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**Rapid Communication****Further records of introduced *Semisulcospira* snails in Japan (Mollusca, Gastropoda): implications for these snails' correct morphological identification**Naoto Sawada<sup>1,\*</sup>, Haruhiko Toyohara<sup>1</sup>, Takuto Miyai<sup>2</sup> and Takafumi Nakano<sup>3</sup><sup>1</sup>Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan<sup>2</sup>16-4, Oshikiri, Ichikawa City, Chiba 272-0107, Japan<sup>3</sup>Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606-8502, JapanAuthor e-mails: [sawada.naoto.82w@st.kyoto-u.ac.jp](mailto:sawada.naoto.82w@st.kyoto-u.ac.jp) (NS), [toyohara@kais.kyoto-u.ac.jp](mailto:toyohara@kais.kyoto-u.ac.jp) (HT), [tm-0381@softbank.ne.jp](mailto:tm-0381@softbank.ne.jp) (TM), [nakano@zoo.zool.kyoto-u.ac.jp](mailto:nakano@zoo.zool.kyoto-u.ac.jp) (TN)

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**Received:** 19 December 2019**Accepted:** 21 March 2020**Published:** 24 April 2020**Handling editor:** Tricia Goulding**Thematic editor:** Kenneth Hayes**Copyright:** © Sawada et al.This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).**OPEN ACCESS****Abstract**

Seven species of the freshwater snail genus *Semisulcospira*, which are indigenous taxa of the largest lake in Japan, Lake Biwa, have been introduced into 17 localities, including five newly recorded localities. Among these species, *S. dilatata* Watanabe and Nishino, 1995, *S. habei* Davis, 1969, and *S. rugosa* Watanabe and Nishino, 1995 were firstly recorded as introduced species. The alien *Semisulcospira* snails could be identified based on the combination of three morphological characters: longitudinal ribs number of penultimate whorl of adult shell, spire angle of adult shell, and shell height of the largest embryonic shell. Accordingly, morphological identification of introduced *Semisulcospira* populations recorded in previously published studies was revisited. The introductions of *Semisulcospira* snails have been suggested to have occurred due to contamination of seedlings of other shellfish and fishes at most sites. Taking into account the potential impacts of accidental species introductions, seedlings should be carefully managed so as not to unintentionally introduce *Semisulcospira* snails.

**Key words:** aquatic, contamination, introduction, diagnostic character, Lake Biwa, past records

**Introduction**

Invasions of aquatic organisms are one of the serious problems in ecosystems around the world, since alien species may cause local extinction of native species, or may affect the structure of trophic cascades (Maezono and Miyashita 2003; Miller et al. 2014). Invasions of freshwater molluscs may also pose grave threats to local biodiversity. For example, the introduced freshwater snail *Pomacea canaliculata* (Lamarck, 1822) was revealed to be associated with changes in ecosystem state and function by causing the loss of aquatic plants, high nutrient concentrations, and high phytoplankton biomass (Carlsson et al. 2004).

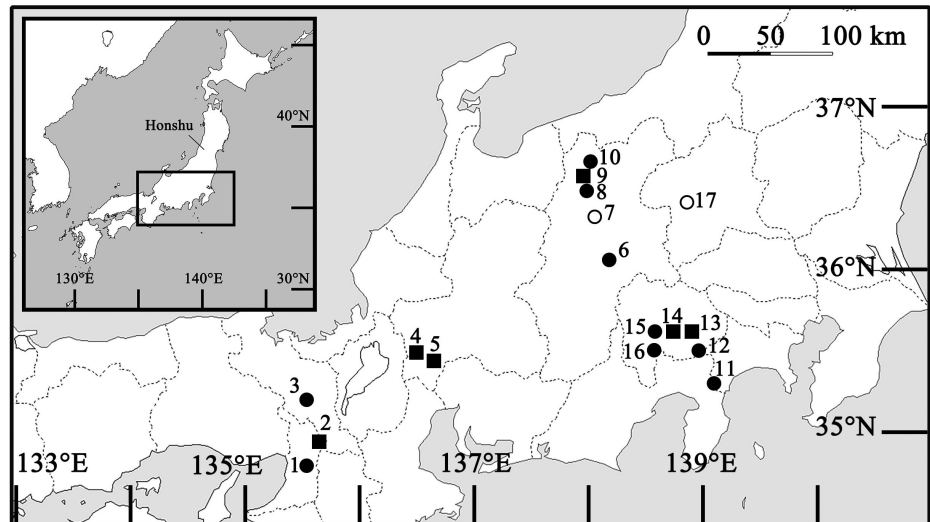
Lake Biwa is the largest lake in Japan and located in the central part of Honshu Island (Satoguchi and Nagahashi 2012). The lake has many endemic species of fish and invertebrates, including freshwater snails of the

genus *Semisulcospira*, which is the most species-rich metazoan group in the lake (Nishino and Watanabe 2000). Some endemic fishes of the lake (e.g., *Carassius cuvieri* Temminck and Schlegel, 1846) have been known as high-value fishery species, and therefore have been introduced into many other (fresh-) water bodies in Japan for culture fishery (Nature Conservation Bureau of Japanese Environment Agency 1993). Additionally, one of the endemic freshwater bivalves, *Corbicula sandai* Reinhardt, 1878, was also introduced into other lakes for cultivation (Nature Conservation Bureau of Japanese Environment Agency 1993). Although *Semisulcospira* snails, which are common in Lake Biwa, are not harvested for fisheries, they have been unintentionally introduced into other (fresh-) water bodies in Japan together with introduced fishes and bivalves (Fujita 1984; Hajimu Kihira, personal communication). Moreover, these snails were also introduced into other inland water habitats, e.g., ponds, small streams, and canals, as a food resource of the firefly *Luciola cruciata* Motschulsky, 1854 (Matsuda and Kihira 1996; Kawase et al. 2012).

Extant *Semisulcospira* snails endemic to Lake Biwa and its water system are currently classified into 15 species (Watanabe and Nishino 1995). They have occasionally been given a distinct subgeneric status as *Semisulcospira* (*Biwamelania*) (Watanabe and Nishino 1995). Recently, however, they were revealed to be divided into two species groups—*habei*-group and *decipiens*-group—that were defined by molecular phylogenetic relationships, and moreover, “*Biwamelania*” was shown not to be a monophyletic group (Kamiya et al. 2011; Miura et al. 2019).

*Semisulcospira libertina* (Gould, 1859), which is an ex-“*Biwamelania*” species widely distributed in East Asia, showed high geographic variation in the shell morphology (Davis 1972). It was revealed that intraspecific variation observed in this species was mainly due to environmental factors as well as heritability of the shell characters (Urabe 1998). On the other hand, such high intraspecific variation has rarely been reported in “*Biwamelania*” species; each of the 15 species was well-defined by morphological characters of the adult and embryonic shells (Davis 1969; Watanabe and Nishino 1995). Although previous phylogenetic analyses based on mitochondrial DNA sequences of *Semisulcospira* species revealed incongruence between morphology-based species classification and snails’ mitochondrial lineages (Miura et al. 2013; Köhler 2016), a phylogenomic study using nuclear restriction-site associated DNA sequencing nevertheless recovered monophyly of the most of each morphology-based “*Biwamelania*” species (Miura et al. 2019). Those results suggest the possibility that molecular identification using mitochondrial DNA markers would sometimes lead to misidentification of “*Biwamelania*” species in contrast to identification based on their shell characters and/or nuclear DNA markers.

In previous studies, non-native “*Biwamelania*” species were found at many places in Japan (Kurasawa et al. 1981; Nature Conservation Bureau



**Figure 1.** Map of Honshu Island, Japan, showing 17 localities of non-indigenous populations of *Semisulcospira* (“*Biwamelania*”) species. Open circles, localities for *S. decipiens*-group species; closed circles, *S. habei*-group species; and closed squares, locations where both groups were found. Locality details and species collected are shown in Table S1.

of Japanese Environment Agency 1993; Uenishi 1994; Matsuda and Kihira 1996; Kurozumi 2007; Kihira et al. 2009; Kawase et al. 2012). However, several of the previous records seem to have been based on misidentification of the species. During field surveys conducted by the first and third authors of the present report, non-native “*Biwamelania*” snails were found at new localities in Japan. Examining those specimens of introduced snails enabled us to determine the most appropriate morphological characters for identifying “*Biwamelania*” species. The records presented in this report about introduced “*Biwamelania*” species contribute to an accurate assessment of the current condition of anthropogenic introductions of freshwater gastropods.

## Materials and methods

“*Biwamelania*” snails were newly found at 14 localities in Honshu Island, Japan, in 2016–2019 by the first and third authors (Figure 1; Supplementary material Table S1). Specimens were separated into shells and soft parts after being boiled in hot water at 95 °C for 30 seconds. Embryos which were extracted from brood pouches of adult females, were dissected and treated with 12% sodium hypochlorite at 26 °C for 1 day to remove soft parts. Also, when necessary, adult shells were treated with 12% sodium hypochlorite at 26 °C to decompose deposits on the shell’s surface following the procedures of Watanabe and Nishino (1995). The spire angle of adult shells was obtained from digital images using ImageJ 1.51k. (Schneider et al. 2012). Specimens were photographed using a Nikon D7100 camera with a Tamron SP 90 mm f/2.8 1:1 macro lens for Nikon. Shell height of embryonic shells was measured with a caliper which could read to precision of 0.1 mm. All newly collected samples were identified following Davis (1969) and

Watanabe and Nishino (1995), and have been deposited in the Zoological Collection of Kyoto University (KUZ).

Abbreviations of the morphological characters used in the present study: RN, longitudinal ribs number of penultimate whorl of adult shell; SA, spire angle of adult shell; and SHE, shell height of the largest embryonic shell.

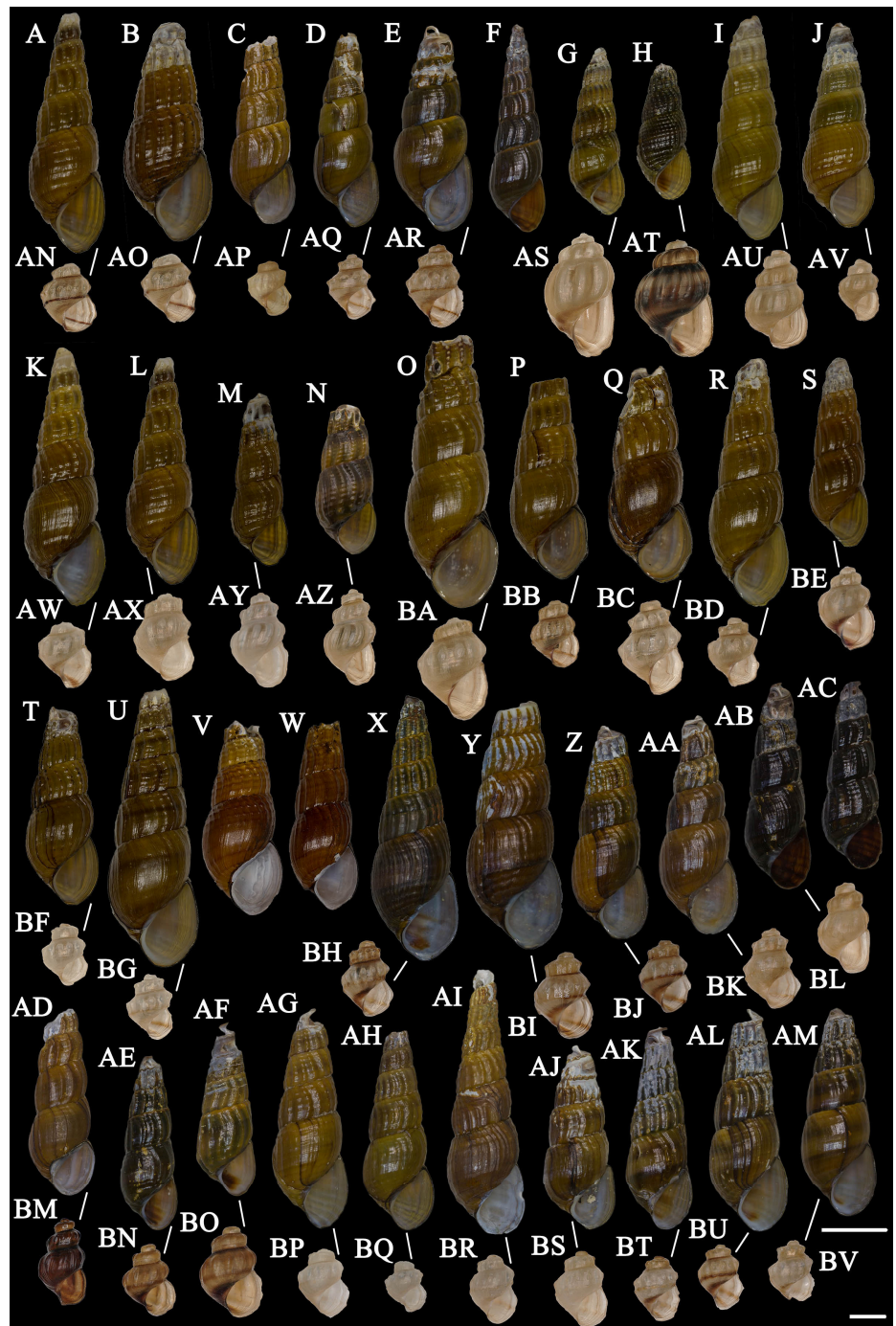
## Results

In total, five “*Biwamelania*” species were newly recorded from 14 localities (Figure 2; Table S1). Three *habei*-group species, *S. dilatata* Watanabe and Nishino, 1995, *S. habei* Davis, 1969, and *S. rugosa* Watanabe and Nishino, 1995 were collected at 12 locations. Two species belonging to the *decepiens*-group, *S. decepiens* (Westerlund, 1883) and *S. multigranosa* (Boettger, 1886), were sampled from six localities. Morphological characters of adult shells of the newly collected samples corresponded to those of “*Biwamelania*” species shown in previous studies (Table 1).

Introduced *habei*-group species were similar with native specimens from Lake Biwa in having adult shells with or without longitudinal ribs on the whorls, large SA, and sizes of embryonic shells. *Semisulcospira dilatata* (KUZ Z2664) was newly recorded in Muro Pond (locality #2 in Figure 1), and possessed a smooth surface on the penultimate whorl of the adult shells, SA  $17.5 \pm 2.0^\circ$  (mean  $\pm$  SD), and middle-sized embryonic shells (SHE  $2.2 \pm 0.3$  mm). *Semisulcospira rugosa* (KUZ Z2676) was newly sampled in Lake Motosu (#16); the specimens had a smooth surface up to the penultimate whorl of the adult shells, SA  $17.1 \pm 0.2^\circ$ , and small embryonic shells (SHE  $1.8 \pm 0.1$ ). *Semisulcospira habei* was the most common introduced taxon among the *habei*-group species, and was collected from 12 new localities: Nagase River (#1; KUZ Z2662), Muro Pond (#2; KUZ Z2663), Lake Suwa (#6; KUZ Z2677), Lake Kizaki (#8; KUZ Z2679), Nogu River (#9; KUZ Z2680), Lake Nakatsuna (#10; KUZ Z2682), Lake Ashino (#11; KUZ Z2668), Lake Yamanaka (#12; KUZ Z2669), Lake Kawaguchi (#13; KUZ Z2670), Lake Sai (#14; KUZ Z2672), Lake Shoji (#15; KUZ Z2674), and Lake Motosu (#16; KUZ Z2675). Although there were some deviations in all characteristics evaluated, multiple morphological characters of the present specimens overlapped those observed in previous studies (RN 13.8–27.5; SA 14.6–21.8°; SHE 1.5–2.7 mm).

The present specimens of the *decepiens*-group species, *S. decepiens* and *S. multigranosa*, also showed similar character states to those of non-introduced populations of species mainly in RN and SA. *Semisulcospira decepiens* was collected from five localities that include three new records: Muro Pond (#2, new locality; KUZ Z2665), Lake Misuzu (#7, new locality; KUZ Z2678), Nogu River (#9, new locality; KUZ Z2681), Lake Kawaguchi (#13; KUZ Z2671), Lake Sai (#14; KUZ Z2673). They possess low RN (13.2–18.7), small to middle SA (12.1–18.4°) and middle-sized embryonic





**Figure 2.** Shells of introduced *Semisulcospira* (“*Biwamelania*”) species collected from 14 localities. A–B, AN–AO: *S. habei* from Nagase River (KUZ Z2662). C, AP: *S. habei* from Muro Pond (KUZ Z2663). D–E, AQ–AR: *S. dilatata* from Muro Pond (KUZ Z2664). F: *S. decipiens* from Muro Pond (KUZ Z2665). G–H, AS–AT: *S. multigranosa* from Muro Pond (KUZ Z2666). I–J, AU–AV: *S. multigranosa* from Gifu City (KUZ Z2667). K–L, AW–AX: *S. habei* from Lake Suwa (KUZ Z2677). M–N, AY–AZ: *S. decipiens* from Lake Misuzu (KUZ Z2678). O–P, BA–BB: *S. habei* from Lake Kizaki (KUZ Z2679). Q–R, BC–BD: *S. habei* from Nogu river (KUZ Z2680). S, BE: *S. decipiens* from Nogu River (KUZ Z2681). T–U, BF–BG: *S. habei* from Lake Nakatsuna (KUZ Z2682). V–W: *S. habei* from Lake Ashino (KUZ Z2668). X–Y, BH–BI: *S. habei* from Lake Yamanaka (KUZ Z2669). Z–AA, BJ–BK: *S. habei* from Lake Kawaguchi (KUZ Z2670). AB–AD, BL–BM: *S. decipiens* from Lake Kawaguchi (KUZ Z2671). AE–AF, BN–BO: *S. decipiens* from Lake Sai (KUZ Z2672). AG–AH, BP–BQ: *S. habei* from Lake Sai (KUZ Z2673). AI–AJ, BR–BS: *S. habei* from Lake Shoji (KUZ Z2674). AK–AL, BT–BU: *S. habei* from Lake Motosu (KUZ Z2675). AM, BV: *S. rugosa* from Lake Motosu (KUZ Z2676). A–AM Adult shells, AN–BV Embryonic shells. Scale bars: 10 mm (A–AM), 1 mm (AN–BV).

**Table 1.** Morphometric characters of *Semisulcospira* (“*Biwamelania*”) species collected in this study. Measurements and counts: mean  $\pm$  SD [except for Watanabe and Nishino (1995)]; mean  $\pm$  95% confidence limits [Watanabe and Nishino (1995)].

Locality name / Reference	Number of specimens studied (adult / embryonic shell)	RN	SA (°)	SHE (mm)
<i>Semisulcospira dilatata</i>				
Muro Pond	11 / 10	–	17.5 $\pm$ 2.0	2.2 $\pm$ 0.3
Watanabe and Nishino 1995	30 / 10	–	20.4 $\pm$ 0.6	2.16 $\pm$ 0.18
<i>Semisulcospira rugosa</i>				
Lake Motosu	2 / 2	–	17.1 $\pm$ 0.2	1.8 $\pm$ 0.1
Watanabe and Nishino 1995	30 / 10	11.5 $\pm$ 0.7	19.1 $\pm$ 0.8	1.70 $\pm$ 0.23
<i>Semisulcospira habei</i>				
Nagase River	10 / 10	16.3 $\pm$ 1.6	20.1 $\pm$ 1.7	1.9 $\pm$ 0.3
Muro Pond	11 / 10	19.1 $\pm$ 2.9	18.6 $\pm$ 2.0	2.0 $\pm$ 0.2
Lake Suwa	8 / 4	17.1 $\pm$ 3.2	19.3 $\pm$ 1.9	1.9 $\pm$ 0.4
Lake Kizaki	3 / 1	15.3 $\pm$ 0.6	16.5 $\pm$ 1.2	1.8
Nogu River	9 / 8	18.8 $\pm$ 2.8	16.0 $\pm$ 1.4	2.3 $\pm$ 0.3
Lake Nakatuna	7 / 7	17.9 $\pm$ 2.9	16.3 $\pm$ 1.6	2.3 $\pm$ 0.4
Lake Ashino	3 / 0	16.3 $\pm$ 2.5	16.9 $\pm$ 2.2	–
Lake Yamanaka	9 / 9	23.7 $\pm$ 3.8	19.3 $\pm$ 2.0	2.2 $\pm$ 0.4
Lake Kawaguchi	8 / 7	19.5 $\pm$ 2.6	19.4 $\pm$ 1.9	2.2 $\pm$ 0.3
Lake Sai	10 / 6	18.6 $\pm$ 2.4	18.3 $\pm$ 2.1	2.0 $\pm$ 0.3
Lake Shoji	7 / 6	21.9 $\pm$ 3.0	18.6 $\pm$ 2.0	2.1 $\pm$ 0.2
Lake Motosu	8 / 1	18.9 $\pm$ 2.0	17.2 $\pm$ 1.5	1.8
Davis 1969	11 / 0	18.0 $\pm$ 3.14	14.5 $\pm$ 2.25	–
Watanabe and Nishino 1995	30 / 10	15.3 $\pm$ 0.8	16.4 $\pm$ 0.5	2.10 $\pm$ 0.17
<i>Semisulcospira decipiens</i>				
Muro Pond	5 / 1	16.2 $\pm$ 1.3	16.3 $\pm$ 0.9	1.5
Lake Misuzu	9 / 7	15.8 $\pm$ 2.4	14.7 $\pm$ 2.6	2.2 $\pm$ 0.6
Nogu River	5 / 4	16.8 $\pm$ 1.9	16.4 $\pm$ 1.8	2.1 $\pm$ 0.1
Lake Kawaguchi	6 / 5	14.8 $\pm$ 1.2	14.4 $\pm$ 1.2	2.2 $\pm$ 0.8
Lake Sai	10 / 7	14.8 $\pm$ 1.6	16.8 $\pm$ 1.6	2.3 $\pm$ 0.2
Davis 1969	20 / 0	16.0 $\pm$ 3.00	12.4 $\pm$ 1.42	–
Watanabe and Nishino 1995	30 / 10	16.9 $\pm$ 0.9	12.2 $\pm$ 0.6	2.41 $\pm$ 0.50
<i>Semisulcospira multigranosa</i>				
Muro Pond	2 / 2	22.0 $\pm$ 2.8	16.8 $\pm$ 2.8	3.3 $\pm$ 0.1
Gifu City	10 / 10	23.2 $\pm$ 3.6	15.7 $\pm$ 2.0	2.4 $\pm$ 0.4
Davis 1969	25 / 0	23.2 $\pm$ 2.42	14.2 $\pm$ 3.11	–
Watanabe and Nishino 1995	30 / 10	–	14.6 $\pm$ 0.5	4.87 $\pm$ 0.43

shells (SHE 1.4–3.0 mm). *Semisulcospira multigranosa* was found at Gifu City (#5; KUZ Z2667) and newly collected from Muro Pond (#2; KUZ Z2666); the specimens showed large RN (19.2–26.8), small SA (13.7–19.6°) and middle to large embryonic shells (SHE 2.0–3.4 mm).

## Discussion

### *Morphological identification of introduced “Biwamelania” species*

The morphological definition of each “*Biwamelania*” species was well established based on characters of adult and embryonic shells (Davis 1969; Watanabe and Nishino 1995): surface structure of the adult shell, basal cord number, spiral cord number, RN, SA, embryonic shell number, SHE, whorl number of embryonic shells, and color patterns of embryonic shells. Among these morphological characters, three morphological characters, RN, SA and SHE, of adult and embryonic shells can lead us to accurate identification of the introduced populations of “*Biwamelania*” species.

*Semisulcospira habei* was exceptional in showing a wide range of the three characters in the introduced populations that deviated from those in its native population. Since monophyly of *S. habei* was not supported by the previous molecular phylogenetic study (Miura et al. 2019), the present “*habei*” may consist of independent taxonomic units, and thus its introduced populations should be identified advisedly taking into account its morphological variability.

Our observations of this combination of three morphological characters, i.e., RN, SA and SHE, can help to re-evaluate the taxonomic identity of introduced “*Biwamelania*” snails reported in previous studies. For example, the snail identified as *S. multigranosa* by Matsuda and Kihira (1996: fig. 1) might belong to *S. habei*, because the specimens of these snails show large SA (more than 20). Since the snail specimen reported as *S. decipiens* by Kawase et al. (2012: figs. 2–9) had RN of more than 20, that individual could presumably be identified as *S. multigranosa*; since *S. multigranosa* is distinguishable from the *S. decipiens* in having a large RN. Moreover, *S. multigranosa* described in Kawase et al. (2012: figs. 2–10) might have been *S. reticulata* Kajiyama and Habe, 1961, according to its possession of larger SA (about 19°), which discriminates *S. reticulata* from *S. multigranosa*. It is possible that other past reports about introduced “*Biwamelania*” species, which lacked figures showing or depicting the snails they saw in their works themselves, may contain records based on misidentified specimens. *Semisulcospira multigranosa* from Lake Kizaki (Kurata 1969) and from Fuji Five Lakes—Lake Yamanaka, Lake Kawaguchi, Lake Sai, Lake Shoji and Lake Motosu—(Kurozumi 2007) might belong to other congeneric species, because *S. multigranosa* could not be found by the authors in the present investigation. In future studies, therefore, taxonomic accounts of introduced “*Biwamelania*” populations recorded in previous studies should be re-evaluated according to the appropriate combination of morphological characters based on RN, SA and SHE.

#### *Possible pathways of introduced “Biwamelania” snails*

In addition to the four previously known species, *S. niponica*, *S. reticulata*, *S. decipiens* and *S. multigranosa*, the present study confirmed that three additional species, *S. dilatata*, *S. habei*, and *S. rugosa*, were introduced to localities outside their native range. Additionally, the “*Biwamelania*” snails were collected from five localities—Nagase River; Muro Pond; Lake Misuzu; Nogu River; Lake Ashino—for the first time. According to the present results and previous studies (with the exception of aforementioned questionable records), seven species were found at 17 sites in previous studies: *S. dilatata* [#2, present study (PS)]; *S. niponica* (#3, Kihira et al. 2009); *S. reticulata* (#4, Matsuda and Kihira 1996; #5, Kawase et al. 2012);



*S. reticulata* (#4, Matsuda and Kihira 1996); *S. rugosa* (#16, PS); *S. habei* (#1, 2, 6, 8–16, PS); *S. decipiens* (#2, 7, 9, 13, 14, PS; #4, Matsuda and Kihira 1996; #5, Kawase et al. 2012; #6, Kurasawa et al. 1981; #8, 10, Fujita 1984; #12–16, Kurozumi 2007); *S. multigranosa* (#2, 5, PS; #4, Matsuda and Kihira 1996; #5, Kawase et al. 2012; #8, Kurata 1969; #12–16, Kurozumi 2007).

Those *Semisulcospira* species were introduced for primarily two reasons: 1) unexpected contamination of them to seedlings of fishes and bivalves; and, 2) intentional introduction as a food resource of fireflies. At most studied sites, introductions seemed to have occurred for the first reason, according to the records of seedling stocks. For example, seedlings of *Carassius cuvieri*, which is an indigenous fish of Lake Biwa, have been introduced into Lake Suwa (locality #6) since 1590 for culture fishery (Deguchi 1984). Additionally, an endemic bivalve of Lake Biwa, *Corbicula sandai*, was also brought into Lake Suwa (Yasuki 2013) and Lake Kizaki (locality #8, Fujita 1984). It is possible that the introductions of those seedlings may be the primary reason for introductions of *Semisulcospira* snails because, to our knowledge, there are no records of stocking of “*Biwamelania*” snails into Lake Suwa or Lake Kizaki. In addition to the introduced “*Biwamelania*” populations found in the two lakes, “*Biwamelania*” populations may have been introduced into other lakes along with seedlings of taxa endemic to Lake Biwa (Nature Conservation Bureau of Japanese Environment Agency 1993): *Carassius cuvieri* into seven localities (#10–15, 17); *Carassius buergeri grandoculis* Temminck and Schlegel, 1846 into Lake Shoji (#15); *Gnathopogon caerulescens* Sauvage, 1883 into Lake Kawaguchi (#13); *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) into Lake Sai (#14).

As noted in previous studies (Matsuda and Kihira 1996; Kawase et al. 2012), “*Biwamelania*” snails found in Ogaki (#4) and Gifu (#5) cities may have been introduced as a food resource of fireflies; *Semisulcospira* species are known to be a major prey of this coleopteran insect (Miyake et al. 2003). It remains uncertain how “*Biwamelania*” snails were introduced to the other four sites (#1, 2, 3, 7) because there are no records of the introduction of seedlings or the snails themselves.

In the investigations by the present authors and in previously published investigations (Nature Conservation Bureau of Japanese Environment Agency 1993; Uenishi 1994; Kurozumi 2007), the introduced “*Biwamelania*” populations from nine localities (#1, 2, 5, 6, 10, 11, 13, 14, 17) co-occurred together with a *Semisulcospira* species that is indigenous at these localities. Taking into account the potential impact of introduced species on native species, the seedlings should be carefully managed so as not to spread “*Biwamelania*” snails into additional habitats.

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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Details of introduced *Semisulcospira* (“*Biwamelania*”) species collected from 14 localities during the investigation conducted in 2016–2019, and the previous studies.

This material is available as part of online article from:

[http://www.reabic.net/journals/bir/2020/Supplements/BIR\\_2020\\_Sawada\\_etal\\_Table\\_S1.xlsx](http://www.reabic.net/journals/bir/2020/Supplements/BIR_2020_Sawada_etal_Table_S1.xlsx)