

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

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Supplementary material

TABLE S1 Morphometric characters of *Semisulcospira niponica* and *S. fuscata* examined in the present study. Measurements and counts: minimum–maximum value (mean $\pm$ SD)

Species	<i>Semisulcospira niponica</i>					<i>Semisulcospira fuscata</i>					
Locality	Oura Port	Nagahama Port	Iso	Kitakomatsu Port	Kitakomatsu Beach	Katata Port	Otsu Port	Nango	Lake Yogo	Oura	Nihonmatsu
specimen number of shells (female / male / embryo)	15 / 1 / 10	15 / 15 / 10	12 / 5 / 8	8 / 0 / 3	0 / 1 / 0	15 / 10 / 10	15 / 14 / 13	15 / 10 / 10	15 / 2 / 10	9 / 1 / 9	14 / 2 / 10
specimen number of radulae	5	5	5	3	0	5	5	5	5	5	5
specimen number of reproductive organs (female / male)	8 / 1	8 / 6	8 / 5	3 / 0	0 / 1	10 / 6	7 / 8	10 / 6	10 / 2	9 / 1	10 / 3
Morphological characters of adult female shells											
Aperture height (AH)	9.6–11.4 (10.9 $\pm$ 0.5)	9.1–10.6 (9.7 $\pm$ 0.5)	9.3–12.6 (10.6 $\pm$ 1.0)	9.2–11.9 (10.6 $\pm$ 1.1)	-	7.7–10.8 (8.9 $\pm$ 0.9)	9.3–11.6 (10.4 $\pm$ 0.6)	9.3–11.2 (10.3 $\pm$ 0.6)	8.4–10.7 (9.3 $\pm$ 0.5)	9.8–11.5 (10.7 $\pm$ 0.5)	10.6–11.7 (11.2 $\pm$ 0.4)
Aperture length (AL)	10.9–12.3 (11.7 $\pm$ 0.4)	9.7–11.0 (10.3 $\pm$ 0.4)	9.5–12.9 (11.4 $\pm$ 1.0)	9.9–12.3 (11.0 $\pm$ 0.9)	-	8.3–11.2 (9.5 $\pm$ 0.9)	10.1–12.2 (11.2 $\pm$ 0.6)	9.9–12.1 (10.9 $\pm$ 0.6)	8.5–10.8 (9.7 $\pm$ 0.6)	9.9–11.5 (10.9 $\pm$ 0.6)	10.9–12.1 (11.4 $\pm$ 0.5)
Aperture swell length (ASL)	0–0.12 (0.07 $\pm$ 0.04)	0–0.09 (0.06 $\pm$ 0.03)	0–0.13 (0.06 $\pm$ 0.05)	0–0.11 (0.06 $\pm$ 0.05)	-	0–0.13 (0.07 $\pm$ 0.04)	0.03–0.14 (0.08 $\pm$ 0.03)	0–0.12 (0.04 $\pm$ 0.04)	0–0.12 (0.07 $\pm$ 0.04)	0–0.10 (0.03 $\pm$ 0.03)	0–0.04 (0.01 $\pm$ 0.01)
Aperture slenderness ratio (ASR)	1.40–1.74 (1.6 $\pm$ 0.1)	1.45–1.81 (1.6 $\pm$ 0.1)	1.34–1.79 (1.6 $\pm$ 0.1)	1.55–1.84 (1.7 $\pm$ 0.1)	-	1.56–1.80 (1.7 $\pm$ 0.1)	1.45–1.74 (1.6 $\pm$ 0.1)	1.60–1.84 (1.7 $\pm$ 0.1)	1.63–1.88 (1.8 $\pm$ 0.1)	1.67–1.79 (1.7 $\pm$ 0.1)	1.6–1.91 (1.8 $\pm$ 0.1)
Aperture width (AW)	6.5–8.2 (7.3 $\pm$ 0.4)	5.7–7.1 (6.5 $\pm$ 0.4)	5.8–7.9 (7.1 $\pm$ 0.7)	5.6–7.9 (6.5 $\pm$ 0.8)	-	5.0–6.8 (5.8 $\pm$ 0.4)	6.4–8.0 (7.0 $\pm$ 0.5)	5.7–7.2 (6.3 $\pm$ 0.4)	5.0–5.9 (5.5 $\pm$ 0.2)	5.6–6.9 (6.3 $\pm$ 0.4)	5.7–7.3 (6.5 $\pm$ 0.4)
Basal cord number (BCN)	3–5 (4.1 $\pm$ 0.5)	2–4 (3.1 $\pm$ 0.7)	3–5 (4.3 $\pm$ 0.7)	2–3 (2.5 $\pm$ 0.5)	-	2–4 (2.4 $\pm$ 0.7)	2–5 (3.5 $\pm$ 0.7)	2–4 (3 $\pm$ 0.8)	2–5 (3.7 $\pm$ 1.0)	2–5 (4.4 $\pm$ 1.1)	2–6 (3.8 $\pm$ 1.0)
Body whorl length (BWL)	17–19.8 (18.8 $\pm$ 0.7)	16.1–18.9 (16.8 $\pm$ 0.8)	15.5–21.2 (17.9 $\pm$ 1.6)	15.6–21.1 (18.4 $\pm$ 2.2)	-	12.9–17.1 (14.9 $\pm$ 1.2)	16.5–19.3 (17.8 $\pm$ 0.9)	16.1–18.6 (17.3 $\pm$ 0.9)	13.9–17.1 (15.5 $\pm$ 0.9)	15.6–19.4 (17.9 $\pm$ 1.1)	16.7–19.8 (18.6 $\pm$ 0.9)
Fourth whorl length (FWL)	3.1–5.2 (3.8 $\pm$ 0.6)	3.1–4.1 (3.5 $\pm$ 0.3)	2.9–4.0 (3.5 $\pm$ 0.3)	2.8–4.6 (3.8 $\pm$ 0.7)	-	2.4–3.4 (2.9 $\pm$ 0.3)	3.2–4.6 (3.8 $\pm$ 0.4)	2.9–3.9 (3.5 $\pm$ 0.2)	2.4–3.8 (3.1 $\pm$ 0.3)	3.4–4.8 (3.9 $\pm$ 0.5)	2.9–4.6 (3.9 $\pm$ 0.5)
Penultimate whorl length (PWL)	6.2–7.7 (7 $\pm$ 0.4)	5.2–6.6 (6.0 $\pm$ 0.4)	5.2–7.6 (6.3 $\pm$ 0.7)	5.4–8.2 (6.9 $\pm$ 1.1)	-	4.4–6.4 (5.2 $\pm$ 0.5)	6.2–7.3 (6.6 $\pm$ 0.4)	5.4–6.8 (6.2 $\pm$ 0.4)	4.9–6.9 (5.5 $\pm$ 0.5)	5.3–7.4 (6.5 $\pm$ 0.6)	5.5–7.5 (6.7 $\pm$ 0.6)

Longitudinal rib number of penultimate whorl (RN)	12–18 (15.3 ± 1.5)	11–16 (12.7 ± 1.3)	13–20 (15 ± 2.3)	9–15 (11.8 ± 2)	-	10–14 (11.9 ± 1.1)	10–17 (13.1 ± 1.8)	9–17 (11.9 ± 2)	9–14 (11.0 ± 1.8)	14–18 (15.7 ± 1.5)	11–18 (15.8 ± 2.0)
Spire angle (SA)	18.8–28.9 (24 ± 2.8)	18.6–26.1 (22.4 ± 2.0)	23.2–28.9 (25.9 ± 2)	19.0–25.9 (21.6 ± 3.3)	-	18.2–28.0 (23.4 ± 2.5)	19.9–27.0 (22.9 ± 1.8)	16.5–26.6 (21.7 ± 2.8)	16.6–24.7 (20.6 ± 2.3)	16.2–20.4 (18.4 ± 1.5)	15.6–20.7 (18.1 ± 1.4)
Spiral cord number of penultimate whorl (SCN)	4–6 (4.6 ± 0.7)	3–4 (3.9 ± 0.4)	4–5 (4.4 ± 0.5)	4–5 (4.8 ± 0.4)	-	3–4 (3.6 ± 0.5)	4–6 (4.7 ± 0.9)	3–5 (4 ± 0.8)	4–6 (4.8 ± 0.8)	5	4–7 (5.3 ± 1.0)
Shell height (SH)	27.2–34.1 (30.1 ± 2.3)	23.3–28.6 (26.2 ± 1.3)	24.0–31.2 (27.3 ± 2.6)	26.1–36.4 (31.1 ± 3.8)	-	21.6–30.1 (24.4 ± 2.7)	26.1–30.0 (28.2 ± 1.2)	25.8–30.7 (28.6 ± 1.6)	21.1–29.6 (25.6 ± 2.1)	26.8–34.8 (31.2 ± 3.2)	28.0–35.0 (32.3 ± 2.2)
Shell width (SW)	12–13.9 (13 ± 0.6)	11.1–12.8 (11.7 ± 0.4)	10.8–14.1 (12.6 ± 1.0)	10.4–13.8 (12.2 ± 1.4)	-	9.1–11.8 (10.5 ± 0.7)	11.8–14.7 (12.9 ± 0.8)	10.2–13.1 (11.4 ± 0.8)	9.1–10.8 (10.0 ± 0.5)	9.9–11.6 (11.0 ± 0.6)	10.5–12.6 (11.4 ± 0.7)
Third whorl length (TWL)	4.2–6.4 (5.1 ± 0.5)	3.8–5.1 (4.5 ± 0.4)	3.7–5.5 (4.7 ± 0.5)	4.0–6.1 (5.1 ± 0.9)	-	3.8–5.1 (4.3 ± 0.4)	4.6–6.0 (5.1 ± 0.4)	4.2–5.3 (4.7 ± 0.3)	3.7–5.2 (4.4 ± 0.3)	4.6–5.8 (5.2 ± 0.4)	4.6–5.8 (5.2 ± 0.3)
Whorl elongation ratio (WER)	2.15–3.52 (2.9 ± 0.4)	2.58–3.17 (2.8 ± 0.2)	2.79–3.41 (3.1 ± 0.2)	2.30–3.32 (2.8 ± 0.4)	-	2.80–3.56 (3.1 ± 0.2)	2.42–3.24 (2.7 ± 0.2)	2.58–3.42 (3.0 ± 0.2)	2.54–3.49 (3.0 ± 0.2)	2.39–3.00 (2.7 ± 0.2)	2.31–3.64 (2.9 ± 0.4)
Whorl number (WN)	3.50–5.25 (4.3 ± 0.5)	3.00–4.50 (4.0 ± 0.4)	3.00–5.00 (4.0 ± 0.5)	3.75–6.25 (5.0 ± 1.0)	-	3.50–5.50 (4.6 ± 0.6)	3.25–5.00 (4.2 ± 0.4)	3.75–5.50 (4.7 ± 0.5)	4.00–5.50 (4.7 ± 0.5)	4.00–5.50 (4.9 ± 0.6)	4.25–6.00 (5.2 ± 0.6)
Sculpture type (node / rib / smooth)	0% / 100% / 0%	93% / 7% / 0%	100% / 0% / 0%	83% / 17% / 0%	-	100% / 0% / 0%	100% / 0% / 0%	67% / 33% / 0%	20% / 80% / 0%	0% / 11% / 89%	0% / 31% / 69%
Morphological characters of adult male shells											
Aperture height (AH)	9	8.0–9.8 (8.8 ± 0.5)	7.9–10.9 (9.7 ± 1.1)	-	9.5	6.4–9.1 (7.9 ± 1.0)	8.3–9.4 (8.7 ± 0.3)	8.1–10.1 (9.0 ± 0.6)	8.1–8.2 (8.2 ± 0.1)	9.4	9.5–9.8 (9.6 ± 0.3)
Aperture length (AL)	9.9	8.7–10.1 (9.3 ± 0.5)	8.8–11.9 (10.6 ± 1.1)	-	9.8	6.8–9.8 (8.4 ± 1.1)	9.2–10.2 (9.6 ± 0.3)	8.7–10.8 (9.5 ± 0.7)	8.7–8.8 (8.8 ± 0.1)	9.7	9.4–9.5 (9.5 ± 0.1)
Aperture swell legth (ASL)	0.08	0–0.12 (0.08 ± 0.03)	0.05–0.14 (0.10 ± 0.04)	-	0.09	0–0.19 (0.09 ± 0.06)	0.05–0.13 (0.10 ± 0.03)	0.02–0.13 (0.07 ± 0.03)	0.09	0	0
Aperture slenderness ratio (ASR)	1.68	1.36–1.71 (1.6 ± 0.1)	1.5–1.91 (1.7 ± 0.1)	-	1.55	1.48–1.79 (1.6 ± 0.1)	1.56–1.85 (1.7 ± 0.1)	1.54–1.82 (1.7 ± 0.1)	1.74–1.77 (1.8 ± 0.1)	1.63	1.85–1.88 (1.9 ± 0.1)
Aperture width (AW)	5.9	5.3–7.3 (5.8 ± 0.5)	5.3–6.9 (6.1 ± 0.7)	-	6.3	4.1–6.3 (5.2 ± 0.7)	5.2–6.0 (5.7 ± 0.2)	4.8–6.4 (5.6 ± 0.5)	5.0	5.9	5.0–5.1 (5.1 ± 0.1)
Basal cord number (BCN)	4	2–4 (3.2 ± 0.9)	3–5 (3.8 ± 0.8)	-	4	2–4 (2.6 ± 0.8)	2–4 (3.3 ± 0.8)	2–4 (3.1 ± 0.9)	3	3	3
Body whorl length (BWL)	14.7	14.0–17.7 (14.9 ± 0.9)	14.1–17.7 (16.2 ± 1.4)	-	15.4	10.6–15.4 (13.3 ± 1.6)	14.1–15.6 (14.8 ± 0.4)	13.3–16.5 (15.1 ± 1.1)	13.6–14.0 (13.8 ± 0.3)	16.0	15.9–16.1 (16.0 ± 0.2)
Fourth whorl length (FWL)	2.4	2.6–3.7 (3.0 ± 0.3)	2.5–3.4 (3.0 ± 0.3)	-	3.5	2.0–3.6 (2.7 ± 0.5)	2.4–3.6 (2.9 ± 0.3)	2.7–3.8 (3.2 ± 0.3)	2.5–2.8 (2.7 ± 0.2)	3.9	3.2–3.9 (3.5 ± 0.5)

Penultimate whorl length (PWL)	4.8	4.8–7.2 (5.4 ± 0.5)	5.2–6.0 (5.5 ± 0.4)	-	5.4	3.8–5.7 (4.8 ± 0.6)	5.0–6.2 (5.5 ± 0.3)	4.5–6.4 (5.4 ± 0.5)	5.0–5.2 (5.1 ± 0.2)	6.1	5.7–5.9 (5.8 ± 0.1)
Longitudinal rib number of penultimate whorl (RN)	12	9–14 (11.5 ± 1.4)	12–18 (14.6 ± 2.6)	-	16	9–13 (11.0 ± 1.4)	10–15 (12.3 ± 1.7)	9–12 (10.6 ± 1.1)	9–10 (9.5 ± 0.7)	14	10–12 (11 ± 1.4)
Spire angle (SA)	29.7	19.0–26.3 (22.7 ± 2.4)	25.5–28.2 (26.7 ± 1.0)	-	23.5	19.0–24.8 (21.7 ± 2.0)	19.0–26.6 (22.9 ± 2.1)	18.2–23.7 (20.8 ± 1.7)	17.9–18.4 (18.1 ± 0.4)	17.4	14.3–16.4 (15.3 ± 1.5)
Spiral cord number of penultimate whorl (SCN)	4	3–5 (3.7 ± 0.6)	3–5 (4.4 ± 0.9)	-	4	3–5 (3.6 ± 0.7)	3–5 (4.2 ± 0.8)	3–5 (3.6 ± 0.8)	5	-	6
Shell height (SH)	22.2	21.8–26.7 (23.3 ± 1.3)	23.1–28.4 (25.6 ± 2.3)	-	24.0	17.5–26.2 (22.1 ± 3.0)	22.1–27.4 (24.2 ± 1.6)	22.0–27.1 (25.1 ± 1.4)	21.8–23.2 (22.5 ± 1.0)	29.2	28.1–28.2 (28.1 ± 0.1)
Shell width (SW)	10.6	9.5–12.6 (10.5 ± 0.8)	9.9–12.1 (11.2 ± 0.9)	-	10.9	7.3–10.4 (9.2 ± 1.0)	9.8–11.2 (10.3 ± 0.4)	8.9–11.1 (9.9 ± 0.7)	8.4–8.8 (8.6 ± 0.2)	9.9	9.1–9.3 (9.2 ± 0.1)
Third whorl length (TWL)	3.7	3.7–5.0 (4.2 ± 0.4)	3.8–4.4 (4.1 ± 0.3)	-	4.3	3.1–4.7 (3.9 ± 0.5)	3.7–5.0 (4.1 ± 0.4)	3.9–4.6 (4.2 ± 0.3)	3.8–4.0 (3.9 ± 0.1)	4.9	4.5–4.7 (4.6 ± 0.2)
Whorl elongation ratio (WER)	3.76	2.65–3.18 (2.9 ± 0.2)	3.09–3.46 (3.2 ± 0.1)	-	2.74	2.51–3.31 (2.9 ± 0.2)	2.40–3.71 (3.0 ± 0.3)	2.46–3.13 (2.8 ± 0.2)	2.90–3.19 (3.0 ± 0.2)	2.39	2.44–3.06 (2.7 ± 0.4)
Whorl number (WN)	4.3	3.25–5.00 (3.9 ± 0.5)	3.50–5.00 (4.6 ± 0.6)	-	4.00	3.75–5.25 (4.7 ± 0.5)	3.50–5.75 (4.6 ± 0.5)	4.00–5.25 (4.6 ± 0.4)	4.25–4.50 (4.4 ± 0.2)	5.00	4.75–5.00 (4.9 ± 0.2)
Sculpture type (node / rib / smooth)	71% / 29% / 0%	93% / 7% / 0%	100% / 0% / 0%	-	100% / 0% / 0%	90% / 10% / 0%	79% / 11% / 0%	80% / 20% / 0%	0% / 100% / 0%	0% / 0% / 100%	0% / 50% / 50%
Morphological characters of embryonic shells											
Number of embryos (EN)	35–145 (74.3 ± 28.7)	7–60 (40.8 ± 15.4)	30–84 (57.5 ± 17.8)	87–134 (113.3 ± 24)	-	13–48 (29.9 ± 11.7)	2–53 (30.7 ± 14.5)	36–95 (54.8 ± 15.7)	23–71 (38.5 ± 14.5)	17–80 (45.0 ± 25.4)	33–82 (61.1 ± 14.6)
Longitudinal rib number on body whorl of the largest embryonic shell (RNE)	9–14 (12.8 ± 1.7)	11–13 (12.0 ± 0.7)	11–13 (11.9 ± 0.9)	9–14 (12.0 ± 2.6)	-	10–15 (12.6 ± 1.3)	11–15 (12.8 ± 1.1)	12–15 (12.7 ± 1.3)	8–12 (10.2 ± 1.2)	10	10–15 (12.2 ± 1.8)
Shell height of the largest embryonic shell (SHE)	2.4–4.0 (3.2 ± 0.5)	2.7–3.6 (3.2 ± 0.4)	2.8–3.6 (3.3 ± 0.3)	2.3–2.9 (2.7 ± 0.4)	-	2.5–3.7 (3.1 ± 0.4)	1.9–3.0 (2.4 ± 0.3)	2.1–3.5 (2.8 ± 0.4)	2.1–2.9 (2.5 ± 0.2)	2.2–3.5 (2.9 ± 0.5)	2.5–3.6 (3.2 ± 0.3)
Shell width of the largest embryonic shell (SWE)	1.9–2.7 (2.4 ± 0.3)	2.1–2.7 (2.4 ± 0.2)	2.1–2.6 (2.4 ± 0.2)	1.8–2.3 (2.1 ± 0.3)	-	2.0–2.5 (2.2 ± 0.2)	1.6–2.3 (2.0 ± 0.2)	1.8–2.7 (2.1 ± 0.3)	1.7–2.2 (1.9 ± 0.1)	1.8–2.6 (2.2 ± 0.3)	1.9–2.5 (2.2 ± 0.2)
Whorl number of the largest embryonic shell (WNE)	2.75–3.75 (3.4 ± 0.3)	3.25–3.75 (3.5 ± 0.2)	3.25–3.50 (3.3 ± 0.1)	2.75–3.25 (3.1 ± 0.3)	-	3.00–3.75 (3.3 ± 0.2)	2.25–3.23 (2.9 ± 0.3)	2.75–3.50 (3.1 ± 0.3)	2.75–3.25 (3.0 ± 0.2)	2.50–3.25 (2.9 ± 0.3)	2.75–4.00 (3.3 ± 0.3)
Morphological characters of radulae											
cusp number of rachidian	5–7	5–7	5–7	5–7	-	5–7	5–6	5–7	5–7	5–7	5–8
cusp number of lateral teeth	5–7	5–7	5–7	5–6	-	5–7	5–6	5–7	5–7	5–7	5–7
cusp number of interior marginal teeth	4–6	4–5	4–6	4–6	-	4–6	4–6	4–5	4–5	4–5	4–5

cuspid number of exterior marginal teeth	4-6	4-5	4-6	4-6	-	4-6	4-6	4-5	4-5	4-5	4-5
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TABLE S2 Morphometric characters of three new *Semisulcospira* species and a putative hybrid examined in the present study.

Measurements and counts: minimum–maximum value (mean $\pm$ SD)

Species	<i>Semisulcospira watanabei</i> sp. nov.			<i>Semisulcospira nakanoi</i> sp. nov.			<i>Semisulcospira salebroa</i> sp. nov.		A putative hybrid between <i>S. fuscata</i> and <i>S. watanabei</i> sp. nov.
Locality	Oura	Nihonmatsu	Kitakomatsu Port	Horikiri Port	Onoe Port	Chikubu- shima	Take-shima	Shiraishi-jima	Nihonmatsu
specimen number of shells (female / male / juvenile / embryo)	0 / 0 / 1 / 0	12 / 2 / 0 / 7	9 / 3 / 0 / 7	15 / 10 / 0 / 10	16 / 5 / 0 / 10	15 / 3 / 0 / 8	15 / 10 / 0 / 10	15 / 4 / 0 / 10	1 / 0 / 0 / 1
specimen number of radulae	0	0	5	5	5	5	5	5	0
specimen number of reproductive organs (female / male)	0 / 0	3 / 0	7 / 3	8 / 6	8 / 2	10 / 5	8 / 6	10 / 6	1 / 0
Morphological characters of adult female shells									
Aperture height (AH)	6.9	9.0–11.8 (10.3 $\pm$ 0.9)	8.6–10.0 (9.2 $\pm$ 0.4)	8.3–10.6 (9.2 $\pm$ 0.6)	9.6–11.3 (10.6 $\pm$ 0.6)	10.2–12.3 (11.6 $\pm$ 0.6)	11.4–13.6 (12.4 $\pm$ 0.7)	12.3–14.8 (13.9 $\pm$ 0.7)	10.6
Aperture length (AL)	7.7	9.5–11.9 (10.6 $\pm$ 0.8)	9.0–10.5 (9.7 $\pm$ 0.5)	9.0–10.8 (9.7 $\pm$ 0.5)	10.1–12.0 (11.2 $\pm$ 0.5)	11.2–13.5 (12.5 $\pm$ 0.7)	11.7–14.8 (13.1 $\pm$ 0.8)	12.9–15.6 (14.4 $\pm$ 0.7)	10.7
Aperture swell legth (ASL)	0.10	0–0.14 (0.08 $\pm$ 0.04)	0–0.05 (0.01 $\pm$ 0.02)	0–0.23 (0.08 $\pm$ 0.06)	0.11–0.29 (0.19 $\pm$ 0.05)	0.15–0.31 (0.21 $\pm$ 0.05)	0.27–0.82 (0.43 $\pm$ 0.13)	0.31–0.71 (0.45 $\pm$ 0.11)	0.09
Aperture slenderness ratio (ASR)	1.64	1.61–1.84 (1.7 $\pm$ 0.1)	1.57–1.79 (1.7 $\pm$ 0.1)	1.52–1.95 (1.7 $\pm$ 0.1)	1.54–1.72 (1.6 $\pm$ 0.1)	1.32–1.74 (1.6 $\pm$ 0.1)	1.48–1.90 (1.7 $\pm$ 0.1)	1.47–1.64 (1.6 $\pm$ 0.1)	1.61
Aperture width (AW)	4.7	5.3–7.0 (6.2 $\pm$ 0.5)	5.4–6.1 (5.8 $\pm$ 0.2)	5.3–7.1 (5.8 $\pm$ 0.5)	6.0–7.7 (7.0 $\pm$ 0.5)	7.2–8.6 (8.0 $\pm$ 0.5)	6.7–9.7 (7.9 $\pm$ 0.8)	8.6–10.2 (9.1 $\pm$ 0.4)	6.7
Basal cord number (BCN)	2	2–4 (2.8 $\pm$ 0.8)	2–4 (2.6 $\pm$ 0.7)	2–4 (2.9 $\pm$ 0.9)	2–3 (2.5 $\pm$ 0.5)	2–3 (2.4 $\pm$ 0.5)	2–5 (2.4 $\pm$ 0.8)	2–3 (2.7 $\pm$ 0.5)	3
Body whorl length (BWL)	11.9	15.3–19.3 (17.0 $\pm$ 1.1)	14.6–16.6 (15.6 $\pm$ 0.7)	14.5–18.5 (15.5 $\pm$ 1.0)	15.9–19.4 (18.0 $\pm$ 0.9)	18.4–21.6 (19.8 $\pm$ 1.0)	19.3–22.3 (20.8 $\pm$ 1.0)	20.1–24.4 (22.9 $\pm$ 1.1)	18.3
Fourth whorl length (FWL)	2.3	3.4–4.6 (4.0 $\pm$ 0.4)	2.7–3.8 (3.3 $\pm$ 0.3)	2.6–3.9 (3.3 $\pm$ 0.3)	2.7–4.3 (3.7 $\pm$ 0.4)	3.7–4.6 (4.1 $\pm$ 0.3)	3.8–4.9 (4.3 $\pm$ 0.3)	3.8–5.3 (4.5 $\pm$ 0.4)	3.9
Penultimate whorl length (PWL)	4.4	5.2–6.8 (6.1 $\pm$ 0.4)	4.7–6.4 (5.6 $\pm$ 0.6)	5.0–7.4 (5.6 $\pm$ 0.6)	5.5–7.5 (6.7 $\pm$ 0.5)	6.5–8.0 (7.3 $\pm$ 0.4)	7.1–8.0 (7.5 $\pm$ 0.3)	7.4–9.0 (8.2 $\pm$ 0.4)	6.9

Longitudinal rib number of penultimate whorl (RN)	7	10–15 (11.3 ± 1.6)	11–16 (12 ± 1.7)	9–15 (10.6 ± 1.5)	10–14 (11.7 ± 1.3)	8–13 (10.8 ± 1.2)	10–14 (12.1 ± 1.2)	8–12 (10.1 ± 1.2)	14
Spire angle (SA)	22.5	17.1–21.2 (18.9 ± 1.4)	19.7–28.4 (23.4 ± 2.9)	16.7–24.6 (21.4 ± 2.1)	17.2–25.7 (21.1 ± 2.3)	17.2–26.7 (21.9 ± 3.0)	17.0–27.7 (21.4 ± 2.5)	18.0–26.0 (21.1 ± 2.6)	21.0
Spiral cord number of penultimate whorl (SCN)	5	4–5 (4.4 ± 0.5)	4	4–6 (4.7 ± 0.6)	3–5 (3.7 ± 0.6)	3–5 (3.7 ± 0.6)	3–6 (3.5 ± 0.9)	3–4 (3.3 ± 0.5)	5
Shell height (SH)	20.1	26.5–37.0 (30.0 ± 2.6)	23.6–26.8 (25.3 ± 1.0)	22.9–28.0 (25.6 ± 1.6)	24.0–31.8 (28.6 ± 2.0)	29.6–35.7 (32.6 ± 1.6)	32.2–37.1 (34.8 ± 1.7)	32.8–39.8 (36.5 ± 2.0)	29.0
Shell width (SW)	8.2	9.8–12.4 (11.2 ± 0.8)	9.9–11.5 (10.4 ± 0.6)	9.6–12.8 (10.4 ± 0.8)	11.1–14.4 (12.6 ± 0.8)	13.4–15.7 (14.5 ± 0.6)	12.8–16.3 (14.5 ± 1.0)	14.5–18.2 (16.1 ± 0.9)	11.7
Third whorl length (TWL)	3.5	4.2–6.0 (4.8 ± 0.4)	4.0–5.1 (4.4 ± 0.3)	3.7–5.4 (4.4 ± 0.5)	4.6–5.9 (5.2 ± 0.4)	4.8–6.5 (5.7 ± 0.4)	5.3–7.0 (5.9 ± 0.4)	5.9–7.3 (6.5 ± 0.4)	4.9
Whorl elongation ratio (WER)	3.02	2.3–2.9 (2.6 ± 0.2)	2.38–3.41 (2.8 ± 0.3)	2.61–3.15 (2.8 ± 0.2)	2.47–3.55 (2.9 ± 0.3)	2.51–3.28 (2.8 ± 0.2)	2.52–3.30 (2.9 ± 0.2)	2.63–3.55 (3.1 ± 0.3)	2.71
Whorl number (WN)	5.25	4.25–5.75 (5.0 ± 0.4)	3.50–5.50 (4.2 ± 0.6)	3.25–6.00 (4.6 ± 0.8)	3.50–5.00 (4.0 ± 0.4)	3.50–5.25 (4.5 ± 0.5)	4.00–6.25 (4.7 ± 0.6)	3.25–5.00 (4.1 ± 0.5)	3.75
Sculpture type (node / rib / smooth)	0% / 100% / 0%	17% / 83% / 0%	0% / 100% / 0%	33% / 67% / 0%	100% / 0% / 0%	100% / 0% / 0%	100% / 0% / 0%	100% / 0% / 0%	0% / 100% / 0%
Morphological characters of adult male shells									
Aperture height (AH)	-	8.6–10.1 (9.4 ± 1.0)	9.2–9.4 (9.3 ± 0.1)	7.7–11.1 (8.8 ± 0.9)	6.8–9.6 (8.3 ± 0.9)	9.4–10.7 (9.9 ± 0.7)	8.2–13.3 (10.2 ± 1.9)	8.4–9.6 (9.0 ± 0.5)	-
Aperture length (AL)	-	9.2–10.2 (9.7 ± 0.7)	9.4–9.6 (9.5 ± 0.1)	8.2–11.8 (9.2 ± 1.1)	7.3–9.5 (8.8 ± 0.8)	10.1–11.5 (10.6 ± 0.8)	8.5–13.9 (10.7 ± 2.0)	9.4–10.5 (9.8 ± 0.5)	-
Aperture swell legth (ASL)	-	0.08–0.12 (0.10 ± 0.03)	0–0.07 (0.02 ± 0.04)	0–0.12 (0.08 ± 0.04)	0.16–0.29 (0.22 ± 0.04)	0.28–0.35 (0.32 ± 0.04)	0.20–0.75 (0.33 ± 0.16)	0.20–0.39 (0.33 ± 0.09)	-
Aperture slenderness ratio (ASR)	-	1.72–1.79 (1.8 ± 0.1)	1.74–1.83 (1.8 ± 0.1)	1.68–1.82 (1.8 ± 0.1)	1.47–1.67 (1.6 ± 0.1)	1.56–1.67 (1.6 ± 0.1)	1.41–1.79 (1.6 ± 0.1)	1.59–1.76 (1.7 ± 0.1)	-
Aperture width (AW)	-	5.3–5.7 (5.5 ± 0.3)	5.3–5.4 (5.3 ± 0.1)	4.5–6.6 (5.2 ± 0.6)	4.7–6.0 (5.5 ± 0.4)	6.4–6.9 (6.6 ± 0.2)	5.2–9.9 (6.8 ± 1.7)	5.3–6.5 (6.0 ± 0.5)	-
Basal cord number (BCN)	-	3	2–3 (2.3 ± 0.6)	2–5 (3.1 ± 1.0)	2–4 (2.3 ± 0.7)	2–3 (2.3 ± 0.6)	2–3 (2.3 ± 0.5)	2–3 (2.3 ± 0.5)	-
Body whorl length (BWL)	-	14.4–15.9 (15.2 ± 1.1)	14.6–15.4 (15.1 ± 0.4)	13.2–18.5 (14.7 ± 1.6)	12.0–16.0 (14.1 ± 1.3)	16.1–18.1 (16.8 ± 1.1)	13.9–22.4 (17.3 ± 3.1)	14.4–15.9 (15.0 ± 0.6)	-

Fourth whorl length (FWL)	-	3.2–3.3 (3.2 ± 0.1)	2.8–3.1 (3.0 ± 0.2)	2.6–3.8 (3.0 ± 0.4)	1.8–3.2 (2.6 ± 0.5)	2.9–3.8 (3.2 ± 0.5)	2.6–4.6 (3.6 ± 0.7)	2.0–2.4 (2.3 ± 0.2)	-
Penultimate whorl length (PWL)	-	5.2–5.3 (5.3 ± 0.1)	4.8–5.6 (5.3 ± 0.4)	4.7–6.7 (5.2 ± 0.6)	4.4–6.1 (5.2 ± 0.6)	5.9–6.5 (6.1 ± 0.3)	5.2–8.3 (6.4 ± 1.1)	5.1–5.7 (5.3 ± 0.3)	-
Longitudinal rib number of penultimate whorl (RN)	-	9–10 (9.5 ± 0.7)	10–11 (10.7 ± 0.6)	7–12 (10.0 ± 1.5)	8–12 (9.3 ± 1.2)	8–11 (9.7 ± 1.5)	10–15 (10.9 ± 1.5)	9–10 (9.8 ± 0.5)	-
Spire angle (SA)	-	22.0–22.7 (22.4 ± 0.5)	20.4–21.5 (20.9 ± 0.6)	13.3–22.8 (20.0 ± 2.9)	17.0–27.7 (22.1 ± 3.3)	16.7–23.7 (19.3 ± 3.8)	16.7–25.1 (19.4 ± 2.7)	23.3–33.3 (26.6 ± 4.6)	-
Spiral cord number of penultimate whorl (SCN)	-	4	4	4–5 (4.6 ± 0.5)	3–4 (3.5 ± 0.5)	3	3–4 (3.3 ± 0.5)	3	-
Shell height (SH)	-	24.6–28.0 (26.3 ± 2.4)	24.6–27.1 (25.4 ± 1.5)	22.0–30.9 (24.6 ± 2.4)	18.0–25.4 (21.9 ± 2.2)	26.6–27.3 (27.0 ± 0.3)	23.9–34.9 (29.4 ± 4.6)	22.1–24.7 (23.1 ± 1.1)	-
Shell width (SW)	-	9.8–10.6 (10.2 ± 0.6)	9.5–9.7 (9.6 ± 0.2)	8.2–11.9 (9.4 ± 1.0)	8.8–11.2 (10.0 ± 0.7)	11.9–13.0 (12.3 ± 0.6)	9.0–16.7 (11.7 ± 2.6)	10.2–11.0 (10.6 ± 0.4)	-
Third whorl length (TWL)	-	3.5–4.3 (3.9 ± 0.6)	4.0–4.6 (4.2 ± 0.3)	3.6–4.8 (4.1 ± 0.4)	2.9–4.7 (3.9 ± 0.6)	4.7–5.2 (4.9 ± 0.3)	4.0–6.4 (5.0 ± 0.8)	3.5–4.1 (3.8 ± 0.3)	-
Whorl elongation ratio (WER)	-	2.65–3.14 (2.9 ± 0.4)	2.95–3.29 (3.1 ± 0.2)	2.80–3.25 (3.0 ± 0.2)	2.87–3.84 (3.3 ± 0.3)	2.58–3.66 (3.1 ± 0.5)	2.35–3.17 (2.8 ± 0.3)	3.47–4.54 (4.0 ± 0.4)	-
Whorl number (WN)	-	5.50–6.00 (5.8 ± 0.4)	4.25–5.50 (4.9 ± 0.6)	4.00–5.50 (4.9 ± 0.5)	3.50–5.25 (4.2 ± 0.5)	4.25–4.50 (4.3 ± 0.1)	4.00–6.00 (4.9 ± 0.6)	4.75–5.25 (5.1 ± 0.2)	-
Sculpture type (node / rib / smooth)	-	100% / 0% / 0%	0% / 100% / 0%	10% / 90% / 0%	70% / 30% / 0%	100% / 0% / 0%	100% / 0% / 0%	100% / 0% / 0%	-
Morphological characters of embryonic shells									
Number of embryos (EN)	-	21–53 (31.29 ± 10.4)	43–90 (72.7 ± 18.9)	28–72 (44.7 ± 15.5)	10–52 (25.7 ± 13.8)	21–62 (38.9 ± 15.1)	15–68 (28.9 ± 15.1)	19–56 (35.1 ± 10.4)	17
Longitudinal rib number on body whorl of the largest embryonic shell (RNE)	-	10–11 (10.6 ± 0.5)	10–12 (11.3 ± 0.8)	9–12 (11.0 ± 1.1)	8–13 (10.3 ± 1.6)	9–14 (11.4 ± 1.3)	10–13 (11.5 ± 1.3)	10–13 (11.9 ± 1.2)	-
Shell height of the largest embryonic shell (SHE)	-	2.2–2.7 (2.4 ± 0.2)	1.5–2.7 (2.1 ± 0.4)	2.2–3.7 (2.7 ± 0.4)	1.8–2.6 (2.2 ± 0.3)	2.1–3.2 (2.6 ± 0.3)	2.2–3.6 (2.8 ± 0.5)	2.5–3.9 (3.0 ± 0.4)	2.9
Shell width of the largest embryonic shell (SWE)	-	1.8–2.3 (1.9 ± 0.2)	1.4–2.0 (1.7 ± 0.2)	1.7–2.7 (2.0 ± 0.3)	1.7–2.2 (2.0 ± 0.2)	1.9–2.6 (2.3 ± 0.2)	2.0–2.7 (2.4 ± 0.2)	2.3–3.0 (2.6 ± 0.2)	2.1
Whorl number of the largest embryonic shell (WNE)	-	2.50–3.25 (2.9 ± 0.3)	2.25–3.25 (2.7 ± 0.4)	2.75–3.50 (3.1 ± 0.3)	2.25–3.00 (2.7 ± 0.3)	2.50–3.25 (2.9 ± 0.2)	2.50–3.75 (2.9 ± 0.3)	2.50–3.75 (3.0 ± 0.3)	3.25
Morphological characters of radulae									

cuspid number of rachidian	-	-	5-7	5-7	5-7	5-8	5-9	5-9	-
cuspid number of lateral teeth	-	-	5-7	5-8	5-7	5-8	4-9	5-8	-
cuspid number of interior marginal teeth	-	-	4-6	4-6	4-6	4-6	4-5	4-5	-
cuspid number of exterior marginal teeth	-	-	4-6	4-6	4-6	4-6	4-5	4-5	-

TABLE S3 Result of the ADMIXTURE analysis showing averages, standard deviations, and minimums of the cross-validation
(CV) error value in 1–10 genetic populations (K)

K	Average of the CV error value	Standard deviation of the CV error value	Minimum of the CV error value
1	0.3876	0.00246	0.3815
2	0.3771	0.0039	0.3694
3	0.3679	0.00349	0.3604
4	0.3661	0.00316	0.3588
5	0.3767	0.00385	0.3675
6	0.3915	0.00592	0.3781
7	0.4093	0.00651	0.3951
8	0.4276	0.00726	0.4120
9	0.4455	0.00782	0.4299
10	0.4672	0.00882	0.4496

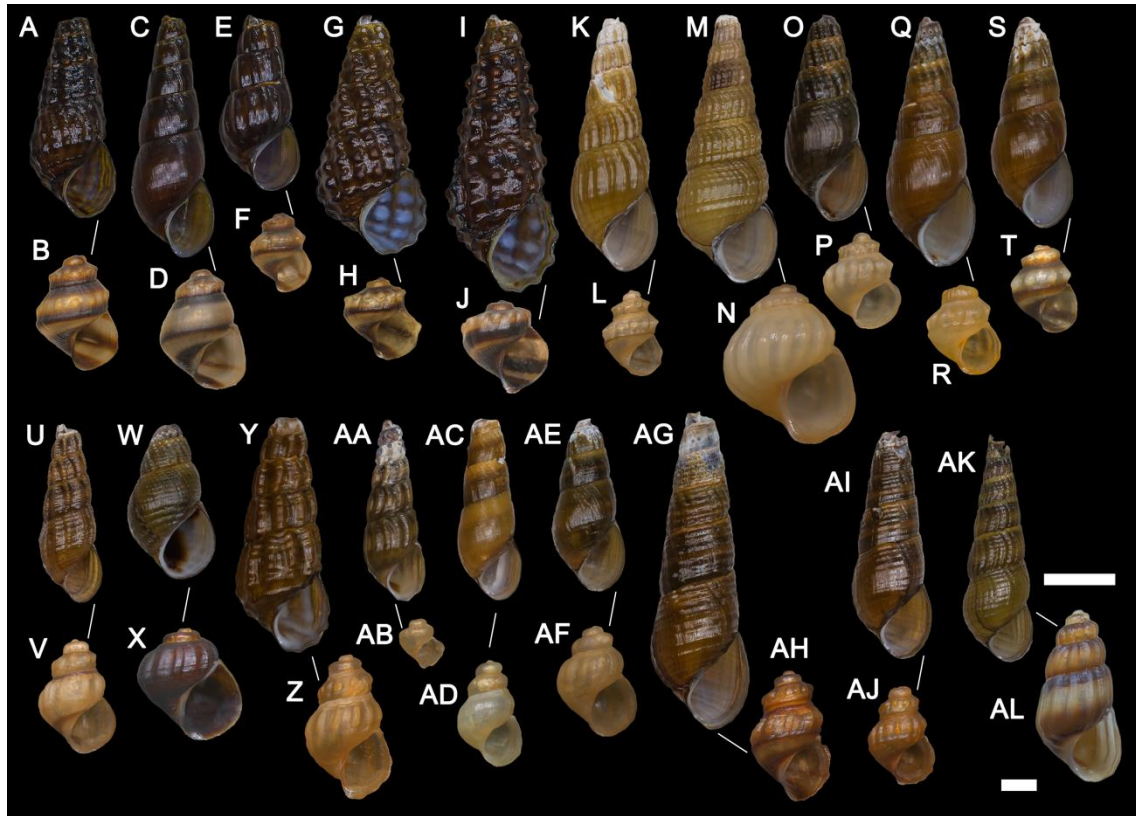


FIGURE S1 Typical shells of five *Semisulcospira niponica*-group species and 13 lacustrine congeners. A–T, *S. habei*-group species. U–AL, *S. decipiens*-group species. A, B, *S. niponica* (Smith, 1876), KUZ Z4093. C, D, *S. fuscata* Watanabe & Nishino, 1995, KUZ Z4105. E, F, *S. watanabei* sp. nov., KUZ Z4109. G, H, *S. nakanoi* sp. nov., KUZ Z4122. I, J, *S. salebrosa* sp. nov., KUZ Z4131. K, L, *S. multigranosa* (Boettger, 1886), KUZ Z4210. M, N, *S. reticulata* Kajiyama & Habe, 1961, KUZ Z4229. O, P, *S. habei* Davis, 1969, KUZ Z4224. Q, R, *S. dilatata* Watanabe & Nishino, 1995, KUZ Z4217. S, T, *S. rugosa* Watanabe & Nishino, 1995, KUZ Z2494. U, V, *S. decipiens*

(Westerlund, 1883), KUZ Z4236. W, X, *S. nakasekoe* Kuroda, 1929, KUZ Z4243. Y, Z, *S. morii* Watanabe, 1984, KUZ Z4255. AA, AB, *S. ourense* Watanabe & Nishino, 1995, KUZ Z4248. AC, AD, *S. arenicola* Watanabe & Nishino, 1995, KUZ Z4235. AE, AF, *S. fluvialis* Watanabe & Nishino, 1995, KUZ Z4242. AG, AH, *S. shiraishiensis* Watanabe & Nishino, 1995, KUZ Z4256. AI, AJ, *S. takeshimensis* Watanabe & Nishino, 1995, KUZ Z4257. AK, AL, *S. davisi* Sawada & Nakano, 2021, KUZ Z3680. Scale bars: 10 mm (adult shell, A, C, E, G, I, K, M, O, Q, S, U, W, Y, AA, AC, AE, AG, AI, AK), 1 mm (embryonic shell, B, D, F, H, J, L, N, P, R, T, V, X, Z, AB, AD, AF, AH, AJ, AL).