWL, SA, SCN, WER, WN, Sculpture Type,
233 presence/absence of longitudinal ribs on teleoconch, PN, SLP, RNP, WNP, Node
234 Number, and Keel Type.

235 The second RF classification was conducted for *S. kurodai* and *S. masudai* sp. nov.,
236 given the low identification rate for *S. masudai* sp. nov. in the first RF. The proximity
237 measure among the specimens was transformed into 2D coordinates using the Euclidean
238 distance to estimate the morphological relationships between the populations of the two
239 species. Subsequently, the RF was trained using specimens of the two species obtained
240 from around the type locality of *S. kurodai* (localities #20, #21, #23–#26, #28, and #29),
241 which had a small morphological overlap with the specimens of *S. masudai* sp. nov.
242 After training the RF model, the morphological similarity of the holotype of *S. kurodai*
243 to newly obtained specimens of *S. kurodai* and *S. masudai* sp. nov. was predicted.

244 In the discriminant analysis, three variables were randomly sampled for each split as
245 candidates, and 100,000 trees were generated, given that the out-of-bag error rate fully

246 decreased with a large number of trees. Only female specimens were used because of
247 the sexual size differences within each species and the small number of male specimens
248 (see Results section). RF analyses were conducted using the package randomForest
249 v4.6–14 (Andy & Matthew, 2002) for R.

250 Variations in the central cusp morphology of the rachidian and lateral teeth of the
251 radula were examined within and among *S. kurodai*, *S. masudai* sp. nov., *S. praecursa*
252 sp. nov., and *S. miurai* sp. nov., following Sawada & Fuke (2022).

253

254 **Genetic and morphological variation of *S. kurodai* populations**

255 Morphological and genetic relationships between *S. kurodai* populations were
256 investigated to infer the factors of morphological variation in the species and its
257 colonisation history in riverine habitats. Genetic relationships among *S. kurodai*
258 populations were estimated by PCA using PLINK. In addition, the tree topology of *S.*
259 *kurodai* populations was estimated using 6,915 SNPs based on multi-species
260 coalescence using singular value decomposition quartets (SVDquartets) (Chifman &
261 Kubatko, 2014) implemented in PAUP* v4.0a (Swofford, 2003). The branch support for
262 the SVDquartets tree was assessed using 200 bootstrap replicates. All other parameters
263 were set to default settings. Based on the phylogenetic relationships between *S. kurodai*,

S. masudai sp. nov., and Lake Biwa endemics of the *S. niponica* group (Morita et al., 2024), these endemics were used as outgroups.

The general relationships between genetic and morphological variations in *S. kurodai* populations were estimated. Morphological variation was calculated by another PCA conducted for ASR, BCN, BWL, SA, SCN, WER, WN, Sculpture Type, PN, RNP, SHP, WNP, Node Number, and Keel Type using R. Only female specimens were used for PCA and populations with fewer than five specimens (localities #19, 27, and 34) were excluded from the analysis. Subsequently, correlations between the population average values of the PC1 scores of the SNP data and morphological characters were tested using a two-sided Kendall rank correlation coefficient using R. Variations in radular morphology were also compared among populations.

Results

Phylogenetic network and population structure

All specimens

After de-multiplexing, 73.5K–1.46M reads were obtained for each specimen. Average coverage per specimen at each locus was 28.9. The filtering pipeline detected 11,434 SNPs with 15.8% missing data on average in 168 specimens.

282 The PC1 and 2 generated by PCA based on 1,425 SNPs explained 32.1 and 12.5%
283 of the total variation, respectively. The first PC primarily separated the specimens into
284 the *S. niponica* group and the remainder (Fig. S1). Variations in the Lake Biwa
285 endemics of the *S. nakasekoe* group, *S. miurai* sp. nov., and the three clades of *S.*
286 *libertina* were explained by PC2 scores.

287

288 ***Semisulcospira niponica* group**

289 The Neighbor-net reconstructed based on 10,006 SNPs estimated well-branched splits
290 of *S. kurodai*, *S. masudai* sp. nov., *S. praecursor* sp. nov., and Lake Biwa endemics,
291 respectively (Fig. 2). Specimens from the same population belonged to common splits
292 except for *S. kurodai* specimens from Meiwa (locality #17). The network also estimated
293 closer relationships among the *S. kurodai* populations, mainly from the eastern part of
294 its distribution, and among those geographically close to each other: Gifu, Kaizu,
295 Meiwa (partial), Hikone, Takashima, Kameoka, Settsu, and Anan (localities #14, #15,
296 #17–#21, and #37); Fukuchiyama and Ono (#23 and #28); Tambasayama and Sanda
297 (#24–#27); and Okayama and Kurashiki (#32, #34, and #36).

298 ADMIXTURE analysis conducted for the *S. niponica* group specimens, based on
299 2,217 SNPs, estimated lower mean cross-validation error values for five to seven

genetic clusters (Table S2). The results of the ADMIXTURE and phylogenetic network analyses were consistent. *Semisulcospira praecursa* sp. nov. and Lake Biwa endemics were clearly divided into independent clusters with K values greater than two. Specimens of *S. kurodai* from Himeji (locality #29) were estimated to partially possess a genetic structure common to *S. masudai* sp. nov. at $K = 4-6$ (Fig. 3). The two species were completely separated with K values of seven and larger. The analysis also detected several genetic clusters within *S. kurodai* with K values greater than four.

Semisulcospira nakasekoe* group and *S. libertina

The phylogenetic network calculated based on 7,280 SNPs distinctly separated *S. miurai* sp. nov., Lake Biwa endemics of the *S. nakasekoe* group, and the three clades of *S. libertina* with well-branched splits (Fig. 4). One specimen obtained from Okayama (locality #32) was positioned between *S. miurai* sp. nov. and the node of the splits of Lake Biwa endemics and *S. libertina* in the Neighbor-net.

Lower mean cross-validation error values were estimated for four or more genetic clusters in the ADMIXTURE analysis based on 1,462 SNPs (Table S3). Corresponding to the results of Neighbor-net, *S. miurai* sp. nov., Lake Biwa endemics of the *S. nakasekoe* group, and *S. libertina* were clearly divided with K values of three and

larger (Fig. 5). The three clades of *S. libertina* were separated into different clusters with K values greater than four. The genetic structures of *S. miurai* sp. nov., Lake Biwa endemics, and *S. libertina* were estimated for a snail with intermediate positions in the phylogenetic network at $K = 4$ and 5 (arrowed in Fig. 5). This putative hybrid contained genetic structures of 70% *S. miurai* sp. nov., 20% *S. libertina* and 10% Lake Biwa endemics at $K = 5$.

Sexual dimorphism of shell morphology

Significant sexual differences were detected by the Wilcoxon rank-sum test for the shell size of *S. kurodai*, *S. masudai* sp. nov., *S. praecursa* sp. nov., and *S. miurai* sp. nov. (Table 2). Sex-related differences in the PC1 scores generated using the eight teleoconch characters were statistically significant in *S. kurodai* and non-significant in the other three species. Among the eight nonlinear characters of teleoconch, Sculpture Type strongly contributed to variations in the PC1 scores (Table S4).

Interspecific morphological comparison

RF classification based on 15 teleoconch and protoconch characters correctly classified 89.2% of the specimens into the four species. Bootstrap tests identified 98% *S. kurodai*,

336 53% *S. masudai* sp. nov., 80% *S. praecursa* sp. nov., and 93% *S. miurai* sp. nov. The
337 mean decrease in the Gini coefficient was larger for PN, SNP, Node Number, ASR,
338 RNP, and BWL (Table S5). The mean decrease in accuracy was greater for the
339 following morphological characters: Node Number, SCN, and ASR in *S. kurodai*; PN,
340 RNP, and Keel Type in *S. masudai* sp. nov.; ASR, BWL, and PN in *S. praecursa* sp.
341 nov.; PN, SCN, RNP, and Node Number in *S. miurai* sp. nov.

342 Focusing on these characters, the specimens of *S. kurodai* possessed a teleoconch
343 with a less elongated aperture and fewer spiral cords, and protoconchs with fewer nodes
344 in the four species (Table 3; Figs 6–9). The specimens of *S. masudai* sp. nov. showed
345 fewer protoconchs with a medium number of ribs and distinct sutural spiral cords. A
346 large teleoconch with a more elongated aperture and a larger number of protoconchs
347 characterised *S. praecursa* sp. nov. The specimens of *S. miurai* sp. nov. represented a
348 teleoconch with a greater number of spiral cords, and fewer protoconchs with fewer
349 ribs. In addition to the RF results, *S. miurai* sp. nov. was identified by its globose
350 protoconchs without pointed nodes on the longitudinal ribs. No shell morphological
351 differences were observed between the putative hybrid and sympatric *S. miurai* sp. nov.
352 specimens (Fig. 9P, R, AJ, AL)

The subsequent RF analysis conducted to estimate the morphological relationships between *S. kurodai* and *S. masudai* sp. nov., correctly identified 99% *S. kurodai* and 53% *S. masudai* sp. nov. Like the results of the first RF analysis, the mean decrease in accuracy was greater for the following morphological characters: ASR, Node Number, and Sculpture Type in *S. kurodai*; PN, RNP, and Sculpture Type in *S. masudai* sp. nov. (Table S6). According to proximities among specimens, the shell morphology of a large part of *S. kurodai* specimens obtained from Kaizu and Anan (localities #15 and #37) overlapped those of Toyooka specimens (#30) of *S. masudai* sp. nov. (Fig. S2). However, the larger SH and smaller RNE values of these *S. kurodai* populations discriminated them from *S. masudai* sp. nov., although these characteristics were less significant for species-level classification.

The above comparison of the two species showed that the *S. kurodai* populations around the type locality of the species were discriminated from *S. masudai* sp. nov. with relatively high accuracy. Another RF classification trained with the specimens of these *S. kurodai* populations and *S. masudai* sp. nov. correctly identified 96% of *S. kurodai* and 77% of *S. masudai* sp. nov. and predicted the holotype to be *S. kurodai* with 75.2% probability.

370 Radular morphological differences were not detected between *S. kurodai* and *S.*
371 *masudai* sp. nov. because of the large intraspecific variation in *S. kurodai* (Figs 10, 11).
372 *Semisulcospira praecursa* sp. nov. and *S. miurai* sp. nov. tended to possess wider and
373 flatter cusps on their rachidians and lateral teeth than the other two species. The central
374 cusps of rachidians were less tapering in *S. praecursa* sp. nov. than in *S. miurai* sp. nov.

375

376 **Genetic and morphological variation of *S. kurodai* populations**

377 The first two PCs generated by PCA based on 1,922 SNPs explained 25.5% of the total
378 variation. Four genetic subgroups were detected by the first two PCs (Fig. 12A). These
379 subgroups were approximately geographically structured: KS-II in Hyogo and northern
380 Kyoto Prefectures (localities # 23–#28); KS-III in Okayama Prefecture (localities #32,
381 #34, and #36); and KS-IV in the east and Shikoku (#14, #15, #17–#21, and #37).
382 However, the subgroup KS-I, that was consisted of specimens from Himeji (locality
383 #29), were separated from KS-II despite their short geographical distances. These PCA
384 results were consistent with results of the phylogenetic network and ADMIXUTRE
385 analyses conducted for the *S. niponica* group species (Figs 2, 3).

386 An SVDquartets tree based on 6,915 SNPs robustly supported the monophyly of *S.*
387 *kurodai*, *S. masudai* sp. nov., and a clade including the two species (BS = 100) (Fig.

12B). Monophyly of the clades of the subgroups KS-III and KS-IV was also strongly supported (BS = 100). The subgroup KS-II clade was recovered with moderate support (BS = 85). The sister relationships between the subgroup KS-III clade and the clade consisting of the subgroups KS-II and KS-IV were estimated. The subgroup KS-I were positioned in the outermost of the *S. kurodai* populations.

The two PC1s, calculated based on 1,922 SNPs and morphological traits, explained 14.2% and 20.3% of the total variation, respectively. The contribution of morphological traits to the PC1 scores was greater in ASR, RN, SA, WER, and Keel Type (Table S7). Large morphological variations were observed within the subgroups (Fig. S3). The correlation coefficients indicated a non-significant correlation between the average values of the *S. kurodai* populations for the two PC1 scores ($\tau = 0.147$, $p = 0.44$).

The central cusps of the rachidian and lateral teeth were wider, flatter, and less tapered in Meiwa, Kameoka, Ono, Himeji, and Kurashiki (localities #17, #20, #28, #29, #36), where snails were obtained from sandy gravel or piled rock substrates, compared to the other localities (Figs 10C, F, L, M, 11A).

Taxonomy

Family **Semisulcospiridae** Morrison, 1952

406 Genus *Semisulcospira* Boettger, 1886

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

408

409 *Semisulcospira kurodai* Kajiyama & Habe, 1961

410 (Tables 3, S8; Figs 6A–CM, 10A–O, 11A–B, 13)

411 Japanese name: Kuroda-kawanina

412

413 *Semisulcospira kurodai* Kajiyama & Habe, 1961: 168–171, figs 1, 2, 1a, 2a [part]

414 (original description); Habe, 1961: 25 [part]; Abe, 1981: 50; Habe & Kosuge, 1967:

415 28 [part]; Habe, 1968: 36, pl. 11, fig. 10 [part]; Davis, 1969: 230–235, pl. 2, figs 1–

416 3, pl. 8, figs 16–20; Habe, 1969: 58 [part]; Habe, 1986: 322–323, pl. 17, fig. 9

417 [part]; Goto, 1994: 100–101, fig. 1; Kihira & Matsuda, 1990: 8–9, unnumbered

418 figures [part]; Minato 1991: 79 [part] (misidentification in unnumbered figure);

419 Takami, 1991: 219–229 [part]; Kihira, 1994: 176, unnumbered plate, fig. 3 [part];

420 Takami, 1997: 309–313, pl. 1, figs 1, 3–8, 10–12 [part]; Sawatani, 2005: 29–31, pl.

421 C, fig. 20 [part]; Kihira et al., 2009: 16, unnumbered figures [part]; Masuda &

422 Uchiyama, 2010: 91, unnumbered figures [part]; Takami, 2013: 100, figs 2A, 3.

423 *Semisulcospira (Biwamelania) kurodai* – Miura et al., 2019: 48 (partial).

424 *Semisulcospira kurodai* K1 – Morita et al., 2024: 5–8.

425

426 **Material examined. *Holotype*.** NSMT-Mo39877, adult female, collected from

427 “Yakamishin, Joto-mura, Taki-gun, Hyogo Prefecture, Japan” (currently Hioki,

428 Tambasasayama, Hyogo Prefecture, Japan; locality #24 or 25).

429 ***Additional materials.*** KUZ Z5225, 10 females, Z5226, 5 males, collected from Gifu

430 (locality #14) on 21 February 2021; KUZ Z5227, 9 females, Z5228, 5 males, collected

431 from Kaizu (#15) on 10 September 2023; KUZ Z5229, 10 females, Z5230, 3 males,

432 collected from Meiwa (#17) on 21 February 2021; KUZ Z5231, 10 females, Z5232, 5

433 males, collected from Hikone (#18) on 14 October 2023; KUZ Z5233, 3 females,

434 Z5234, 3 males, collected from Takashima (#19) on 6 February 2021; KUZ Z5235, 10

435 females, Z5236, 5 males, collected from Kameoka (#20) on 22 August 2023; KUZ

436 Z5237, 6 females, Z5238, 3 males, collected from Settsu (#21) on 5 August 2023; KUZ

437 Z5239, 2 females, Z5240, 3 males, collected from Fukuchiyama (#23) on 22 August

438 2023; KUZ Z5241, 7 females, Z5242, 2 males, collected from Fukuchiyama (#23) on 25

439 November 2023; KUZ Z5243, 10 females, Z5244, 5 males, collected from

440 Tambasasayama (#24) on 26 January 2020; KUZ Z5145, Z5245, 9 females, Z5246, 5

441 males, collected from Tambasasayama (#25) on 22 August 2023; KUZ Z5247, 10

442 females, Z5248, 5 males, collected from Tambasayama (#26) on 22 August 2023;
 443 KUZ Z5249, 2 females, Z5250, 1 male, collected from Sanda (#27) on 16 August 2023;
 444 KUZ Z5251, 10 females, Z5252, 3 males, collected from Ono (#28) on 12 February
 445 2021; KUZ Z5253, 10 females, Z5254, 5 males, collected from Himeji (#29) on 12
 446 February 2021; KUZ Z5255, 3 females, collected from Okayama (#32) on 12 February
 447 2021; KUZ Z5256, 5 females, Z5257, 2 males, collected from Okayama (#34) on 5 July
 448 2023; KUZ Z5258, 2 females, collected from Okayama (#34) on 5 July 2023; KUZ
 449 Z5259, 10 females, Z5260, 4 males, collected from Kurashiki (#36) on 5 July 2023;
 450 KUZ Z5261, 10 females, Z5262, 5 males, collected from Anan (#37) on 2 July 2023.

451

452 **Emended diagnosis.** Viviparous. Teleoconch medium to large sized for the genus [SH
 453 30.6 ± 4.6 (mean $\pm$ SD) (female), 27.6 ± 4.2 (male) mm; BWL 18.0 ± 2.2 , 16.4 ± 2.2
 454 mm], moderately elongated (SA 18.3 ± 2.4 , 19.2 ± 2.5 degrees); outer lip of aperture
 455 simple, smooth; aperture pear-shaped, slightly to moderately elongated (ASR 1.7 ± 0.1 ,
 456 1.7 ± 0.1); basal cords in medium number (BCN 5.6 ± 1.0 , 5.7 ± 1.1); longitudinal ribs
 457 indistinct on penultimate whorl in most specimens (RN 17.0 ± 2.1 , 15.2 ± 2.3); spiral
 458 cords in medium number (SCN 9.3 ± 1.2 , 9.2 ± 1.1); colour in olive brown to light
 459 brown background. Protoconch medium sized for the genus (SLP 2.3 ± 0.4 mm, WNP

460 3.4 ± 0.4); longitudinal ribs typically prominently granulated in 1 row; colour in light
461 beige, with or without 1–3 dark reddish-brown bands.

462

463 **Description of holotype (NSMT-Mo39877; Fig. 6A–D). *Teleoconch*.** AH 11.2 mm,
464 AL 11.5 mm, ASR 1.86, AW 6.2 mm, BCN 6, BWL 19.1 mm, FWL 3.7 mm, PWL 6.9
465 mm, SA 18.7 degrees, SCN 11, SH 31.7 mm, SW 11.2 mm, TWL 4.7 mm, WER 3.04,
466 WN 5.50. Shell moderately elongated. Spires slightly broadened laterally, moderately
467 convex. Suture slightly undulating; outer lip of aperture simple, smooth. Aperture thin,
468 pear-shaped, moderately elongated. Apex of shell eroded. Longitudinal ribs oblique,
469 opisthocyrte, irregular, indistinct on body to fourth whorls; almost regular, distinct on
470 fifth whorl. Spiral cords regular, weak, partially carving longitudinal ribs on body to
471 penultimate whorls. Shell coloured light yellowish-brown background near aperture.
472 Shell surface brown to blackish brown with deposits.

473 ***Operculum*.** 8.3 mm in long diameter. Oval, paucispiral, comprising around 3
474 whorls. Nucleus subcentral.

475

476 **Variation (Figs 6, 10, 11, 13). *Teleoconch*.** Measurements and counts shown in Table
477 S8. Females larger than males in shell size. Spires slightly to moderately broadened.

478 Longitudinal ribs orthocline to opisthocyrt; typically regular, distinct on upper whorls;
479 fade on third to body whorls; slightly granulated at intersection with spiral cords;
480 sometimes distinct on penultimate to body whorls. Spiral cords regular, usually
481 indistinct. Shell coloured olive brown to light brown with irregular, narrow, dark brown
482 longitudinal stripes. Colour bands thick, dark brown, sometimes present on central
483 and/or lower part of whorls and/or basal part of shell.

484 ***Operculum.*** 4.5–7.9 mm in long diameter.

485 ***Protoconch.*** Measurements and counts shown in Table S8. Shell slightly to
486 moderately elongated. Suture prominently depressed by discrepancy between adjacent
487 whorls. Longitudinal ribs regular, remarkable, pointedly granulated on central part of
488 whorls with spiral cords in 1–3 rows. Sutural spiral cord distinct or weak on upper and
489 lower part of whorls. Shell coloured light beige to light brown in background; sutural
490 spiral cord on lower part of body to penultimate whorls coloured dark reddish brown;
491 another band rarely on basal part of shell; bands sometimes absent.

492 ***Radula.*** Taenioglossate. Rachidian teeth consisting of central denticle and 2–4 small
493 pointed triangular cusps on each side; tip of rachidian central denticle triangular, 2.0–
494 4.0 times longer than other triangular cusps; central denticle typically wide, flatter in
495 sandy and gravel substrate, narrow, pointed on muddy bottoms. Lateral teeth consisting

496 of large central denticle and 2–4 inner and outer pointed cusps; central cusp of lateral
497 teeth narrow, pointed, triangular, 2.0–3.5 times longer. Interior and exterior marginal
498 teeth spoon-shaped with 4–6 rounded denticles.

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

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

510

511 **Distribution and ecology.** According to previous and present investigations, *S. kurodai*
512 had certainly recorded at Aichi, Gifu, Mie, Shiga, Kyoto, Osaka, Hyogo, Okayama, and
513 Tokushima Prefectures (Kimura 1994; Takami 1997; Table 1; Fig. 1). In addition, an

uncertain record of this species included Hiroshima Prefecture (Japan Wildlife Research Center 2002). The previous record from Wakayama likely corresponds to this species, given the known distribution of *S. kurodai* (Ikebe, 2008). However, this species was not found in Aichi and Wakayama Prefectures in this study. *Semisulcospira kurodai* has typically been found on sandy mud to muddy bottoms of creeks, rivers, and ponds at a depth of 0–1 m. The species inhabits piled rock or concrete bottoms at Kameoka, Sanda, Ono, Himeji, and Kurashiki (localities #20, #27–#29, and #36). This species was collected with other congeners: *S. decipiens* (Westerlund, 1883) at Hikone (locality #18); *S. habei* and *S. nakasekoe* at Settsu (#21); *S. miurai* sp. nov. at Okayama and Kurashiki (#32, #34, and #36); *S. libertina* at all habitats.

Remarks. *Semisulcospira kurodai* can be discriminated from other congeners, including sympatric *S. libertina*, by the combination of an elongated teleoconch with a medium number (around six) of basal cords and a medium-sized, elongated, granulated, beige-coloured protoconch. However, these traits are not useful for distinguishing *S. kurodai*, *S. masudai* sp. nov., and *S. praecursa* sp. nov. Most populations of *S. kurodai* can be identified from *S. masudai* sp. nov. by their smaller ASR and larger PN, whereas *S. kurodai* specimens from Kaizu and Anan are similar to the other new species in these

532 characteristics. The larger SH and smaller RNE of these *S. kurodai* populations
533 discriminate them from *S. masudai* sp. nov. *Semisulcospira kurodai* represents smaller
534 ASR, BWL, and PN values than *S. praecursa* sp. nov.

535

536 *Semisulcospira masudai* Sawada & Miura sp. nov.

537 (Tables 3, S8; Figs 7A–T, 11C–E)

538 New Japanese name: Tajima-kawanina (タジマカワニナ)

539

540 *Semisulcospira kurodai* – Masuda & Uchiyama, 2010: 91 [part].

541 *Semisulcospira* (*Semisulcospira*) *kurodai* – Kamiya et al., 2011: 25.

542 *Semisulcospira kurodai* K2 – Morita et al., 2024: 5–8.

543

544 **ZooBank registration LSID.** urn:lsid:zoobank.org:act:...

545

546 **Material examined. *Holotype*.** KUZ Z5263, adult female, collected from sandy gravel

547 bottom at depths of 0–1 m Roppo River in Kamihachiyama, Toyooka, Hyogo

548 Prefecture, Japan (locality #30) on 22 August 2023 by Naoto Sawada.

549 **Paratypes.** KUZ Z5146, Z5264, Z5165, 3 adult females, Z5166, Z5167, 2 adult
550 males collected with holotype.

551 **Additional materials.** KUZ Z5147, Z5268, 11 females, Z5269, 1 male, collected
552 with holotype; KUZ Z5270, 15 females, Z5271, 10 males, collected from Iwami
553 (locality #31) on 22 August 2023.

554

555 **Diagnosis.** Viviparous. Teleoconch medium to large sized for the genus [SH 29.2 ± 2.1 ,
556 26.5 ± 3.5 mm; BWL 17.5 ± 1.2 , 16.1 ± 1.6 mm], moderately elongated (SA 18.0 ± 2.6 ,
557 18.5 ± 1.4 degrees); outer lip of aperture simple, smooth; aperture pear-shaped, slightly
558 elongated (ASR 1.8 ± 0.1 , 1.8 ± 0.1); basal cords in medium number (BCN 6.2 ± 0.9 ,
559 6.0 ± 0.9); longitudinal ribs indistinct on penultimate whorl in most specimens (RN 16.5
560 ± 1.9 , 15.7 ± 1.9); spiral cords in medium number (SCN 9.6 ± 0.9 , 9.5 ± 0.6); colour in
561 olive brown to light brown background. Protoconch medium sized for the genus (SLP
562 2.4 ± 0.3 mm, WNP 3.6 ± 0.3), in small number (PN 32.4 ± 14.9); longitudinal ribs in
563 medium number (RNP 14.7 ± 1.3), prominently granulated; lower sutural spiral cord
564 distinct; colour in light beige, with or without 1–3 dark reddish-brown bands.

565

566 **Description of holotype (KUZ Z5263; Figs 7A–G, 11C). *Teleoconch*.** AH 12.9 mm,
567 AL 12.9 mm, ASR 1.63, AW 7.9 mm, BCN 5, BWL 21.3 mm, FWL 4.0 mm, PWL 7.7
568 mm, SA 21.0 degrees, SH 35.8 mm, SW 13.7 mm, TWL 5.1 mm, WER 3.26, WN 5.50.
569 Shell moderately elongated. Spires slightly broadened laterally, slightly convex. Suture
570 slightly undulating. Outer lip of aperture simple, smooth. Aperture thick, pear-shaped,
571 slightly elongated. Apex of shell eroded. Longitudinal ribs oblique, opisthocyrt,
572 irregular, indistinct on body to third whorls; regular, distinct on fourth to fifth whorls.
573 Spiral cord indistinct, partially carving longitudinal ribs on body to penultimate whorls.
574 Shell coloured light yellowish-brown background on penultimate to fifth whorls; light
575 brown on body to penultimate whorls, with one narrow dark brown colour band on
576 lower part of penultimate to fifth whorls.
577 ***Operculum*.** 7.4 mm in long diameter. Oval, paucispiral, comprising around 3
578 whorls. Nucleus subcentral.
579 ***Protoconch*.** PN 55, RNP 14, SLP 2.6 mm, SWP 1.8 mm, WNP 3.75. Shell globose.
580 Suture prominently depressed by discrepancy between adjacent whorls. Longitudinal
581 ribs regular, remarkable, prominently granulated with spiral cords in 3 rows on central
582 part of whorls. Sutural spiral cord distinct on upper and lower parts of whorls. Shell

583 coloured light beige in background; sutural spiral cord on lower part of body to
584 penultimate whorls coloured dark reddish brown.

585 ***Radula.*** Taenioglossate. Rachidian teeth consisting of central denticle and 3–4 small
586 pointed triangular cusps on each side; tip of rachidian central denticle narrow, rounded,
587 triangular, 3.0–3.5 times longer than other triangular cusps. Lateral teeth consisting of
588 large central denticle and 2–3 inner and outer pointed cusps; central cusp of lateral teeth
589 narrow, rounded, triangular, 2.5 times longer. Interior and exterior marginal teeth
590 spoon-shaped with 4–5, 5–6 rounded denticles, respectively.

591 ***Reproductive organ. Female.*** Characteristics of reproductive organs similar to those
592 of *S. kurodai* (Fig. 13). Oviduct long, narrow, emerging from ovary entering near
593 seminal receptacle on ventral side of soft body; protrusions of seminal receptacle short.
594 Sperm gutter extending from spermatophore bursa towards mantle cavity, curved
595 inward along whorls. Brood pouch extending along dorsal side of spermatophore bursa
596 and sperm gutter, dorsally inflated, separated into many chambers, including eggs and
597 embryos; eggs coloured beige, radially developing from base of brood pouch near
598 seminal receptacle.

599

600 **Variation (Figs 7, 11). *Teleoconch*.** Measurements and counts shown in Table S8.

601 Females larger than males in shell size. Spires slightly to moderately broadened.

602 Longitudinal ribs opisthocyrt, typically regular, distinct on upper whorls; fade on

603 penultimate to body whorls; weakly granulated at intersection with spiral cords;

604 sometimes distinct on penultimate to body whorls. Spiral cords regular, usually

605 indistinct. Shell coloured olive brown to light brown with irregular, narrow, dark brown

606 longitudinal stripes; colour bands usually thick, dark brown, distinct on lower part of

607 whorls and/or basal part of shell; indistinct on central part of whorls; bands often absent.

608 ***Operculum*.** 4.2–7.4 mm in long diameter.

609 ***Protoconch*.** Measurements and counts shown in Table S8. Shell slightly to

610 moderately elongated. Longitudinal ribs regular, pointedly granulated with spiral cords

611 on central part of whorls in 1–3 rows. Sutural spiral cord distinct or weak on upper and

612 lower part of whorls. Sutural spiral cord on lower part of body to penultimate whorls

613 typically coloured dark reddish brown; bands rarely distinct on central spiral cord and

614 basal part of shell; all bands sometimes absent.

615 ***Radula*.** Rachidian teeth consisting of 5–8 triangular cusps; tip of rachidian central

616 denticle typically narrow, pointed, rarely rounded, 2.5–3.5 times longer than other

617 triangular cusps. Central cusp of lateral teeth typically narrow, rounded, rarely pointed.

618 Interior and exterior marginal teeth spoon-shaped with 4–5, 4–6 rounded denticles,
619 respectively.

620 **Reproductive organ. Female.** Protrusions of seminal receptacle rarely short.

621 **Male.** Characteristics of reproductive organs similar to those of *S. kurodai* (Fig. 13).

622 Vas deferens long, narrow, emerging from testes, entering posterior end of prostate.

623 Prostate without penis elongated, inflated in posterior ventral part, with deep groove,
624 forming U-shape in transverse section, anterior narrowly opening to mantle cavity.

625

626 **Etymology.** The specific name is dedicated to Osamu Masuda, who first discovered the
627 new species and investigated its distribution in Hyogo Prefecture (Masuda, 2014;
628 Masuda & Uchiyama, 2010). The new Japanese name refers to the main distribution of
629 this species.

630

631 **Distribution and ecology.** The distribution of *S. masudai* sp. nov. is restricted to the
632 northern part of Hyogo and Tottori Prefectures (around Tajima Region) (Table1; Fig.
633 1). This species was found on sandy gravel and sandy bottoms of a creek and river at a
634 depth of 0–1 m. *Semisulcospira masudai* sp. nov. was estimated to have been recorded
635 as *S. kurodai* from the Maruyamagawa and Yatagawa River systems in northern Hyogo

636 Prefecture (Masuda, 2014). The new species was collected with *S. libertina* from all
637 habitats. *Semisulcospira masudai* sp. nov. was not collected from Tambasasayama
638 (locality #25), where the species was previously recorded (Morita et al., 2024).

639

640 **Remarks.** The traits of an elongated teleoconch with a medium number (around six) of
641 basal cords and a medium-sized, elongated, granulated, beige-coloured protoconch
642 identify this new species, *S. kurodai*, and *S. praecursa* sp. nov. in the genus.
643 *Semisulcospira masudai* sp. nov. can be distinguished from *S. kurodai* by the
644 combination of larger ASR and RNE, and smaller SH and PN. The characteristics of a
645 smaller BWL, PN, and RNP discriminate this new species from *S. praecursa* sp. nov.

646

647 *Semisulcospira praecursa* Sawada & Miura sp. nov.

648 (Tables 3, S8; Figs 8A–AA, 11F–I)

649 New Japanese name: Sakigake-kawanina (サキガケカワニナ)

650

651 *Semisulcospira kurodai* –Kajiyama & Habe, 1961: 168–171, figs 3, 3a [part]; Habe,
652 1961: 25 [part]; Habe & Kosuge, 1967: 28 [part]; Habe, 1968: 36 [part]; Habe,
653 1969: 58 [part]; Habe, 1986: 322–323 [part]; Kihira & Matsuda, 1990: 8–9 [part];

654 Minato, 1991: 79 [part]; Takami, 1991: 219–229 [part]; Kihira, 1994: 176 [part];
655 Takami, 1997: 309–313, pl. 1, figs 2, 9 [part]; Sawatani, 2005: 29–31, pl. B, figs
656 14–18 [part]; Kihira et al., 2009: 16 [part]; Masuda & Uchiyama, 2010: 91 [part];
657 Kurozumi, 2019: 86–87, fig. 3.

658 *Semisulcospira kurodai* K3 – Morita et al., 2024: 5–8.

659

660 **ZooBank registration LSID.** urn:lsid:zoobank.org:act:...

661

662 **Material examined. *Holotype*.** KUZ Z5272, adult female, collected from piled rock
663 bottom at depths of 0–1 m, Ichiunsai River in Hiramatsu, Iwata, Shizuoka Prefecture,
664 Japan (locality #1) on 21 February 2021 by Naoto Sawada.

665 ***Paratypes*.** KUZ Z5273–Z5275, 3 adult females, Z5276, Z5277, 2 adult males
666 collected with holotype.

667 ***Additional materials*.** KUZ Z5278, 10 females, Z5279, 4 males, collected with
668 holotype; KUZ Z5280, 14 females, Z5281, 7 males, collected from Hamamatsu (locality
669 #2) on 21 February 2021; KUZ Z5282, 14 females, Z5283, 8 males, collected from
670 Toyokawa (#5) on 21 February 2021; KUZ Z5284, 2 females collected from Toyota
671 (#7) on 9 September 2023.

672

673 **Diagnosis.** Viviparous. Teleoconch large for the genus [SH 33.6 ± 2.9 , 29.6 ± 2.4 mm;
674 BWL 20.8 ± 1.7 , 18.5 ± 1.6 mm], moderately elongated (SA 18.1 ± 2.5 , 19.0 ± 1.9
675 degrees); outer lip of aperture simple, smooth; aperture pear-shaped, moderately to
676 greatly elongated (ASR 1.9 ± 0.1 , 1.8 ± 0.1); basal cords in medium number (BCN $6.8 \pm$
677 1.0 , 7.0 ± 1.0); longitudinal ribs indistinct on penultimate whorl (RN 20.7 ± 2.3); spiral
678 cords in medium number (SCN 9.6 ± 1.2 , 10.3 ± 1.2); colour in olive brown to light
679 brown background. Protoconch medium sized for the genus (SLP 2.5 ± 0.3 mm, WNP
680 3.5 ± 0.4), in large number (PN 105.3 ± 44.5); longitudinal ribs prominently granulated;
681 colour in light beige, with or without 1–3 dark reddish-brown bands. Central cusps of
682 rachidian and lateral teeth wide, flatter; central cusps of rachidian less tapering.

683

684 **Description of holotype (KUZ Z5272; Figs 8A–G, 11F). *Teleoconch*.** AH 13.5 mm,
685 AL 13.6 mm, ASR 1.76, AW 7.8 mm, BCN 5, BWL 22.7 mm, FWL 3.9 mm, PWL 8.6
686 mm, RN 22, SA 16.6 degrees, SCN 9, SH 37.7 mm, SW 14.0 mm, TWL 6.2 mm, WER
687 3.47, WN 5.25. Shell moderately elongated. Spires slightly broadened laterally, slightly
688 convex. Suture slightly undulating. Outer lip of aperture simple, smooth. Aperture thick,
689 pear-shaped, moderately elongated. Apex of shell eroded. Longitudinal ribs oblique,

690 opisthocyrt, irregular, indistinct on body to penultimate whorls; distinct on third to fifth
691 whorls. Spiral cord regular weak, partially carving longitudinal ribs. Shell coloured light
692 brown background, with one thick olive brown colour band distinct on central part
693 whorls and basal part of shell.

694 ***Operculum.*** 6.1 mm in long diameter. Oval, paucispiral, comprising around 3
695 whorls. Nucleus subcentral.

696 ***Protoconch.*** PN 117, RNP 17, SLP 2.5 mm, SWP 1.7 mm, WNP 3.50. Shell slightly
697 elongated. Suture prominently depressed by discrepancy between adjacent whorls.
698 Longitudinal ribs regular, remarkable, prominently granulated with spiral cord in 1 row
699 on central part of whorls. Sutural spiral cord weak on upper parts of whorls; distinct on
700 lower ones. Shell coloured light beige to light brown in background; sutural spiral cord
701 on lower part of body to penultimate whorls coloured dark reddish brown.

702 ***Radula.*** Taenioglossate. Rachidian teeth consisting of central denticle and 3–4 small
703 pointed triangular cusps on each side; tip of rachidian central denticle wide, rounded,
704 triangular, 2.5–3.0 times longer than other triangular cusps. Lateral teeth consisting of
705 large central denticle and 2–3 inner and outer pointed cusps; central cusp of lateral teeth
706 wide, rounded, triangular, 2.5 times longer. Interior and exterior marginal teeth spoon-
707 shaped with 4–5, 5–6 rounded denticles, respectively.

708 **Reproductive organ. Female.** Characteristics of reproductive organs similar to those
709 of *S. kurodai* (Fig. 13). Oviduct long, narrow, emerging from ovary entering near
710 seminal receptacle on ventral side of soft body; protrusions of seminal receptacle long.
711 Sperm gutter extending from spermatophore bursa towards mantle cavity, curved
712 inward along whorls. Brood pouch extending along dorsal side of spermatophore bursa
713 and sperm gutter, dorsally inflated, separated into many chambers, including eggs and
714 embryos; eggs coloured beige, radially developing from base of brood pouch near
715 seminal receptacle.

716

717 **Variation (Figs 8, 11). Teleoconch.** Measurements and counts shown in Table S8.
718 Females larger than males in shell size. Spires slightly to moderately broadened.
719 Longitudinal ribs typically regular, distinct on upper whorls; fade on penultimate to
720 body whorls; weakly granulated at intersection with spiral cords. Spiral cords regular,
721 usually indistinct. Shell coloured olive brown to light brown with irregular, narrow,
722 dark brown longitudinal stripes; colour bands thick, dark brown, distinct on central
723 and/or lower part of whorls and/or basal part of shell.

724 **Operculum.** 5.8–8.7 mm in long diameter.

725 **Protoconch.** Measurements and counts shown in Table S8. Shell slightly to
726 moderately elongated. Longitudinal ribs regular, pointedly granulated with spiral cords
727 on central part of whorls in 1–3 rows. Sutural spiral cord distinct or weak on upper and
728 lower part of whorls. Sutural spiral cord on lower part of body to penultimate whorls
729 typically coloured dark reddish brown; bands rarely distinct on central spiral cord and
730 basal part of shell; all bands sometimes absent.

731 **Radula.** Rachidian teeth consisting of 6–9 triangular cusps; tip of rachidian central
732 denticle 2.0–3.5 times longer than other triangular cusps. Central cusp of lateral teeth
733 2.5–3.0 times longer than other triangular cusps than other triangular cusps. Interior and
734 exterior marginal teeth spoon-shaped with 4–6 rounded denticles.

735 **Reproductive organ. Female.** Protrusions of seminal receptacle rarely short.

736 **Male.** Characteristics of reproductive organs similar to those of *S. kurodai* (Fig. 13).

737 Vas deferens long, narrow, emerging from testes, entering posterior end of prostate.

738 Prostate without penis elongated, inflated in posterior ventral part, with deep groove,

739 forming U-shape in transverse section, anterior narrowly opening to mantle cavity.

740

741 **Etymology.** The specific name is a participle referring that *S. praecursa* sp. nov. has

742 been estimated to be the first species that had advanced into riverine habitats from lakes,

at least in the *S. niponica* group (Morita et al., 2024). The Japanese name indicates the pioneering feature of this new species mentioned above.

Distribution and ecology. The new species is distributed in western Shizuoka and eastern Aichi Prefectures (Table1; Fig. 1). Previous records of *S. kurodai* from Shizuoka and Aichi Prefectures likely include those of this new species (Kawase et al., 2011; Kimura, 1994; Sawatani, 2005; Takami, 1997). This species was found on sandy gravel and sandy bottoms of rivers at a depth of 0–1 m. The new species was collected with *S. libertina* from all habitats.

Remarks. *Semisulcospira praecursa* sp. nov. can be discriminated from the other congeners by the combination of the following traits: an elongated, large teleoconch with medium number (around six) of basal cords and elongated aperture; a medium-sized, elongated, granulated, beige-coloured protoconch in large number; radula with wider, flatter central cusps on rachidian and lateral teeth.

Semisulcospira miurai Sawada sp. nov.

761 (Tables 3, S8; Figs 9A–AL, 11J–O)

762 New Japanese name: Yume-kawanina (ユメカワニナ)

763

764 *Semisulcospira kurodai* – Kihira & Matsuda, 1990: 8–9 [part]; Kihira, 1994: 176 [part];

765 Kihira et al., 2009: 16 [part].

766 *Semisulcospira kurodai* K4 – Morita et al., 2024: 5–8.

767 *Semisulcospira* cf. *kurodai* – Fukuda, 2024.

768

769 **ZooBank registration LSID.** urn:lsid:zoobank.org:act:...

770

771 **Material examined. *Holotype*.** KUZ Z5285, adult female, collected from muddy

772 bottom at depths of 0–1 m, Sasagase River in Fujita, Minami-ku, Okayama, Okayama

773 Prefecture, Japan (locality #34) on 5 July 2023 by Naoto Sawada.

774 ***Paratypes*.** KUZ Z5286–Z5288, 3 adult females, Z5289, Z5290, 2 adult males

775 collected with holotype.

776 ***Additional materials*.** KUZ Z5291, 13 females, Z5292, 8 males, collected with

777 holotype; KUZ Z5293, 1 female collected from Okayama (locality #32) on 12 February

778 2021; KUZ Z5294, 13 females, Z5295, 3 males, collected from Okayama (#32) on 5

779 July 2023; KUZ Z5296, 11 females, Z5297, 10 males, collected from Okayama (#33)
780 on 5 July 2023; KUZ Z5298, 7 females, Z5299, 7 males, collected from Okayama (#35)
781 on 5 July 2023; KUZ Z5300, 7 females, Z5301, 2 males, collected from Kurashiki (#36)
782 on 5 July 2023.

783

784 **Diagnosis.** Viviparous. Teleoconch small to medium sized for the genus [SH 28.1 ± 3.3 ,
785 24.9 ± 2.4 mm; BWL 16.8 ± 1.7 , 15.0 ± 1.5 mm], moderately to strongly elongated (SA
786 18.8 ± 3.8 , 19.1 ± 2.4 degrees); outer lip of aperture simple, smooth; aperture pear-
787 shaped, moderately to greatly elongated (ASR 1.7 ± 0.1 , 1.7 ± 0.1); basal cords in
788 medium number (BCN 5.7 ± 0.8 , 5.8 ± 1.1); longitudinal ribs indistinct on penultimate
789 whorl; spiral cords weak, in large number (SCN 11.0 ± 1.2 , 10.3 ± 1.3); colour in light
790 olive brown to light brown background. Protoconch medium sized for the genus (SLP
791 2.5 ± 0.6 mm, WNP 3.3 ± 0.3), in small number (PN 18.9 ± 10.3); longitudinal ribs in
792 small number (RNP 11.3 ± 1.1), smooth or weakly granulated; colour in light beige,
793 with or without 1–3 dark reddish-brown bands. Central cusps of rachidian and lateral
794 teeth wide, rounded.

795

796 **Description of holotype (KUZ Z5285; Figs 9A–G, 11J). *Teleoconch*.** AH 11.8 mm,
797 AL 11.7 mm, ASR 1.66, AW 7.1 mm, BCN 6, BWL 19.5 mm, FWL 3.3 mm, PWL 7.2
798 mm, SA 15.2 degrees, SCN 12, SH 33.3 mm, SW 11.8 mm, TWL 5.1 mm, WER 3.53,
799 WN 6.50. Shell moderately elongated. Spires slightly broadened laterally, slightly
800 convex. Suture slightly undulating. Outer lip of aperture simple, smooth. Aperture thick,
801 pear-shaped, moderately elongated. Apex of shell eroded. Longitudinal ribs oblique,
802 opisthocyrt, irregular indistinct on body to fifth whorls; regular, distinct on sixth to
803 seventh whorls. Spiral cord regular, weak. Shell coloured light yellowish-brown
804 background, with regular, narrow, dark brown longitudinal stripe, without distinct
805 colour band.

806 ***Operculum*.** 6.4 mm in long diameter. Oval, paucispiral, comprising around 3
807 whorls. Nucleus subcentral.

808 ***Protoconch*.** PN 19, RNP 10, SLP 2.5 mm, SWP 2.0 mm, WNP 3.25. Shell slightly
809 elongated. Suture prominently depressed by discrepancy between adjacent whorls.
810 Longitudinal ribs regular, remarkable, weakly granulated on central part of whorls.
811 Sutural spiral cord weak on upper parts of whorls; thick, distinct on lower ones. Shell
812 coloured light beige in background without colour band.

813 **Radula.** Taenioglossate. Rachidian teeth consisting of central denticle and 2–3 small
814 pointed triangular cusps on each side; tip of rachidian central denticle wide, rounded,
815 triangular, 2.5 times longer than other triangular cusps. Lateral teeth consisting of large
816 central denticle and 2–3 inner and outer pointed cusps; central cusp of lateral teeth wide,
817 rounded, triangular, 2.5 times longer. Interior and exterior marginal teeth spoon-shaped
818 with 5 rounded denticles.

819 **Reproductive organ. Female.** Characteristics of reproductive organs similar to those
820 of *S. kurodai* (Fig. 13). Oviduct long, narrow, emerging from ovary entering near
821 seminal receptacle on ventral side of soft body; protrusions of seminal receptacle short.
822 Sperm gutter extending from spermatophore bursa towards mantle cavity, curved
823 inward along whorls. Brood pouch extending along dorsal side of spermatophore bursa
824 and sperm gutter, dorsally inflated, separated into many chambers, including eggs and
825 embryos; eggs coloured beige, radially developing from base of brood pouch near
826 seminal receptacle.

827

828 **Variation (Figs 9, 11). Teleoconch.** Measurements and counts shown in Table S8.
829 Females larger than males in shell size. Spires slightly to moderately broadened.
830 Longitudinal ribs regular, distinct on upper whorls; fade on most remaining whorls.

831 Spiral cords weak or indistinct. Shell coloured olive brown to light brown with regular
832 or irregular, narrow, dark brown longitudinal stripes; colour bands thick, dark brown,
833 distinct on central and/or lower part of whorls and/or basal part of shell.

834 ***Operculum.*** 4.0–6.4 mm in long diameter.

835 ***Protoconch.*** Measurements and counts shown in Table S8. Shell globose or slightly
836 elongated. Longitudinal ribs smooth or weakly granulated with in 1 row. Sutural spiral
837 cord distinct or weak on upper and lower part of whorls. Sutural spiral cord on lower
838 part of body to penultimate whorls and basal part of shell rarely coloured dark reddish
839 brown.

840 ***Radula.*** Rachidian teeth consisting of 5–8 triangular cusps; tip of rachidian central
841 denticle typically narrow, pointed, rarely rounded, 2.5–4.0 times longer than other
842 triangular cusps. Central cusp of lateral teeth typically wide, rounded, rarely narrow,
843 2.5–3.0 times longer than other triangular cusps than other triangular cusps; central
844 denticle typically wide, rounded in gravel substrate, narrow, pointed on muddy bottoms.
845 Interior and exterior marginal teeth spoon-shaped with 4–5, 5–6 rounded denticles,
846 respectively.

847 ***Reproductive organ. Female.*** Protrusions of seminal receptacle rarely short.

848 **Male.** Characteristics of reproductive organs similar to those of *S. kurodai* (Fig. 13).

849 Vas deferens long, narrow, emerging from testes, entering posterior end of prostate.

850 Prostate without penis elongated, inflated in posterior ventral part, with deep groove,

851 forming U-shape in transverse section, anterior narrowly opening to mantle cavity.

852

853 **Etymology.** The specific name is dedicated to Osamu Miura, who first identified this

854 new species as a member of the *S. nakasekoe* group (Morita et al. 2024). The new

855 Japanese name refers to the novelty that the *S. nakasekoe* group species had advanced

856 into riverine habitats from the Lake Biwa system.

857

858 **Distribution and ecology.** The distribution of this new species is restricted to the

859 southern plains of Okayama Prefecture. (Table1; Fig. 1). Previous records of *S. kurodai*

860 from Okayama Prefecture may include those of this new species (Egi, 2022; Fukuda,

861 2003). This species was found on muddy or gravel bottoms of rivers at a depth of 0–1

862 m. The new species was collected with *S. kurodai* at Oyakama and Kurashiki (localities

863 #32, #34, and #36). *Semisulcospira miurai* sp. nov. was observed with *S. libertina* in all

864 habitats.

865

Remarks. This new species can be discriminated from other congeners, including sympatric *S. libertina*, by the combination of an elongated teleoconch with a medium number (around six) of basal cords and a medium-sized, globose, ribbed, beige-coloured protoconch. Although teleoconch morphology of *S. miurai* sp. nov. resembles that of *S. kurodai*, *S. masudai* sp. nov., and *S. praecursa* sp. nov., *S. miurai* sp. nov. is identifiable by its ribbed protoconch. In addition, *S. miurai* sp. nov. and *S. nakasekoe* are similar in protoconch and radula morphology. The basal cord number, teleoconch without longitudinal ribs on penultimate to third whorls, and radula with wider, more rounded central cusps on rachidian and lateral teeth discriminate *S. miurai* sp. nov. from the other species.

Discussion

The results of this study elucidated that *S. kurodai*, *S. masudai* sp. nov., *S. praecursa* sp. nov., and *S. miurai* sp. nov. represent distinct genetic and morphological traits. Due to the similarities in the medium numbers of basal cords on teleoconchs and the morphology of protoconchs, which had been important for the identification of *S. kurodai*, the four species have likely been confused in previous studies (Kajiyama & Habe, 1961; Kawase et al., 2011; Masuda & Uchiyama, 2010; Takami, 1997). However,

884 most specimens of the four species could be identified by the combination of the
885 characteristics of teleoconch, protoconch, and radula. Based on their genetic and
886 morphological distinctiveness, the authors considered these four species to be
887 independent species. Furthermore, the morphological similarity between the type
888 specimen and newly collected materials of *S. kurodai* clarified their conspecifics.

889 The present genetic analysis showed close relationships between *S. kurodai*, *S.*
890 *masudai* sp. nov., *S. praecursa* sp. nov., and Lake Biwa endemics of the *S. niponica*
891 group, and between *S. miurai* sp. nov. and the Lake Biwa *S. nakasekoe* group species,
892 corresponding to the results of Morita et al. (2024). The pointed protoconch nodes of *S.*
893 *kurodai*, *S. masudai* sp. nov., and *S. praecursa* sp. nov. were similar to those of the *S.*
894 *niponica* group species (Sawada & Fuke, 2022), indicating that these three species are
895 members of the *S. niponica* group. Similarly, *S. miurai* sp. nov. was identified as a *S.*
896 *nakasekoe* group species based on its ribbed protoconchs. The results of the
897 morphological comparison in this study were consistent with those of the previous study
898 in that *S. miurai* sp. nov. possessed fewer protoconchs than the other three species
899 (Morita et al., 2024). On the other hand, interspecific differences in teleoconch
900 slenderness and protoconch size, that were observed previously, were not detected in
901 this study. The increased intraspecific variation within each species, associated with the

902 increased sample size in the present study, likely caused these differences. The similar
903 teleoconch morphology of *S. kurodai* and the new species indicated a parallel
904 morphological evolution between the *S. niponica* and *S. nakasekoe* group species in
905 riverine habitats, as suggested in Lake Biwa endemics (Sawada et al., 2024).

906 The known distribution of *S. praecursa* sp. nov. is expanded eastwards into western
907 Shizuoka Prefecture as a result of this study. This new species was obtained from coarse
908 substrates in flowing rivers, and recorded allopatrically with *S. kurodai* across western
909 Aichi and central Gifu Prefectures (between localities #7, #14, and #15). Although
910 several occurrence records have been accumulated in this poorly explored area (Goto,
911 1994; Kimura, 1994; Sawatani, 2005; Takami, 1997; localities #8–#13), neither species
912 have been found in previous and present field surveys (Kimura, 2020). Among these,
913 specimens from Toyota (locality #6), Anjo (#9), and Seki (#13) are identifiable as *S.*
914 *kurodai* based on the morphological traits of a less elongated aperture and ecological
915 features that they were obtained from fine-grained substrates (Goto, 1994; Sawatani,
916 2005; Takami, 1997). Therefore, it is likely that *S. kurodai* and *S. praecursa* sp. nov.
917 had overlapped their distributions in lost habitats in the Yahagi River system in the
918 central Aichi Prefecture (near localities #6 and #7). Most habitats of *S. kurodai* and the
919 three new species have been exposed to anthropogenic impacts, and they are included as

920 *S. kurodai* in the Red List of the country and many prefectures (e.g., Goto, 2010; Kato,
921 2019; Kimura, 2020; Kondo, 2015; Wildlife Division, Nature Conservation Bureau,
922 Ministry of the Environment, 2020). Habitat loss in these species may have reduced
923 their original distribution, hampering the verification of reproductive isolation between
924 *S. kurodai* and *S. praecursa* sp. nov. under natural conditions.

925 The present investigation did not find *S. masudai* sp. nov. around the type locality of
926 *S. kurodai* despite focused surveys around the area, whereas one specimen of *S.*
927 *masudai* sp. nov. was collected with *S. kurodai* there (Morita et al., 2024). Based on
928 previous and present investigations (Morita et al., 2024), the main range of *S. masudai*
929 sp. nov. is likely restricted to the northern part of the Chugoku District by the Chugoku
930 Mountains. As discussed by Morita et al. (2024), the geographical isolation caused by
931 the Chugoku Mountains has been observed in other freshwater organisms in the region
932 (Fujita et al., 2011; Takehana et al., 2003). Therefore, the present field survey increased
933 the probability that the previous study collected a specimen of *S. masudai* sp. nov.,
934 which has accidentally crossed this barrier. At the same time, the present results
935 indirectly indicated reproductive isolation between *S. kurodai* and *S. masudai* sp. nov.
936 because the genetic structure of *S. masudai* sp. nov. was not detected in the *S. kurodai*
937 specimens from around the type locality.

938 Genetic analyses detected four genetic subgroups within *S. kurodai*, and the
939 distribution of each subgroup suggested its different expansion patterns. The genetic
940 divergence between the subgroups KS-II and KS-III of *S. kurodai* in Okayama and
941 Hyogo Prefectures may indicate that the mountains between the two prefectures caused
942 the geographical isolation of *S. kurodai*, although further surveys are needed on the
943 fragmentary records of this species in this mountainous area (Egi, 2022; Masuda, 2014).
944 In contrast, the subgroup KS-IV in the eastern distribution of the species extends
945 eastwards beyond the Suzuka Mountains, which had been uplifted around 1.4 Mya
946 (Kawabe, 1994; Yokoyama, 1969). The clades of *S. kurodai* and *S. masudai* sp. nov.
947 have been estimated to diverge about 0.17 Mya (Morita et al., 2024), far after the uplift
948 of the Suzuka Mountains. Therefore, the subgroup KS-IV had likely overcome this
949 mountain range, which has been known as one of the major geographic barriers for
950 Japanese freshwater species (Nakajima, 2012; Komiya et al., 2014; Watanabe et al.,
951 2014). The distribution of the subgroup KS-II in southern Hyogo and northern Kyoto
952 Prefectures suggests that the subgroup had expanded during river capture in the
953 lowlands of the region (Hikami Corridor), as estimated for riverine fishes (Miyazaki et
954 al., 2018; Saitoh et al., 2010) and shrimp (Fujita et al., 2011).

The subgroup KS-I was positioned in the outermost of the *S. kurodai* populations in the SVDquartets tree. In addition, the ADMIXTURE results with smaller *K* values revealed a genetic similarity between the KS-I and *S. masudai* sp. nov. These results suggest that *S. kurodai* originated not directly around Lake Biwa but in the Chugoku District, where the common ancestor of the two species was isolated by the Chugoku Mountains. It has been estimated that the southern part of the Chugoku District had faced a freshwater system connected to Lake Biwa in the Pliocene and Pleistocene (Kuwashiro, 1959; Yoshida, 1992), and this water system had facilitated the migration of the ancestors of *S. miurai* sp. nov. and *S. libertina* (Miura et al., 2020; Morita et al., 2024). Because the divergence of *S. kurodai*, *S. masudai* sp. nov., and their common ancestor was calculated to be in the late Pleistocene (Morita et al., 2024), the migration of the common ancestor in this water system may also be related to the advancement of the *S. niponica*-group into riverine habitats.

Considerable shell morphological variation was observed within the populations and genetic subgroups of *S. kurodai*. Significant correlations between the overall genetic and shell morphological variations were not detected in the *S. kurodai* populations. In contrast, the relationships between radula morphology and substrates were observed in *S. kurodai* specimens. As is known for other congeners and freshwater molluscs (Bertin

et al., 2012; Rassam et al., 2021; Sawada & Fuke, 2022; Urabe, 1998; Verhaegen et al., 2019), therefore, ontogenetic and local environmental factors may increase morphological variation of *S. kurodai*, making it difficult to identify populations, subgroups, and sister species.

A putative hybrid of *S. miurai* sp. nov. and its congener was collected from Okayama (locality #32). The genetic structures of *S. miurai* sp. nov., Lake Biwa endemics, and *S. libertina* L2 were detected in this specimen by the ADMIXTURE analysis. Given the natural distribution of *S. libertina* L2 in western Japan, including the Okayama Prefecture, this specimen likely resulted from the hybridisation between *S. miurai* sp. nov. and *S. libertina* L2. Historical gene flow between these two species has been estimated (Morita et al., 2024), and they may still maintain their genetic independence with infrequent hybridisation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Data accessibility statement

The raw MIG-seq data generated during this study are available from the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (accession number: PRJDB17676). The summarised morphological data in this study are available in the supplemental materials. Raw morphological data are available from the author upon request.

1009 **Supplemental material**

1010 Supplemental material for this article can be accessed here:

1011

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- 1289

1290 Tables

1291 **Table 1.** Specimen list of *Semisulcospira* species with voucher numbers, collection localities, and DDBJ Sequence Read Archive (DRA)
 1292 accession numbers for the specimens used for genetic analysis. Locality numbers correspond to Fig. 1. Acronym: KUZ, Zoological
 1293 Collection of Kyoto University; NSMT, National Museum of Nature and Science, Japan.

Voucher number	Collection locality (reference)	DRA accession number (reference)
<i>Semisulcospira niponica</i> group		
<i>Semisulcospira kurodai</i>		
Kajiyama & Habe, 1961		
NSMT-Mo39877 (holotype)	Muddy bottom, creek, Hioki, Tambasayama City, Hyogo Prefecture, Japan (locality #22 or #23; Kajiyama & Habe, 1961)	
KUZ Z5225, Z5226	Muddy bottom at depths of 0–1 m, Toba River, Shimotsuchi, Gifu City, Gifu Prefecture, Japan (locality #14)	DRR582760–DRR582763 (this study)
KUZ Z5227, Z5228	Muddy bottom at depths of 0–1 m, Ibi River, Aburajima, Kaizu-cho, Kaizu City, Gifu Prefecture, Japan (locality #15)	DRR582771–DRR582774 (this study)
KUZ Z5229, Z5230	Sandy gravel bottom at depths of 0–1 m, Sasabue River, Saiku, Meiwa-cho, Taki-gun, Mie Prefecture, Japan (locality #17)	DRR582783–DRR582787 (this study)
KUZ Z5231, Z5232	Muddy bottom at depths of 0–1 m, creek, Hassaka-cho, Hikone City, Shiga Prefecture, Japan (locality #18)	DRR582764–DRR582765 (this study)

KUZ Z5233, Z5234	Muddy bottom at depths of 0–1 m, creek, Fukashimizu, Imazu-cho, Takashima City, Shiga Prefecture, Japan (locality #19)	DRR582816–DRR582820 (this study)
KUZ Z5235, Z5236	Piled rock bottom at depths of 0–1 m, Nakayama Pond, Nakayama, Shimoyada-cho, Kameoka City, Kyoto Prefecture, Japan (locality #20)	DRR582775–DRR582778 (this study)
KUZ Z5237, Z5238	Muddy bottom at depths of 0–1 m, Yodo River, Torikainaka, Settsu City, Osaka Prefecture, Japan (locality #21)	DRR582811–DRR582815 (this study)
KUZ Z5239–Z5242	Muddy bottom at depths of 0–1 m, Yura River, Maeda, Fukuchiyama City, Kyoto Prefecture, Japan (locality #23)	DRR582753–DRR582759 (this study)
KUZ Z5243, Z5244	Muddy bottom at depths of 0–1 m, creek, Hioki, Tambasayama City, Hyogo Prefecture, Japan (locality #24)	DRR582802–DRR582804 (this study)
KUZ Z5145, Z5245, Z5246	Muddy bottom at depths of 0–1 m, creek, Hioki, Tambasayama City, Hyogo Prefecture, Japan (locality #25)	DRR537224–DRR537228 (Sawada et al., 2024); DRR582805–DRR582806 (this study)
KUZ Z5247, Z5248	Muddy bottom at depths of 0–1 m, creek, Izumi, Tambasayama City, Hyogo Prefecture, Japan (locality #26)	DRR582807–DRR582810 (this study)
KUZ Z5249, Z5250	Concrete bottom at depths of 0–1 m, Muko River, Miwa, Sanda City, Hyogo Prefecture, Japan (locality #27)	DRR582799–DRR582801 (this study)
KUZ Z5251, Z5252	Piled rock bottom at depths of 0–1 m, Maetani River, Kishi-cho, Ono City, Hyogo Prefecture, Japan (locality #28)	DRR582794–DRR582798 (this study)
KUZ Z5253, Z5254	Piled rock bottom at depths of 0–1 m, Kamo River, Minamiyamada, Himeji City, Hyogo Prefecture, Japan (locality #29)	DRR582766–DRR582770 (this study)
KUZ Z5255–Z5257	Muddy bottom at depths of 0–1 m, creek, Kimitsu, Higashi-ku, Okayama City, Okayama Prefecture, Japan (locality #32)	DRR582789–DRR582793 (this study)

KUZ Z5258	Muddy bottom at depths of 0–1 m, Sasagase River, Fujita, Minami-ku, Okayama City, Okayama Prefecture, Japan (locality #34)	DRR582788 (this study)
KUZ Z5259, Z5260	Concrete bottom at depths of 0–1 m, Takahashi River, Tamashimauwanari, Kurashiki City, Okayama Prefecture, Japan (locality #36)	DRR582779–DRR582782 (this study)
KUZ Z5261, Z5262	Muddy bottom at depths of 0–1 m, creek, Nagata, Nagaïke-cho, Anan City, Tokushima Prefecture, Japan (locality #37)	DRR582748–DRR582752 (this study)
<i>Semisulcospira masudai</i> sp. nov.		
KUZ Z5263 (holotype)	Sandy gravel bottom at depths of 0–1 m, Roppo River, Kamihachiyama, Toyooka City, Hyogo Prefecture, Japan (locality #30)	DRR582821 (this study)
KUZ Z5146, Z5264–Z5267 (paratypes)	Sandy gravel bottom at depths of 0–1 m, Roppo River, Kamihachiyama, Toyooka City, Hyogo Prefecture, Japan (locality #30)	DRR537229 (Sawada et al., 2024); DRR582822 (this study)
KUZ Z5147, Z5268, Z5269	Sandy gravel bottom at depths of 0–1 m, Roppo River, Kamihachiyama, Toyooka City, Hyogo Prefecture, Japan (locality #30)	DRR537230 (Sawada et al., 2024); DRR582823 (this study)
KUZ Z5270, Z5271	Sandy bottom at depths of 0–1 m, Shin River, Honjo, Iwami-cho, Iwami-gun, Tottori Prefecture, Japan (locality #31)	DRR582824–DRR582828 (this study)
<i>Semisulcospira praecursa</i> sp. nov.		
KUZ Z5272 (holotype)	Piled rock bottom at depths of 0–1 m, Ichiunsai River in Hiramatsu, Iwata City, Shizuoka Prefecture, Japan (locality #1)	DRR582831 (this study)
KUZ Z5273–Z5277 (paratypes)	Piled rock bottom at depths of 0–1 m, Ichiunsai River in Hiramatsu, Iwata City, Shizuoka Prefecture, Japan (locality #1)	DRR582829, DRR582833 (this study)
KUZ Z5278, Z5279	Piled rock bottom at depths of 0–1 m, Ichiunsai River in Hiramatsu, Iwata City, Shizuoka Prefecture, Japan (locality #1)	DRR582830, DRR582832 (this study)

KUZ Z5280, Z5281	Sandy gravel bottom at depths of 0–1 m, Miyakoda River, Nakagawa, Hosoe-cho, Hamana-ku, Hamamatsu City, Shizuoka Prefecture, Japan (locality #2)	DRR582834–DRR582837 (this study)
KUZ Z5282, Z5283	Concrete bottom at depths of 0–1 m, Toyo River, Ishiki, Togo-cho, Toyokawa City, Aichi Prefecture, Japan (locality #5)	DRR582838–DRR582841 (this study)
KUZ Z5284	Sandy gravel bottom at depths of 0–1 m, Yahagi River, Fuso-cho, Toyota City, Aichi Prefecture, Japan (locality #7)	DRR582842–DRR582843 (this study)
<i>Semisulcospira niponica</i> (Smith, 1876)		
KUZ Z4093	Concrete brock bottom at depths of 0–1 m, Lake Biwa, Nagahama Port, Nagahama City, Shiga Prefecture, Japan	DRR360330–DRR360334 (Sawada & Fuke, 2023)
<i>Semisulcospira reticulata</i> Kajiya & 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	DRR502630–DRR502631 (Sawada et al., 2024)
<i>Semisulcospira habei</i> Davis, 1969		
KUZ Z4224	Piled rock bottom at depths of 0–0.5 m, Uji River, Oshima, Uji City, Kyoto Prefecture, Japan	DRR398607–DRR398609 (Sawada & Fuke, 2022)
<i>Semisulcospira rugosa</i> Watanabe & Nishino, 1995		
KUZ Z4228	Piled rock bottom at depths of 0–1 m, Lake Biwa, Kitafunaki, Takashima City, Shiga Prefecture, Japan	DRR398496–DRR398599 (Sawada & Fuke, 2022)

Semisulcospira fuscata

Watanabe & Nishino, 1995

KUZ Z4105	Piled rock bottom at depths of 0–1 m, Lake Biwa, Oura, Nagahama City, Shiga Prefecture, Japan	DRR360364–DRR360366 (Sawada & Fuke, 2023)
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Semisulcospira nakasekoe

group

Semisulcospira miurai sp. nov.

KUZ Z5285 (holotype)	Muddy bottom at depths of 0–1 m, Sasagase River in Fujita, Minami-ku, Okayama City, Okayama Prefecture, Japan (locality #34)	DRR582847 (this study)
KUZ Z5286–Z5290 (paratypes)	Muddy bottom at depths of 0–1 m, Sasagase River in Fujita, Minami-ku, Okayama City, Okayama Prefecture, Japan (locality #34)	DRR582848, DRR582849, DRR582851 (this study)
KUZ Z5291, Z5292	Muddy bottom at depths of 0–1 m, Sasagase River in Fujita, Minami-ku, Okayama City, Okayama Prefecture, Japan (locality #34)	DRR582850, DRR582852, DRR582853 (this study)
KUZ Z5293–Z5295	Muddy bottom at depths of 0–1 m, creek, Kimitsu, Higashi-ku, Okayama City, Okayama Prefecture, Japan (locality #32)	DRR582854–DRR582857 (this study)
KUZ Z5296, Z5297	Sandy gravel bottom at depths of 0–1 m, Asahi River, Nakashima, Naka-ku, Okayama City, Okayama Prefecture, Japan (locality #33)	DRR582858–DRR582861 (this study)
KUZ Z5298, Z5299	Muddy bottom at depths of 0–1 m, Ashimori River, Nakanatsugawa, Kita-ku, Okayama City, Okayama Prefecture, Japan (locality #35)	DRR582862–DRR582865 (this study)
KUZ Z5300, Z5301	Concrete bottom at depths of 0–1 m, Takahashi River, Tamashimauwanari, Kurashiki City, Okayama Prefecture, Japan (locality #36)	DRR582844–DRR582846 (this study)

Semisulcospira nakasekoe

(Kuroda, 1929)

KUZ Z4243	Piled rock bottom at depths of 0–1 m, Uji River, Ujiotsukata, Uji City, Kyoto Prefecture, Japan	DRR398612–DRR398614 (Sawada & Fuke, 2022)
<i>Semisulcospira morii</i> Watanabe, 1984		
KUZ Z4255, Z4892	Rocky bottom at depths of 0–3 m, Lake Biwa, Chikubu-shima Island, Nagahama City, Shiga Prefecture, Japan	DRR642740, DRR642742, DRR642744 (Sawada, 2024)
<i>Semisulcospira arenicola</i> Watanabe & Nishino, 1995		
KUZ Z4235	Sandy bottom at depths of 0–1 m, Lake Biwa, Satsuma-cho, Hikone City, Shiga Prefecture, Japan	DRR398590, DRR398592, DRR398593 (Sawada & Fuke, 2022)
<i>Semisulcospira takeshimensis</i> Watanabe & Nishino, 1995		
KUZ Z4257, Z4904	Rocky bottom at depths of 0–3 m, Lake Biwa, Take-shima Island, Hikone City, Shiga Prefecture, Japan	DRR641404–DRR641407 (Sawada, 2024)
<i>Semisulcospira davisi</i> Sawada & Nakano, 2021		
KUZ Z3681, Z3682	Sandy mud bottom at depths of 3–7 m, Lake Biwa, Tsukide, Nishiazai-cho, Nagahama City, Shiga Prefecture, Japan	DRR641409–DRR641411 (Sawada, 2024)
<i>Semisulcospira libertina</i> L1 sensu Miura et al. (2020)		
KUZ Z5302	Piled rock bottom at depths of 0–1 m, creek, Nagata, Shizuka-machi, Yokote City, Akita Prefecture, Japan	DRR582866–DRR582868 (this study)

Semisulcospira libertina L2

sensu Miura et al. (2020)

KUZ Z5303

Piled rock bottom at depths of 0–1 m, Asanishin River, Nazeasanishin-machi, Amami City, Kagoshima Prefecture, Japan

DRR582869–DRR582871 (this study)

Semisulcospira libertina L4

sensu Miura et al. (2020)

KUZ Z5304

Sandy bottom at depths of 0–1 m, creek, Kogochi, Shimizu-ku, Shizuoka City, Shizuoka Prefecture, Japan

DRR582872–DRR582876 (this study)

Putative hybrid between *S.*

miurai sp. nov. and congener

KUZ Z5305

Muddy bottom at depths of 0–1 m, creek, Kimitsu, Higashi-ku, Okayama City, Okayama Prefecture, Japan (locality #32)

DRR582877 (this study)

1294

1295

1296 Table 2. Results of tests of sex differences in body whorl length (BWL) and principal component 1 (PC1) scores in four *Semisulcospira*
 1297 species. Asterisks indicate *p*-values smaller than 0.05.

Species	Test	<i>W</i> value	<i>p</i> -value
<i>S. kurodai</i>	Difference in BWL	7291	< 0.001*
	Difference in PC1 scores	6698	< 0.001*
<i>S. masudai</i> sp. nov.	Difference in BWL	314	0.001*
	Difference in PC1 scores	231	0.352
<i>S. praecursa</i> sp. nov.	Difference in BWL	793	< 0.001*
	Difference in PC1 scores	506	0.545
<i>S. miurai</i> sp. nov.	Difference in BWL	1439	< 0.001*
	Difference in PC1 scores	717	0.096

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1299

1300 **Table 3.** Morphometric characters of female specimens of the four *Semisulcospira* species examined in the present study. Measurements
1301 and counts: minimum–maximum value (mean $\pm$ SD).

Character	<i>S. kurodai</i>	<i>S. masudai</i> sp. nov.	<i>S. praecursa</i> sp. nov.	<i>S. miurai</i> sp. nov.
Aperture slenderness ratio (ASR)	1.42–1.94 (1.7 $\pm$ 0.1)	1.51–1.94 (1.8 $\pm$ 0.1)	1.65–2.11 (1.8 $\pm$ 0.1)	1.34–1.87 (1.7 $\pm$ 0.1)
Basal cord number (BCN)	4–9 (5.6 $\pm$ 0.9)	5–8 (6.2 $\pm$ 0.9)	5–9 (6.8 $\pm$ 1)	4–8 (5.7 $\pm$ 0.8)
Body whorl length (BWL) (mm)	12.8–24.8 (18 $\pm$ 2.2)	15.3–21.3 (17.5 $\pm$ 1.2)	18.2–27.2 (20.8 $\pm$ 1.7)	13.4–20.1 (16.8 $\pm$ 1.7)
Longitudinal rib number of penultimate whorl (RN)	14–21 (17.0 $\pm$ 2.0)	13–19 (16.5 $\pm$ 1.8)	18–22 (20.7 $\pm$ 2.3)	19
Spire angle (SA) (degrees)	13.4–26.1 (18.3 $\pm$ 2.4)	14.2–26.5 (17.9 $\pm$ 2.6)	14.7–24.6 (18.1 $\pm$ 2.5)	10.9–29.7 (18.8 $\pm$ 3.8)
Spiral cord number of penultimate whorl (SCN)	7–12 (9.3 $\pm$ 1.2)	8–11 (9.6 $\pm$ 0.9)	8–13 (9.6 $\pm$ 1.2)	8–13 (11.0 $\pm$ 1.2)
Whorl elongation ratio (WER)	2.66–3.84 (3.1 $\pm$ 0.3)	2.58–3.94 (3.3 $\pm$ 0.3)	2.71–3.89 (3.3 $\pm$ 0.3)	2.64–4.34 (3.2 $\pm$ 0.3)
Whorl number (WN)	3.00–10.25 (5.3 $\pm$ 1.0)	4.50–6.50 (5.2 $\pm$ 0.5)	3.00–6.00 (4.6 $\pm$ 0.6)	4.00–8.25 (5.3 $\pm$ 1.0)
Sculpture Type (granulated rib / smooth rib / spiral cord / smooth) (%)	38 / 4 / 54 / 5	70 / 17 / 10 / 3	36 / 2 / 55 / 7	4 / 16 / 71 / 9
Frequency of longitudinal rib on penultimate whorl (%)	18.1	50.0	6.8	1.9
Number of protoconchs (PN)	6–258 (63.3 $\pm$ 47.4)	15–65 (32.4 $\pm$ 14.9)	19–234 (105.3 $\pm$ 44.5)	3–64 (18.9 $\pm$ 10.2)

Longitudinal rib number on body whorl of the largest protoconch (RNP)	10–19 (13.8 ± 2.2)	13–17 (14.7 ± 1.3)	12–21 (15.3 ± 2.1)	10–15 (11.3 ± 1.1)
Shell length of the largest protoconch (SLP) (mm)	1.1–3.2 (2.3 ± 0.4)	1.9–2.8 (2.4 ± 0.3)	1.9–3.3 (2.5 ± 0.3)	1.3–3.4 (2.5 ± 0.6)
Whorl number of the largest protoconch (WNP)	2.00–4.25 (3.3 ± 0.4)	3.00–4.25 (3.6 ± 0.3)	2.75–4.25 (3.5 ± 0.4)	2.75–4.25 (3.3 ± 0.3)
Number of nodes on body whorl of the largest protoconch (Node Number) (0 / 1 / 2 / 3) (%)	0 / 74 / 17 / 9	0 / 58 / 42 / 0	0 / 70 / 27 / 3	55 / 23 / 9 / 14
Keel type on body whorl of the largest protoconch (Keel Type) (absent / weak / prominent) (%)	0 / 44 / 56	0 / 0 / 100	0 / 3 / 97	0 / 77 / 23
Typical shape of the largest cusp of rachidian tooth	narrow or wide, pointed or rounded	narrow, pointed	wide, rounded	wide, rounded
Typical shape of the largest cusp of lateral tooth	narrow or wide, pointed or rounded	narrow, pointed	wide, rounded	wide, rounded

Figure legends

Fig. 1. Map of central Japan representing 37 localities where *Semisulcospira* snails were investigated. Closed circles, collection localities of *S. kurodai* and/or new species; open circles, localities where *S. kurodai* and/or new species were not found. Symbol colours indicate *S. kurodai* (blue), *S. masudai* sp. nov. (green), *S. praecursor* (orange), and *S. miurai* sp. nov. (red). Symbol shapes of *S. kurodai* show genetic subgroups within the species. The detailed localities are shown in Table 1. This figure was drawn based on data from the Geospatial Information Authority of Japan (2024), Jarvis et al. (2008), and Ministry of Land, Infrastructure, Transport and Tourism of Japan (2020).

Fig. 2. Neighbor-net of *Semisulcospira niponica* group species reconstructed based on uncorrected *p*-distances of 10,006 SNPs. Sample names indicate locality names (numbers) or scientific names.

Fig. 3. Results of the ADMIXTURE analysis based on 2,217 SNPs conducted for *Semisulcospira niponica* group species. The numbers and abbreviations under the bars indicate locality numbers or scientific names: FU, *S. fuscata*; HA, *S. habei*; NI, *S. niponica*; RE, *S. reticulata*; RU, *S. rugosa*.

1321

1322 **Fig. 4.** Neighbor-net of *Semisulcospira nakasekoe* group species, *S. libertina*, and a
1323 putative hybrid reconstructed based on uncorrected *p*-distances of 7,280 SNPs. Sample
1324 names indicate locality names (locality numbers) or scientific names.

1325

1326 **Fig. 5.** Results of ADMIXTURE analysis based on 1,462 SNPs conducted for the
1327 *Semisulcospira nakasekoe* group species, *S. libertina*, and a putative hybrid. The
1328 numbers and abbreviations under the bars indicate locality numbers or scientific names:
1329 AR, *S. arenicola*; DA, *S. davisi*; MO, *S. morii*; NA, *S. nakasekoe*; TA, *S.*
1330 *takeshimensis*.

1331

1332 **Fig. 6.** Shells and opercula of *Semisulcospira kurodai*. **A–D**, Holotype collected from
1333 Tambasasayama (locality #24 or #25), NSMT-Mo39877; **E–I**, Gifu (#14), KUZ Z5225,
1334 Z5226; **J–N**, Kaizu (#15), KUZ Z5227, Z5228; **O–S**, Meiwa (#17), KUZ Z5229,
1335 Z5230; **T–X**, Hikone (#18), KUZ Z5231, Z5232; **Y–AC**, Takashima (#19), KUZ
1336 Z5233, Z5234; **AD–AH**, Kameoka (#20), KUZ Z5235, Z5236; **AI–AM**, Settsu (#21),
1337 KUZ Z5237, Z5238; **AN–AR**, Fukuchiyama (#23), KUZ Z5240, Z5241; **AS–AW**,
1338 Tambasasayama (#24), KUZ Z5243, Z5244; **AX–BB**, Tambasasayama (#25), KUZ

1339 Z5145, Z5246; **BC–BG**, Tambasasayama (#26), KUZ Z5247, Z5248; **BH–BL**, Sanda
 1340 (#27), KUZ Z5249, Z5250; **BM–BQ**, Ono (#28), KUZ Z5251, Z5252; **BR–BV**, Himeji
 1341 (#29), KUZ Z5253, Z5254; **BW–BZ**, Okayama (#32), KUZ Z5255, Z5257; **CA–CC**,
 1342 Okayama (#34), KUZ Z5258; **CD–CH**, Okayama (#36), KUZ Z5259, Z5260; **CI–CM**,
 1343 Anan (#37), KUZ Z5261, Z5262. Scale bars: 10 mm (1), female teleoconch (A–C, E, J,
 1344 O, T, Y, AD, AI, AN, AS, AX, BC, BH, BM, BR, BW, CA, CD, CI), male teleoconch
 1345 (H, M, R, W, AB, AG, AL, AQ, AV, BA, BF, BK, BP, BU, BZ, CG, CL), operculum
 1346 (D, F, I, K, N, P, S, U, X, Z, AC, AE, AH, AJ, AM, AO, AR, AT, AW, AY, BB, BD,
 1347 BG, BI, BL, BN, BQ, BS, BV, BX, CB, CE, CH, CJ, CM); 1 mm (2), protoconch (G, L,
 1348 Q, V, AA, AF, AK, AP, AU, AZ, BE, BJ, BO, BT, CC, CF, CK).

1349

1350 **Fig. 7.** Shells and opercula of *Semisulcospira masudai* sp. nov. **A–G**, Holotype from
 1351 Toyooka (locality #30), KUZ Z5263; **H–O**, Paratypes from Toyooka, KUZ Z5146,
 1352 Z5165, Z5166; **P–T**, Iwami (#31), KUZ Z5270, Z5271. Scale bars: 10 mm (1), female
 1353 teleoconch (A–C, H, K, P), male teleoconch (N, S), operculum (D, I, L, O, Q, T); 1 mm
 1354 (2), protoconch (E–G, J, M, R).

1355

1356 **Fig. 8.** Shells and opercula of *Semisulcospira praecursa* sp. nov. **A–G**, Holotype from
1357 Iwata (locality #1), KUZ Z5272; **H–O**, Paratypes from Iwata, KUZ Z5273, Z5274,
1358 Z5277; **P–T**, Hamamatsu (#2), KUZ Z5280, Z5281; **U–Y**, Toyokawa (#5), KUZ Z5282,
1359 Z5283; **Z–AA**, Toyota (#7), KUZ Z5284. Scale bars: 10 mm (**1**), female teleoconch (A–
1360 C, H, K, P, U, Z), male teleoconch (N, S, X), operculum (D, I, L, O, Q, T, V, Y, AA); 1
1361 mm (2), protoconch (E–G, J, M, R, W).

1362

1363 **Fig. 9.** Shells and opercula of *Semisulcospira miurai* sp. nov. (**A–AI**), and a putative
1364 hybrid (**AJ–AL**). **A–G**, Holotype from Okayama (locality #34), KUZ Z5285; **H–O**,
1365 Paratypes from Okayama (#34), KUZ Z5286, Z5287, Z5289; **P–T**, **AJ–AL**, Okayama
1366 (#32), KUZ Z5293, Z5295, Z5305; **U–Y**, Okayama (#33), KUZ Z5296, Z5297; **Z–AD**,
1367 Okayama (#35), KUZ Z5298, Z5299; **AE–AI**, Kurashiki (#36), KUZ Z5300, Z5301.
1368 Scale bars: 10 mm (**1**), female teleoconch (A–C, H, K, P, U, Z, AE, AJ), male
1369 teleoconch (N, S, X, AC, AH), operculum (D, I, L, O, Q, T, V, Y, AA, AD, AF, AI,
1370 AK); 1 mm (2), protoconch (E–G, J, M, R, W, AB, AG, AL).

1371

1372 **Fig. 10.** Radulae of *Semisulcospira kurodai*. **A**, Gifu (#14), KUZ Z5225; **B**, Kaizu
1373 (#15), KUZ Z5227; **C**, Meiwa (#17), KUZ Z5229; **D**, Hikone (#18), KUZ Z5231; **E**,

1374 Takashima (#19), KUZ Z5233; **F**, Kameoka (#20), KUZ Z5235; **G**, Settsu (#21), KUZ
 1375 Z5237; **H**, Fukuchiyama (#23), KUZ Z5240; **I**, Tambasasayama (#24), KUZ Z5243; **J**,
 1376 Tambasasayama (#26), KUZ Z5247; **K**, Sanda (#27), KUZ Z5249; **L**, Ono (#28), KUZ
 1377 Z5251; **M**, Himeji (#29), KUZ Z5253; **N**, Okayama (#32), KUZ Z5255; **O**, Okayama
 1378 (#34), KUZ Z5258. Scale bars: 100 μ m.

1379

1380 **Fig. 11.** Radulae of *Semisulcospira kurodai* (**A**, **B**), *S. masudai* sp. nov. (**C–E**), *S.*
 1381 *praecursa* sp. nov. (**F–I**), and *S. miurai* sp. nov. (**J–O**). **A**, Okayama (#36), KUZ
 1382 Z5259; **B**, Anan (#37), KUZ Z5261; **C**, Holotype from Toyooka (locality #30), KUZ
 1383 Z5263; **D**, Paratype from Toyooka , KUZ Z5146; **E**, Iwami (#31), KUZ Z5270; **F**,
 1384 Holotype from Iwata (locality #1), KUZ Z5272; **G**, Paratype from Iwata , KUZ Z5274;
 1385 **H**, Hamamatsu (#2), KUZ Z5280; **I**, Toyokawa (#5), KUZ Z5282; **J**, Holotype from
 1386 Okayama (locality #34), KUZ Z5285; **K**, Paratype from Okayama (#34), KUZ Z5288;
 1387 **L**, Okayama (#32), KUZ Z5293; **M**, Okayama (#33), KUZ Z5296; **N**, Okayama (#35),
 1388 KUZ Z5298; **O**, Kurashiki (#36), KUZ Z5300. Scale bars: 100 μ m.

1389

1390 **Fig. 12.** Results of the principal component analysis conducted for *Semisulcospira*
 1391 *kurodai* based on 1,922 SNPs (**A**) and a lineage tree of *S. kurodai*, *S. masudai* sp. nov.,

and Lake Biwa endemics of the *S. niponica*-group species calculated based on 6,915 SNPs by SVDquartets (**B**). The numbers associated with the nodes represent bootstrap values. Legends indicate locality names (locality numbers).

Fig. 13. Schematic drawings indicating generalised features of reproductive organs of *Semisulcospira kurodai* collected from its type locality (locality #24). **A**, Female; **B**, Male. Abbreviations: bp, brood pouch; eg, egg; em, embryo; op, opening of deep groove of prostate; ovi, oviduct; pr, prostate; rcs, seminal receptacle; sg, sperm gutter; spb, spermatophore bursa; vd, vas deferens. Scale bars: 5 mm.

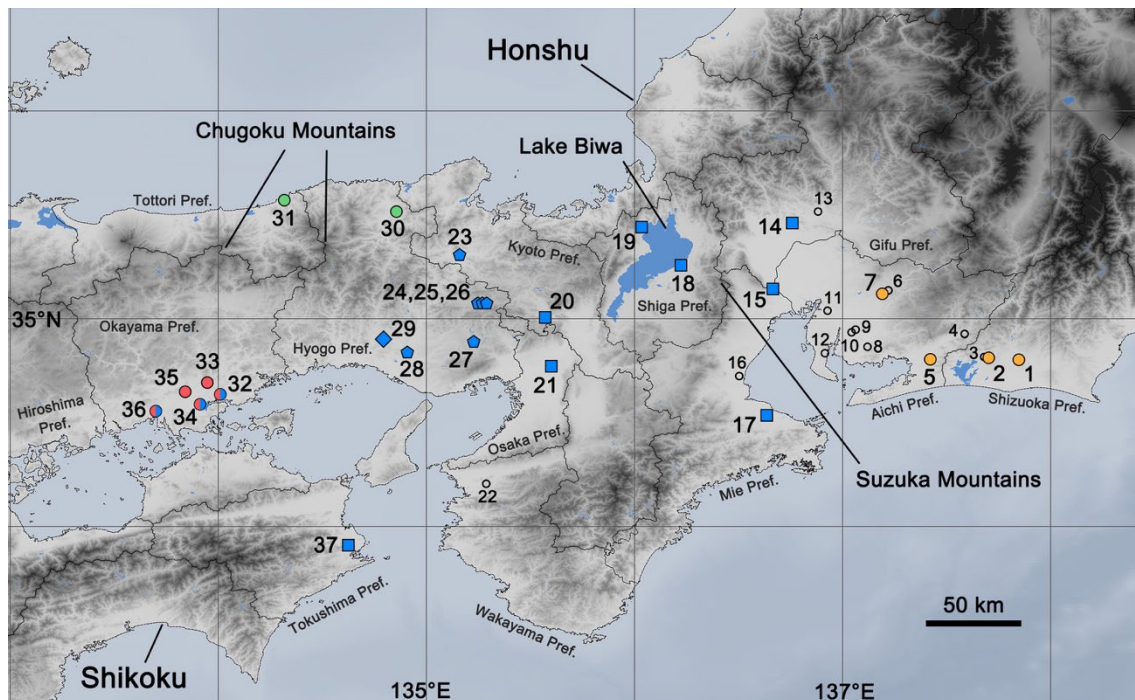


Fig. 1. Map of central Japan representing 37 localities where *Semisulcospira* snails were investigated. Closed circles, collection localities of *S. kurodai* and/or new species; open circles, localities where *S. kurodai* and/or new species were not found. Symbol colours indicate *S. kurodai* (blue), *S. masudai* sp. nov. (green), *S. praecursa* (orange), and *S. miurai* sp. nov. (red). Symbol shapes of *S. kurodai* show genetic subgroups within the species. The detailed localities are shown in Table 1. This figure was drawn based on data from the Geospatial Information Authority of Japan (2024), Jarvis et al. (2008), and Ministry of Land, Infrastructure, Transport and Tourism of Japan (2020).

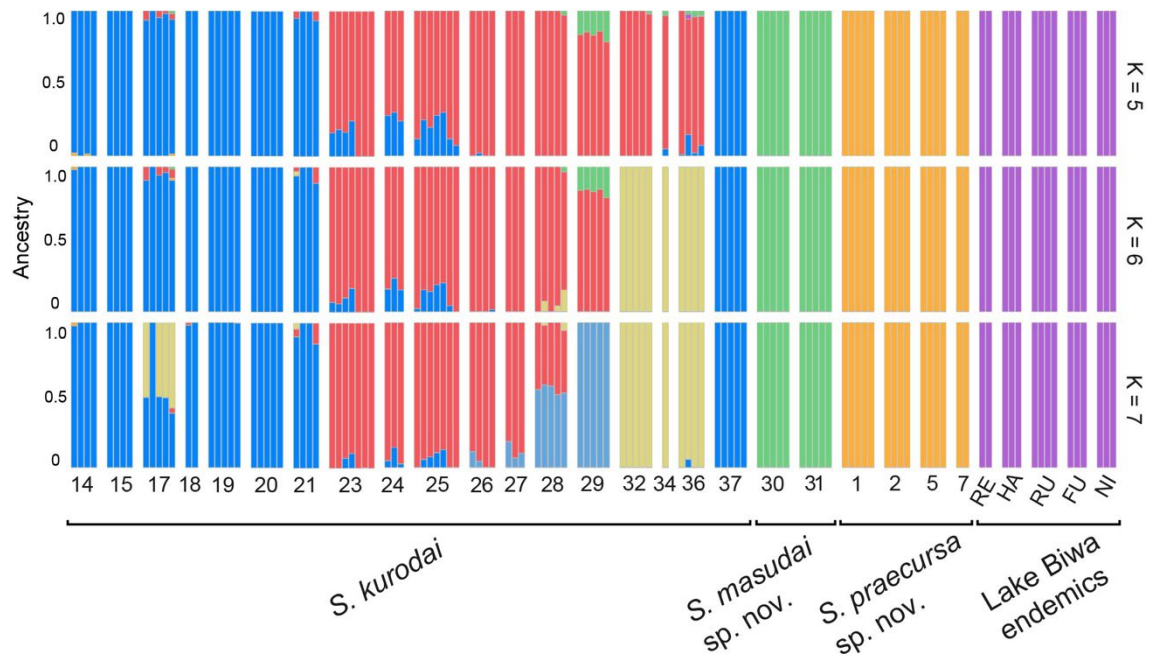


Fig. 3. Results of the ADMIXTURE analysis based on 2,217 SNPs conducted for *Semisulcospira niponica* group species. The numbers and abbreviations under the bars indicate locality numbers or scientific names: FU, *S. fuscata*; HA, *S. habei*; NI, *S. niponica*; RE, *S. reticulata*; RU, *S. rugosa*.

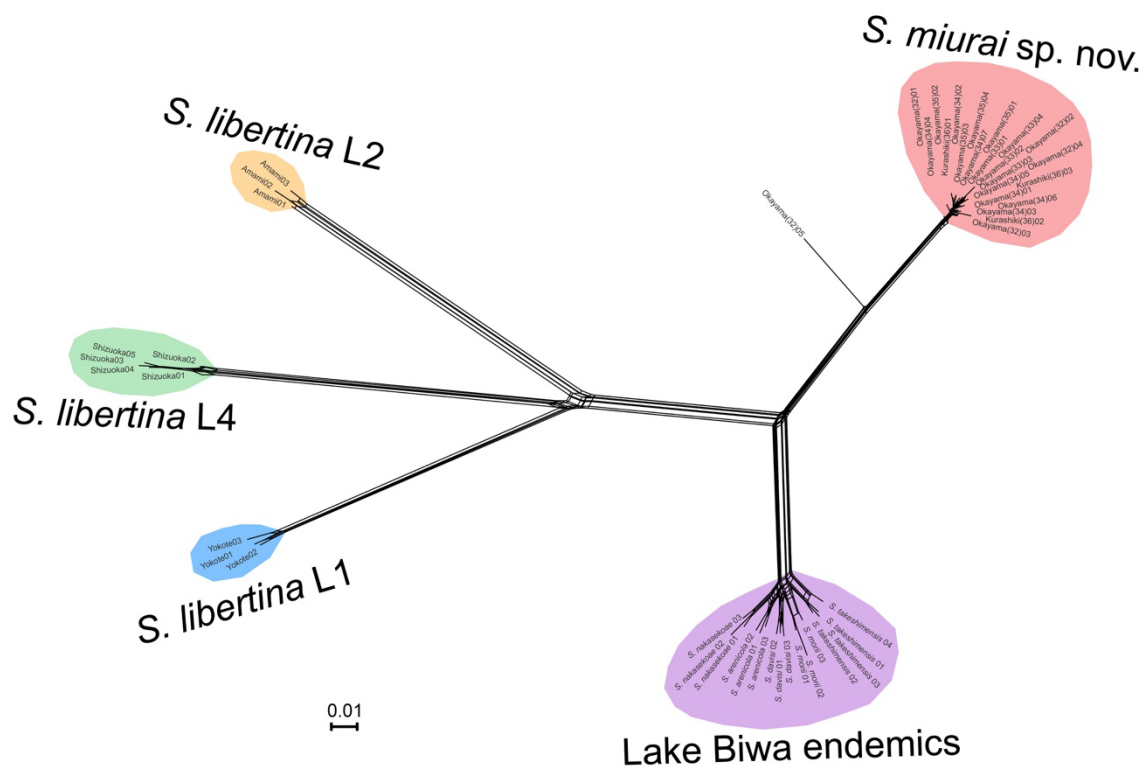


Fig. 4. Neighbor-net of *Semisulcospira nakasekoe* group species, *S. libertina*, and a putative hybrid reconstructed based on uncorrected p -distances of 7,280 SNPs. Sample names indicate locality names (locality numbers) or scientific names.

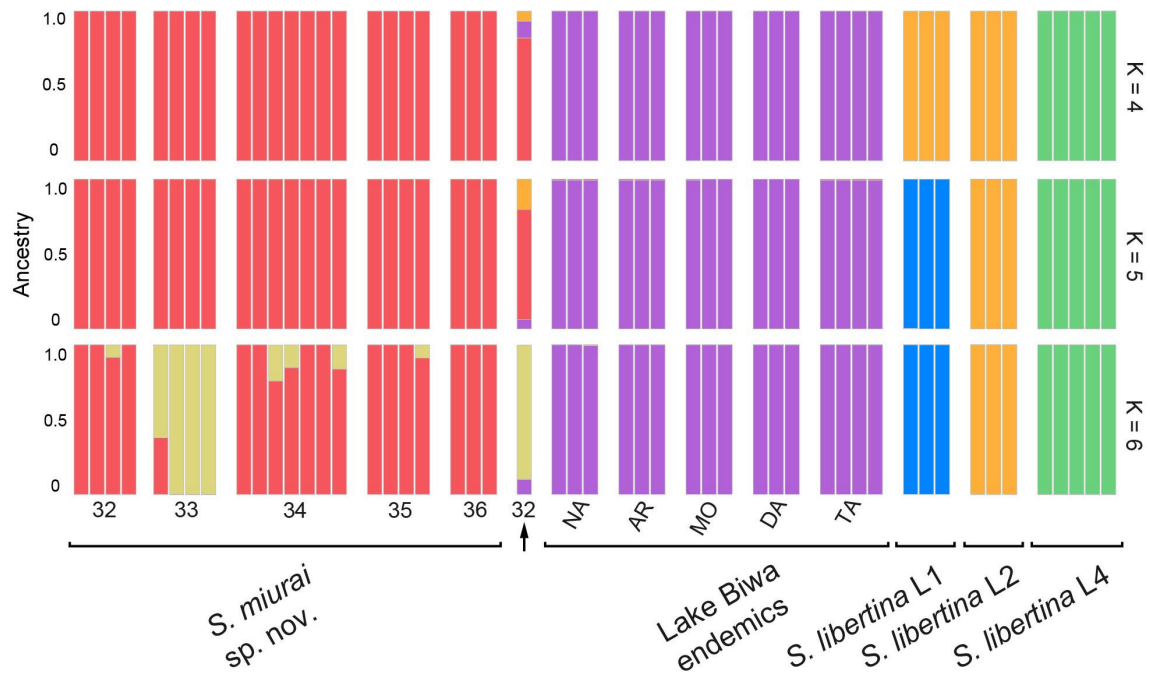


Fig. 5. Results of ADMIXTURE analysis based on 1,462 SNPs conducted for the *Semisulcospira nakasekoe* group species, *S. libertina*, and a putative hybrid. The numbers and abbreviations under the bars indicate locality numbers or scientific names: AR, *S. arenicola*; DA, *S. davisi*; MO, *S. morii*; NA, *S. nakasekoe*; TA, *S. takeshimensis*.

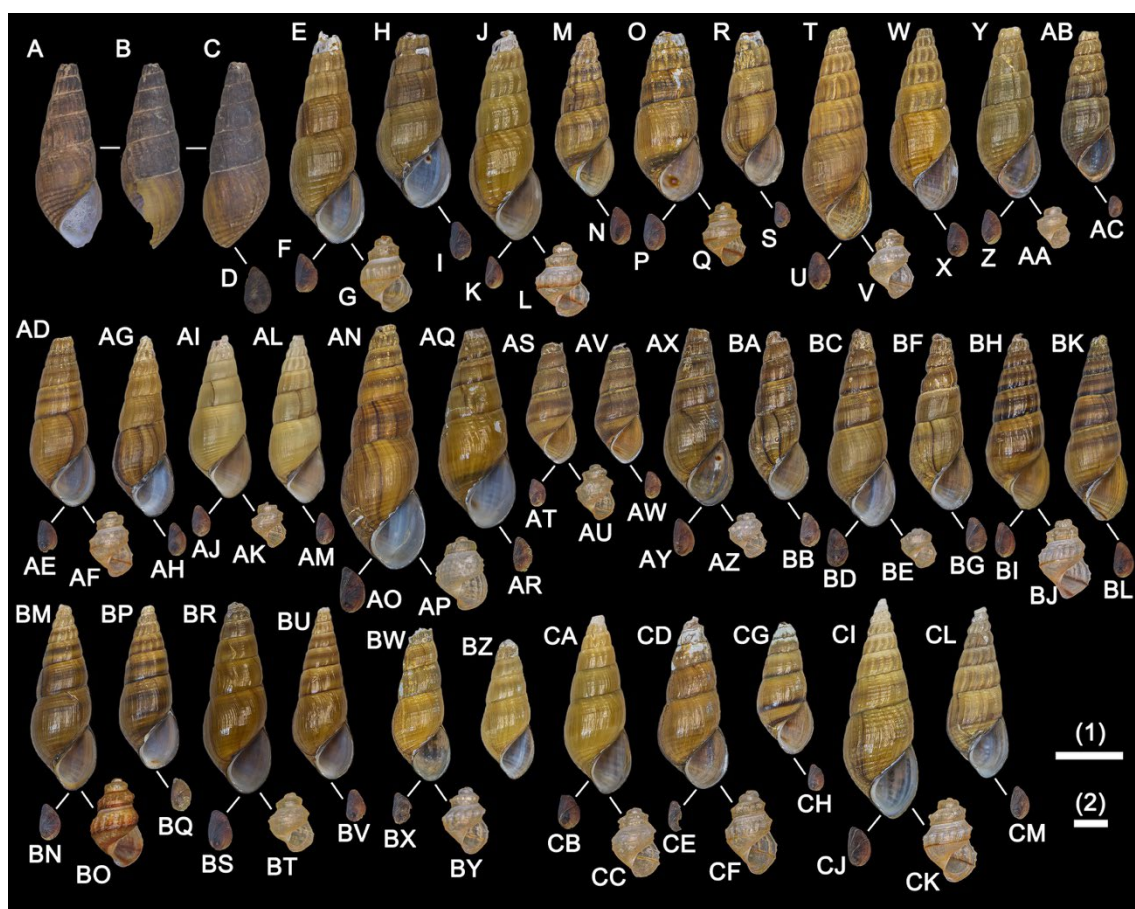


Fig. 6. Shells and opercula of *Semisulcospira kurodai*. **A–D**, Holotype collected from Tambasasayama (locality #24 or #25), NSMT-Mo39877; **E–I**, Gifu (#14), KUZ Z5225, Z5226; **J–N**, Kaizu (#15), KUZ Z5227, Z5228; **O–S**, Meiwa (#17), KUZ Z5229, Z5230; **T–X**, Hikone (#18), KUZ Z5231, Z5232; **Y–AC**, Takashima (#19), KUZ Z5233, Z5234; **AD–AH**, Kameoka (#20), KUZ Z5235, Z5236; **AI–AM**, Settsu (#21), KUZ Z5237, Z5238; **AN–AR**, Fukuchiyama (#23), KUZ Z5240, Z5241; **AS–AW**, Tambasasayama (#24), KUZ Z5243, Z5244; **AX–BB**, Tambasasayama (#25), KUZ Z5145, Z5246; **BC–BG**, Tambasasayama (#26), KUZ Z5247, Z5248; **BH–BL**, Sanda (#27), KUZ Z5249, Z5250; **BM–BQ**, Ono (#28), KUZ Z5251, Z5252; **BR–BV**, Himeji (#29), KUZ Z5253, Z5254; **BW–BZ**, Okayama (#32), KUZ Z5255, Z5257; **CA–CC**, Okayama (#34), KUZ Z5258; **CD–CH**, Okayama (#36), KUZ Z5259, Z5260; **CI–CM**, Anan (#37), KUZ Z5261, Z5262. Scale bars: 10 mm (1), female teleoconch (A–C, E, J, O, T, Y, AD, AI, AN, AS, AX, BC, BH, BM, BR, BW, CA, CD, CI), male teleoconch (H, M, R, W, AB, AG, AL, AQ, AV, BA, BF, BK, BP, BU, BZ, CG, CL), operculum (D, F, I, K, N, P, S, U, X, Z, AC, AE, AH, AJ, AM, AO, AR, AT, AW, AY, BB, BD, BG, BI, BL, BN, BQ, BS, BV, BX, CB, CE, CH, CJ, CM); 1 mm (2), protoconch (G, L, Q, V, AA, AF, AK, AP, AU, AZ, BE, BJ, BO, BT, CC, CF, CK).

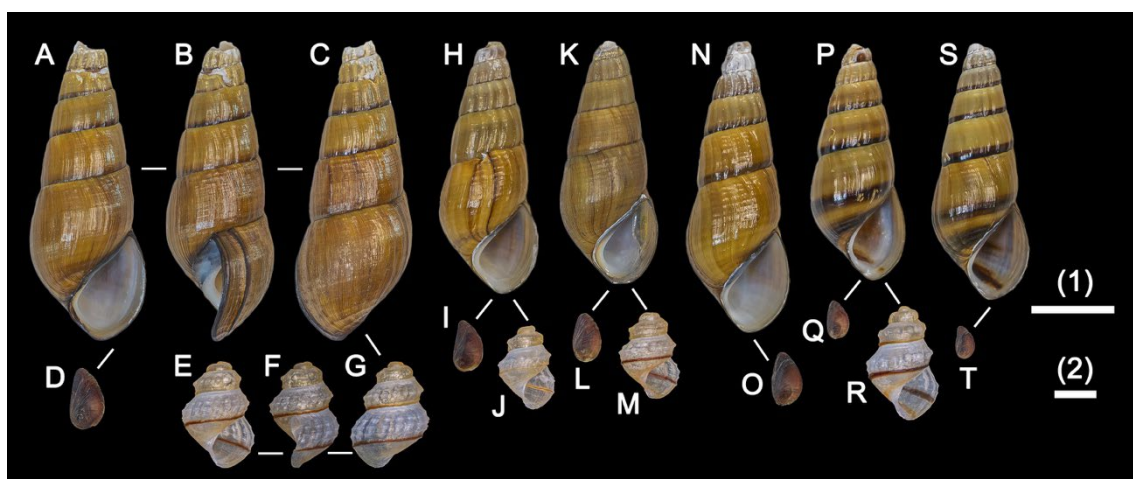


Fig. 7. Shells and opercula of *Semisulcospira masudai* sp. nov. **A–G**, Holotype from Toyooka (locality #30), KUZ Z5263; **H–O**, Paratypes from Toyooka, KUZ Z5146, Z5165, Z5166; **P–T**, Iwami (#31), KUZ Z5270, Z5271. Scale bars: 10 mm (**1**), female teleoconch (A–C, H, K, P), male teleoconch (N, S), operculum (D, I, L, O, Q, T); 1 mm (**2**), protoconch (E–G, J, M, R).

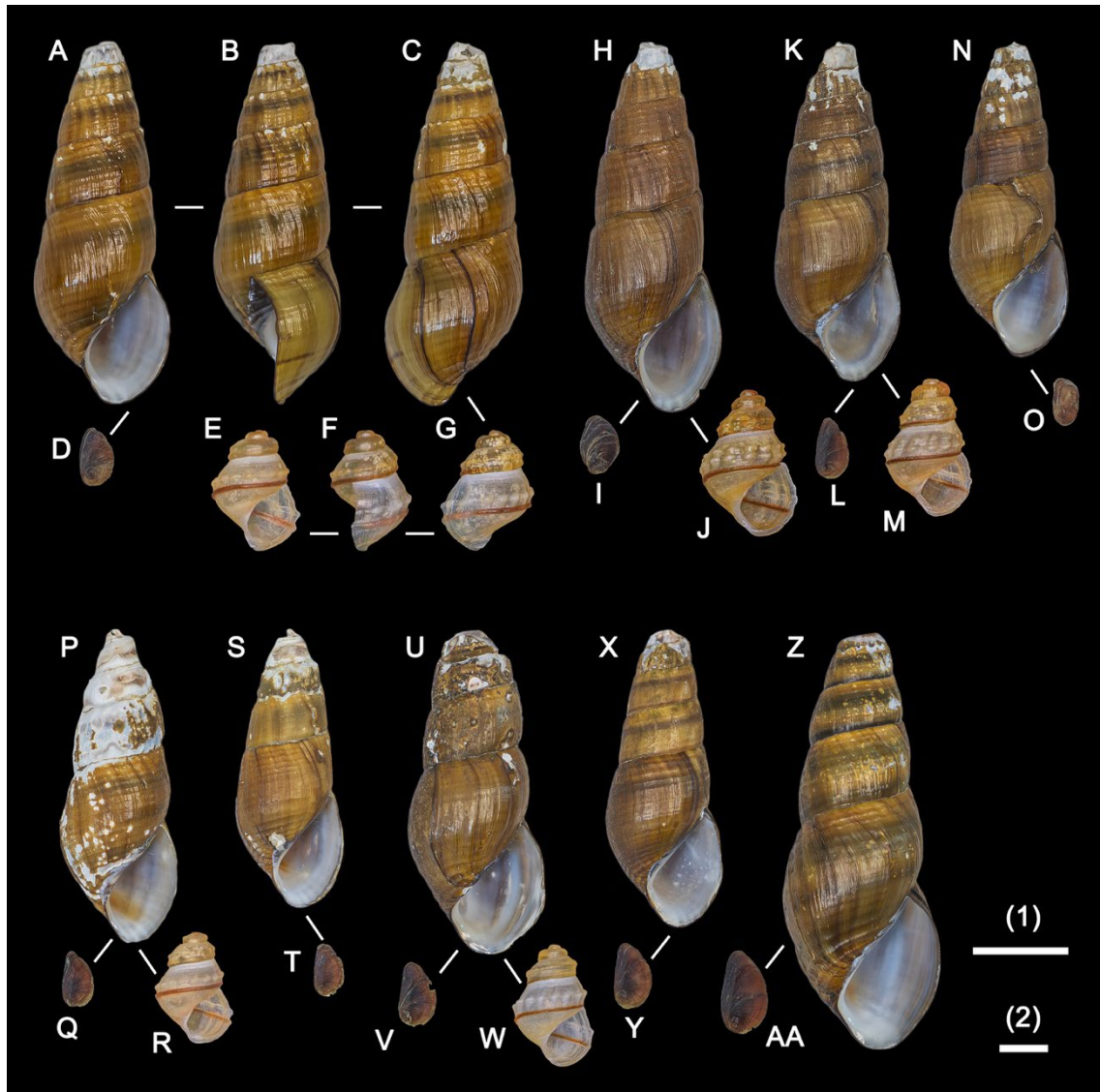


Fig. 8. Shells and opercula of *Semisulcospira praecursa* sp. nov. **A–G**, Holotype from Iwata (locality #1), KUZ Z5272; **H–O**, Paratypes from Iwata, KUZ Z5273, Z5274, Z5277; **P–T**, Hamamatsu (#2), KUZ Z5280, Z5281; **U–Y**, Toyokawa (#5), KUZ Z5282, Z5283; **Z–AA**, Toyota (#7), KUZ Z5284. Scale bars: 10 mm (1), female teleoconch (A–C, H, K, P, U, Z), male teleoconch (N, S, X), operculum (D, I, L, O, Q, T, V, Y, AA); 1 mm (2), protoconch (E–G, J, M, R, W).

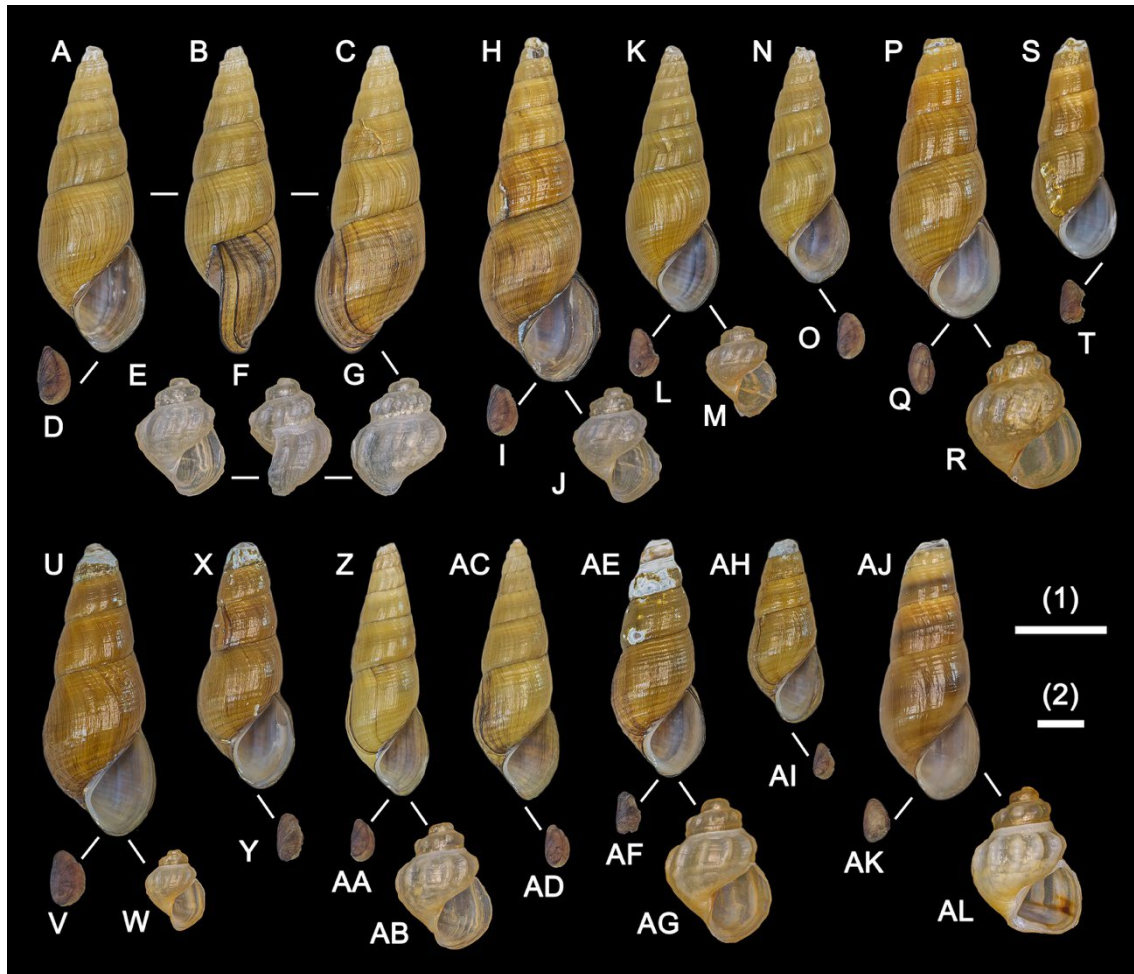


Fig. 9. Shells and opercula of *Semisulcospira miurai* sp. nov. (A–AI), and a putative hybrid (AJ–AL). A–G, Holotype from Okayama (locality #34), KUZ Z5285; H–O, Paratypes from Okayama (#34), KUZ Z5286, Z5287, Z5289; P–T, AJ–AL, Okayama (#32), KUZ Z5293, Z5295, Z5305; U–Y, Okayama (#33), KUZ Z5296, Z5297; Z–AD, Okayama (#35), KUZ Z5298, Z5299; AE–AI, Kurashiki (#36), KUZ Z5300, Z5301. Scale bars: 10 mm (1), female teleoconch (A–C, H, K, P, U, Z, AE, AJ), male teleoconch (N, S, X, AC, AH), operculum (D, I, L, O, Q, T, V, Y, AA, AD, AF, AI, AK); 1 mm (2), protoconch (E–G, J, M, R, W, AB, AG, AL).

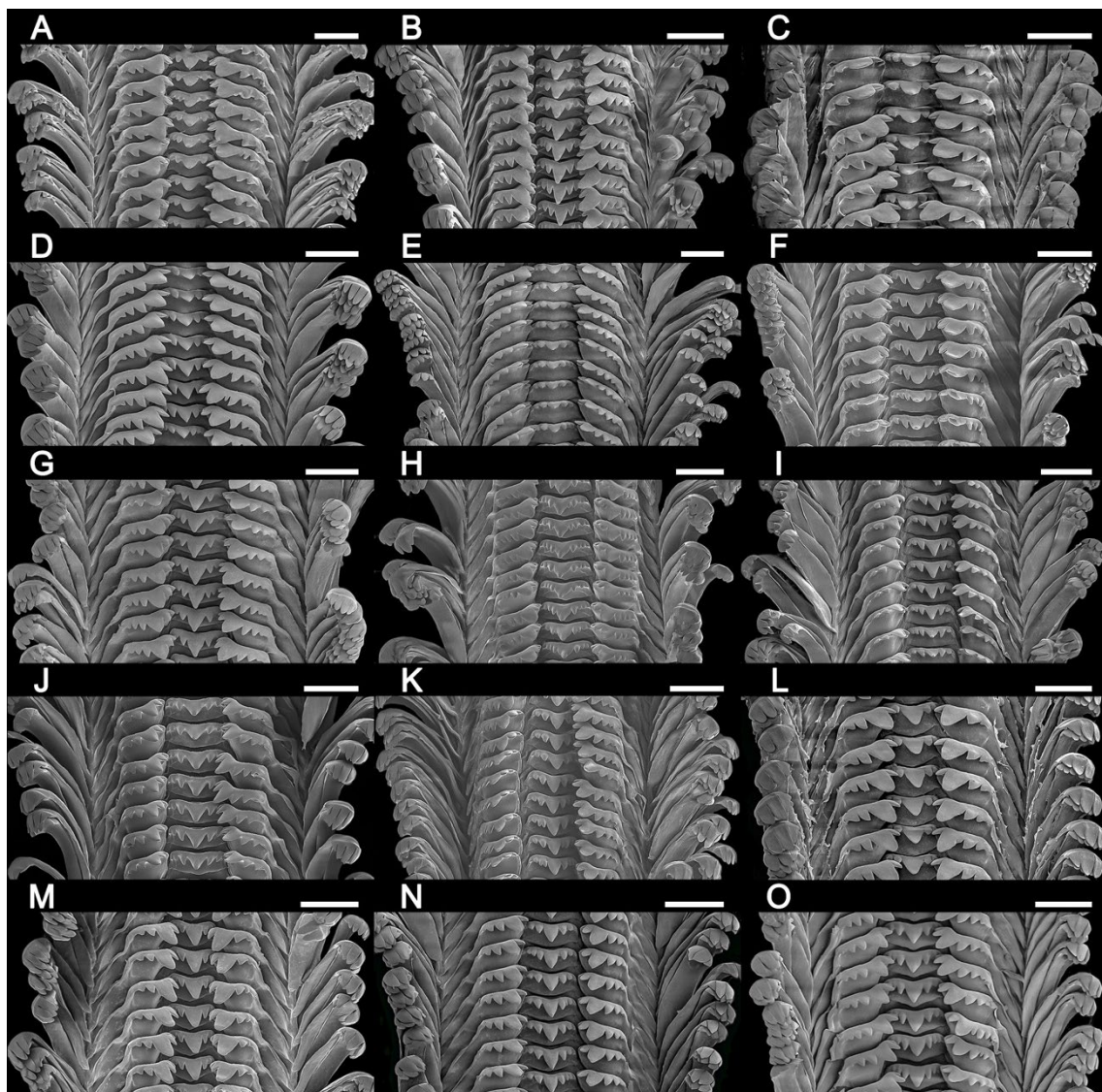


Fig. 10. Radulae of *Semisulcospira kurodai*. **A**, Gifu (#14), KUZ Z5225; **B**, Kaizu (#15), KUZ Z5227; **C**, Meiwa (#17), KUZ Z5229; **D**, Hikone (#18), KUZ Z5231; **E**, Takashima (#19), KUZ Z5233; **F**, Kameoka (#20), KUZ Z5235; **G**, Settsu (#21), KUZ Z5237; **H**, Fukuchiyama (#23), KUZ Z5240; **I**, Tambasasayama (#24), KUZ Z5243; **J**, Tambasasayama (#26), KUZ Z5247; **K**, Sanda (#27), KUZ Z5249; **L**, Ono (#28), KUZ Z5251; **M**, Himeji (#29), KUZ Z5253; **N**, Okayama (#32), KUZ Z5255; **O**, Okayama (#34), KUZ Z5258. Scale bars: 100 μ m.

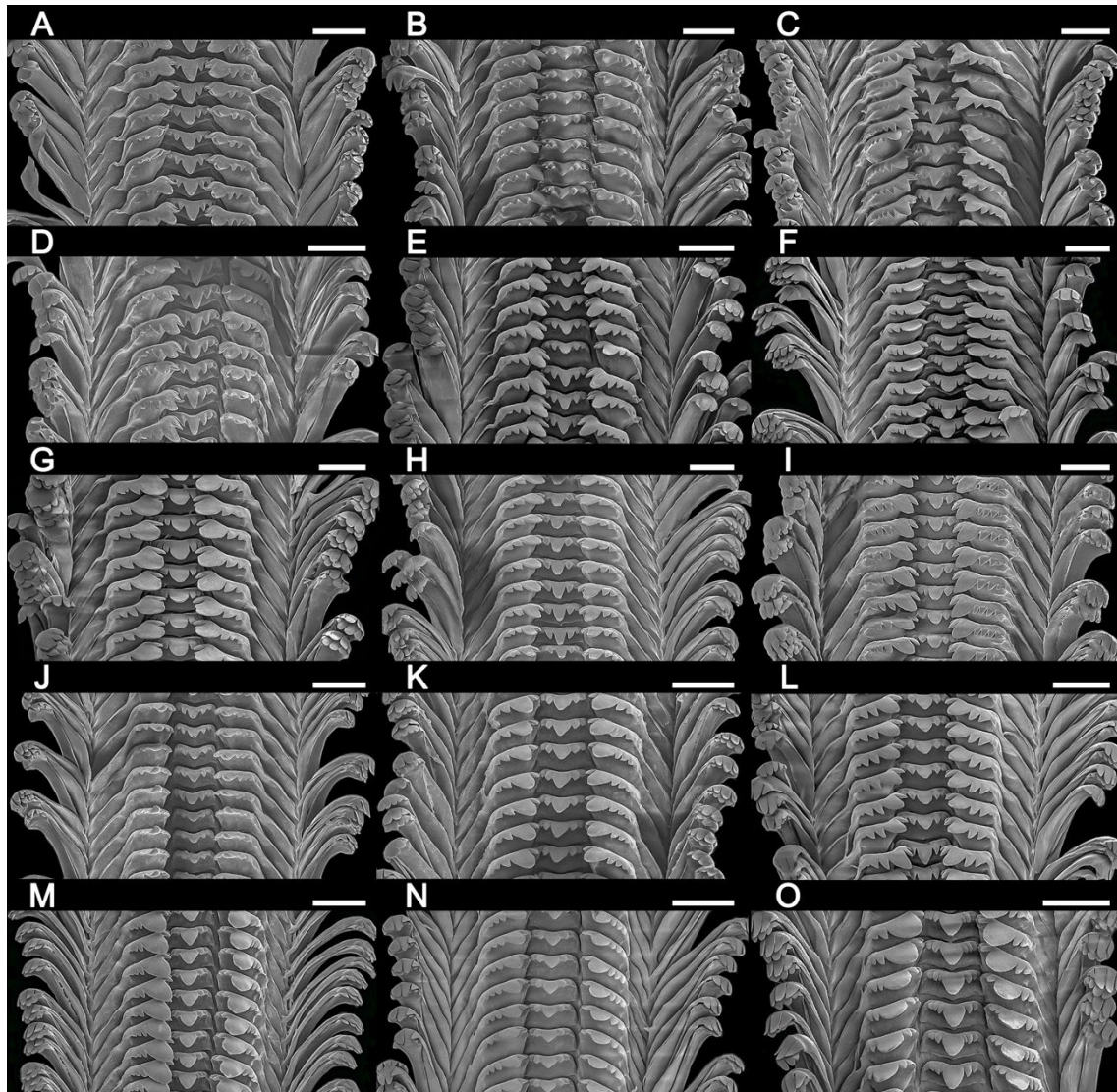


Fig. 11. Radulae of *Semisulcospira kurodai* (A, B), *S. masudai* sp. nov. (C–E), *S. praecursa* sp. nov. (F–I), and *S. miurai* sp. nov. (J–O). A, Okayama (#36), KUZ Z5259; B, Anan (#37), KUZ Z5261; C, Holotype from Toyooka (locality #30), KUZ Z5263; D, Paratype from Toyooka, KUZ Z5146; E, Iwami (#31), KUZ Z5270; F, Holotype from Iwata (locality #1), KUZ Z5272; G, Paratype from Iwata, KUZ Z5274; H, Hamamatsu (#2), KUZ Z5280; I, Toyokawa (#5), KUZ Z5282; J, Holotype from Okayama (locality #34), KUZ Z5285; K, Paratype from Okayama (#34), KUZ Z5288; L, Okayama (#32), KUZ Z5293; M, Okayama (#33), KUZ Z5296; N, Okayama (#35), KUZ Z5298; O, Kurashiki (#36), KUZ Z5300. Scale bars: 100 μ m.

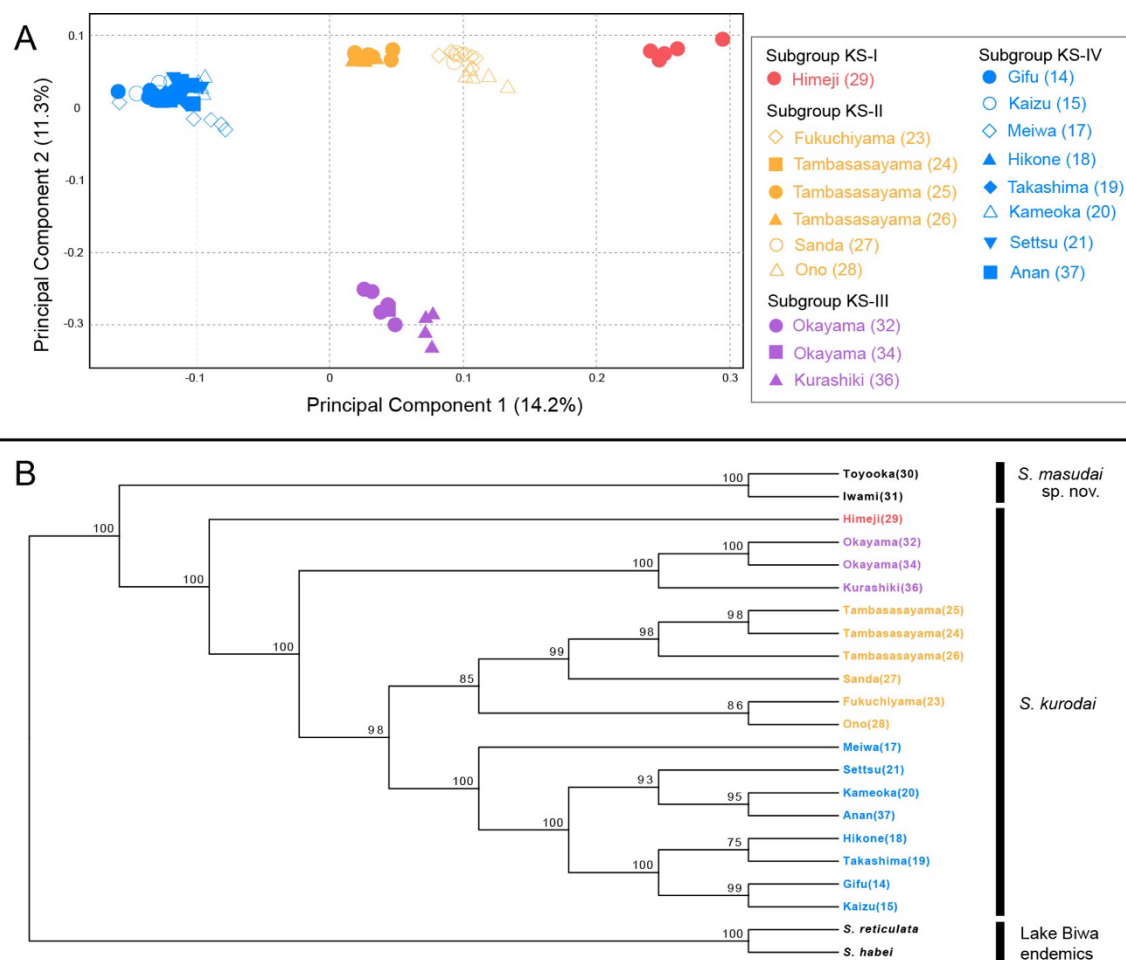


Fig. 12. Results of the principal component analysis conducted for *Semisulcospira kurodai* based on 1,922 SNPs (**A**) and a lineage tree of *S. kurodai*, *S. masudai* sp. nov., and Lake Biwa endemics of the *S. niponica*-group species calculated based on 6,915 SNPs by SVDquartets (**B**). The numbers associated with the nodes represent bootstrap values. Legends indicate locality names (locality numbers).

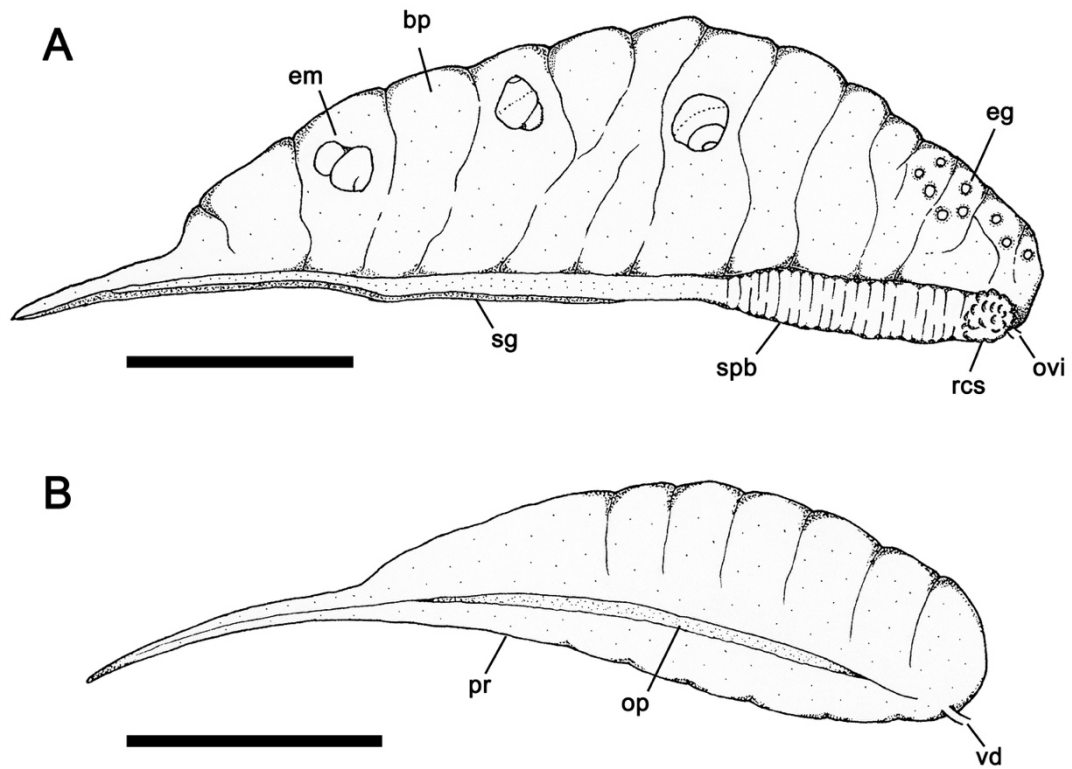


Fig. 13. Schematic drawings indicating generalised features of reproductive organs of *Semisulcospira kurodai* collected from its type locality (locality #24). **A**, Female; **B**, Male. Abbreviations: bp, brood pouch; eg, egg; em, embryo; op, opening of deep groove of prostate; ovi, oviduct; pr, prostate; rcs, seminal receptacle; sg, sperm gutter; spb, spermatophore bursa; vd, vas deferens. Scale bars: 5 mm.