



**Oncomelania hupensis iriomotensis n. ssp.
(Caenogastropoda: Truncatelloidea: Pomatiopsidae)
from Waterfalls on Iriomote Island, Okinawa, Southern
Japan**

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ONCOMELANIA HUPENSIS IRIOMOTENSIS N. SSP. (CAENOGASTROPODA: TRUNCATELLOIDEA: POMATIOPSIDAE) FROM WATERFALLS ON IRIOMOTE ISLAND, OKINAWA, SOUTHERN JAPAN

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ABSTRACT

***Oncomelania hupensis iriomotensis* Fukuda & Sawada, n. ssp.**, is described from around waterfalls on Iriomote Island at the southwestern part of the Ryūkyū Archipelago, Okinawa, Japan. The subspecies has an extremely short sperm duct similar to that of *O. h. nosophora* (Robson, 1915) from mainland Japan, but the shell is ovate and readily distinguishable from the latter. According to the mitochondrial phylogeny, the subspecies is included within a clade of *O. hupensis* Gredler, 1881 and its subspecies. It is known only from very narrow ranges in two localities and can be evaluated as critically endangered (CR) according to the criteria for the Red List by the Japanese government. DNA from *Schistosoma japonicum* (Katsurada, 1904), which uses *O. h. nosophora* as an intermediate host, was not detected in environmental DNA analyses of the snail habitats on Iriomote Island. In addition, cercarial shedding was not observed in infection experiments. However, although no data in our study suggest a potential infection risk, further research is required to evaluate the potential risk of schistosomiasis.

Key words: anatomy, conservation, freshwater snail, parasitology, Ryūkyūs, *Schistosoma japonicum*, schistosomiasis, taxonomy.

摘要

澤田直人・桐木雅史・福田 宏：西表島の滝に産するミゾヒダニナ（新生腹足類：クビキレガイ上科：イツマデガイ科）の1新亜種の記載。沖縄県西表島山間部の滝の周囲から新亜種イリオモテミヤイリガイ ***Oncomelania hupensis iriomotensis* Fukuda & Sawada, n. ssp.** を記載する。本新亜種は雌の精子管が極端に短い点で日本本土産ミヤイリガイ（カタヤマガイ）*O. h. nosophora* (Robson, 1915) と共通するが、殻は卵形を呈する点で容易に区別できる。ミトコンドリアDNAに基づく分子系統解析の結果によると本新亜種はミゾヒダニナ *O. hupensis* Gredler, 1881 とその諸亜種（ミヤイリガイを含む）が形成するクレードに含まれる。本新亜種はわずか2箇所のごく狭い範囲でのみ知られ、環境省レッドリストの選定基準に照らせば絶滅危惧IA類 (CR) と見做しうる。ミヤイリガイを中間宿主とする日本住血吸虫 *Schistosoma japonicum* (Katsurada, 1904) のDNAは今回の産地での環境DNA解析で検出されず、感染実験において感染幼虫の遊出は確認できなかった。現時点で感染リスクを示すデータはないが、住血吸虫症の潜在的リスクを評価するためには引き続き研究が必要である。

INTRODUCTION

Recent taxa of the pomatiopsid genus *Oncomelania* Gredler, 1881, live in freshwater habitats and are distributed mainly in southeastern Asia including China, Taiwan, Japan, the Philippines, and Indonesia. Some of them are widely known as intermediate hosts of *Schistosoma*

japonicum (Katsurada, 1904) 日本住血吸虫, the major infectious agent of schistosomiasis (bilharzia). A series of anatomical studies by the late George M. Davis (e.g., Davis, 1967, 1969a, b, 1979, 1980; Davis & Carney, 1973) greatly contributed to understanding the morphological diversity and evolutionary relationships of the taxa belonging to the genus.

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Two *Oncomelania* taxa have been known from Japan: *O. hupensis nosophora* (Robson, 1915) ミヤイリガイ (カタヤマガイ) from central to western Honshū and northern Kyūshū; and *O. minima* (Bartsch, 1936b) ナタネミズボ from northeastern Honshū on the side bordering the Japan Sea. Both of these taxa are restricted to temperate mainland Japan.

Recently the first author discovered an undescribed taxon of the genus around waterfalls in the mountainous areas of Iriomote Island at the southwestern part of the subtropical Ryūkyū Archipelago. We here describe it as a new subspecies of *Oncomelania hupensis* Gredler, 1881 ミゾヒダニナ. We also report the results of genetic and schistosome analyses, including e-DNA surveys and experimental infection trials.

MATERIALS AND METHODS

Specimen Preparation and Morphological Examination

Live animals were removed from the shell without damage by applying the *niku-nuki* method (Fukuda et al., 2008) of boiling for 22 s in hot water at 95°C. Removal was easy and successful in all specimens on which it was tried. Then muscular tissue (a part of the foot and columellar muscle) was removed from the soft parts and fixed in 99% ethanol (EtOH) for DNA analysis. The remainder of the soft parts were fixed in 5% neutral seawater formalin for the anatomical work in this study.

Anatomical investigation was undertaken by the third author using a Leica stereoscopic microscope with a drawing apparatus as in Fukuda (2023) and Fukuda & Kubo (2024). The methods for the extraction of the radulae were those adopted by Fukuda & Mitoki (1995). The radulae together with the shells and opercula were examined by the first author using a Hitachi TM1000 scanning electron microscope at Kyoto University.

Abbreviations for the specimen depositories are: NSMT, National Museum of Nature and Science, Tokyo, Tsukuba, Japan; OKCAB, Conservation of Aquatic Biodiversity, Faculty of Agriculture, Okayama University, Okayama, Japan; and OMNH, Osaka Museum of Natural History, Ōsaka, Japan.

Genetic Analyses

Genomic DNA was extracted from ten specimens of the new subspecies, and one

specimen of *Oncomelania hupensis nosophora* (Table 1). DNA was extracted from the head-foot following the procedure described by Okamoto et al. (2006) using a protein precipitation solution (Promega Corporation, USA). Fragments of the cytochrome c oxidase subunit I (COI) and 16S rRNA (16S) genes were amplified using a polymerase chain reaction (PCR). The PCR was performed with a GeneAmp PCR System 9700 (Thermo Fisher Scientific) using a TaKaRa Ex Premier DNA Polymerase (Takara Bio) and the following primer sets: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') /HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) and 16S-H (5'-CGCCTGTTTATCAAAAACAT-3') /16S-R (5'-CCGGTCTGAAGTCTCAGATCAGT-3') (Simon et al., 1994). Based on Kameda & Kato (2011), the PCR mixtures were heated to 94°C for 4 min; followed by 40 cycles at 94°C for 30 s, different annealing temperatures for each gene (42°C for COI and 52°C for 16S) for 30 s and 72°C for 2 min; with a final extension at 72°C for 5 min. The cycle sequencing reactions were performed according to Sawada et al. (2021). The sequences obtained in this study were deposited in the International Nucleotide Sequence Database Collaboration through the DNA Databank of Japan (LC860648–LC860667).

The phylogenetic relationships among the 18 newly obtained sequences and the 76 sequences of *Oncomelania minima* and the subspecies of *O. hupensis* retrieved from the INSD were estimated (Table 1). These sequences were aligned using MAFFT v7 (Katoh & Standley, 2013) for each gene. Regions of both genes that are only sequenced less than half and nonconserved regions of 16S were trimmed using Gblocks v. 0.91b (Castresana, 2000). The lengths of the COI and 16S sequences were 638 and 527 bp, respectively.

Phylogenetic trees were reconstructed based on a concatenated dataset of COI and 16S using maximum likelihood (ML) with IQ-TREE v1.6.12 (Nguyen et al., 2014) and Bayesian inference (BI) with the MPI version of MrBayes v3.2.7 (Altekar et al., 2004; Ronquist et al., 2012). The best-fit models were calculated based on the Bayesian information criterion and the greedy algorithm using ModelFinder implemented in IQ-TREE (Kalyaanamoorthy et al., 2017) for the ML and using PartitionFinder v2.1.1 (Lanfear et al., 2016) for the BI. The selected substitution models for each partition are shown in Table 2. The branch support of the ML phylogenetic tree was assessed using

TABLE 1. Specimen list of the present molecular analyses with collection localities, accession numbers of the International Nucleotide Sequence Database (INSD), and references

Taxon	Locality	INSD Accession #		Reference
		COI	16S	
<i>Oncomelania hupensis hupensis</i> Gredler, 1881	Guangde, Anhui, China	JF284686	JF284686	Li et al. (2013)
	Guangde, Anhui, China	JF284687	JF284687	Li et al. (2013)
	Yangzhou, Jiangsu, China	JF284688	JF284688	Li et al. (2013)
	Gong'an, Hubei, China	JF284689	JF284689	Li et al. (2013)
	Jiangling, Hubei, China	JF284690	JF284690	Li et al. (2013)
	China	LC276221	LC276221	unpublished
	China	LC276222	LC276222	unpublished
	China	LC276223	LC276223	unpublished
<i>Oncomelania hupensis quadras</i> (Möllendorff in Quadras & Möllendorff, 1895)	Pu-Yen, Philippines	DQ112287	DQ212862	Wilke et al. (2006)
	Calatrava, Negros Occidental, Philippines	-	KY432667	Chua et al. (2017)
	Calatrava, Negros Occidental, Philippines	-	KY432668	Chua et al. (2017)
	Magsaysay, Bohol, Philippines	-	KY432669	Chua et al. (2017)
	Philippines	LC276227	LC276227	unpublished
	Philippines	DQ112288	-	unpublished
	Philippines	DQ112289	-	unpublished
<i>Oncomelania hupensis formosana</i> (Pilsbry & Y. Hirase, 1906)	Pu-Yen, Taiwan	DQ112283	DQ212861	Wilke et al. (2006)
	Taiwan	DQ112281	-	unpublished
	Taiwan	DQ112282	-	unpublished
<i>Oncomelania hupensis nosophora</i> (Robson, 1915)	Nirasaki, Yamanashi, Japan	AB611787	AB611786	Kameda & Kato (2011)
	Kisarazu, Chiba, Japan	KR002673	KR002676	Attwood et al. (2015)
	Japan	LC276225	LC276225	unpublished
	Japan	LC276226	LC276226	unpublished
	Japan	DQ112284	-	unpublished
	Japan	DQ112285	-	unpublished

(continues)

TABLE 1. Continued.

Taxon	Locality	INSID Accession #		Reference
		COI	16S	
<i>Oncomelania hupensis nosophora</i> (continued)	Japan	DQ112286	-	unpublished
	Nirasaki, Yamanashi, Japan	LC860648	LC860659	This study
<i>Oncomelania hupensis tangi</i> (Bartsch, 1936b)	Daqin Hailiang, Fujian, China	DQ212796	DQ212860	Wilke et al. (2006)
<i>Oncomelania hupensis robertsoni</i> (Bartsch, 1946)	Anning River Valley, Sichuan, China	AF213339	AF212893	Wilke et al. (2000)
	Mianzhu, Sichuan, China	AF531547	AF531545	Attwood et al. (2003)
	Magau, Sichuan, China	DQ212799	DQ212863	Wilke et al. (2006)
	Magau, Sichuan, China	DQ212808	DQ212871	Wilke et al. (2006)
	Minhe, Sichuan, China	DQ212823	DQ212878	Wilke et al. (2006)
	Gucheng, Sichuan, China	DQ212829	DQ212884	Wilke et al. (2006)
	Gucheng, Sichuan, China	DQ212833	DQ212887	Wilke et al. (2006)
	Shuanggou, Sichuan, China	DQ212848	DQ212896	Wilke et al. (2006)
	Zi Ran, Yunnan, China	DQ212857	DQ212902	Wilke et al. (2006)
<i>Oncomelania hupensis chiui</i> (Habe & Miyazaki, 1962)	Taiwan	DQ112271	-	unpublished
	Taiwan	DQ112272	-	unpublished
	Taiwan	DQ112273	-	unpublished
	Taiwan	DQ112274	-	unpublished
	Taiwan	DQ112275	-	unpublished
	Taiwan	DQ112276	-	unpublished
	Taiwan	DQ112277	-	unpublished
	Taiwan	DQ112278	-	unpublished
	Taiwan	DQ112279	-	unpublished
<i>Oncomelania hupensis iriomotensis</i> n. ssp.	Yuchin Falls, Okinawa, Japan	LC860649	LC860660	This study
	Yuchin Falls, Okinawa, Japan	LC860650	LC860661	This study

(continues)

TABLE 1. Continued.

Taxon	Locality	INSD Accession #		Reference
		COI	16S	
<i>Oncomelania hupensis iriomotensis</i> n. ssp.(continued)	Yuchin Falls, Okinawa, Japan	LC860651	LC860662	This study
	Yuchin Falls, Okinawa, Japan	LC860652	LC860663	This study
	Yuchin Falls, Okinawa, Japan	LC860653	LC860664	This study
	near Maryudō Falls, Okinawa, Japan	LC860654	LC860665	This study
	near Maryudō Falls, Okinawa, Japan	LC860655	LC860666	This study
	near Maryudō Falls, Okinawa, Japan	LC860656	-	This study
	near Maryudō Falls, Okinawa, Japan	LC860657	LC860667	This study
	near Maryudō Falls, Okinawa, Japan	LC860658	-	This study
<i>Oncomelania minima</i> (Bartsch, 1936b)	Wajima, Ishikawa, Japan	AB611791	AB611790	Kameda & Kato (2011)
	Sado, Niigata, Japan	AB611795	AB611794	Kameda & Kato (2011)

200 nonparametric bootstrap (BS) replicates. In the BI phylogeny, the four Markov chains in eight independent runs were conducted for ten million generations and the tree was sampled every 100 generations. After confirming the parameter estimates and convergence using Tracer v1.7.2 (Rambaut & Drummond, 2009), the first million trees were discarded. *Oncomelania minima* was set as outgroup for

the present analysis, based on the phylogenetic relationships within the genus (Kameda & Kato, 2011). The mean COI pairwise genetic distances among the *Oncomelania* taxa were estimated using MEGA v11 (Tamura et al., 2021).

Experimental Infection of Snails with *Schistosoma japonicum*

First, we placed snails in 15 cm diameter Petri dishes lined with wiping paper and fern leaves (*Microsorium pteropus* (Blume) Copel, 1929 ミツデヘラシダ; Polypodiaceae ウラボシ科). Next, water was added until the paper was fully submerged. To control evaporation, a 15 cm diameter circular glass plate with a central hole 2 cm in diameter was used as a lid. Snails were maintained at 25°C, and water was replenished as needed to prevent desiccation. Next, experimental infection of snails with *Schistosoma japonicum* (Sj) from Yamanashi Prefecture, Japan, was conducted at Dokkyo Medical University. To do so, miracidia were obtained from eggs sourced from the livers of

TABLE 2. The selected substitution models for each partition in the phylogenetic analyses.

Partitions	Model (ML)	Model (BI)
COI 1st position	TIM3e+G4	HKY+G
COI 2nd position	F81+F	HKY+G
COI 3rd position	TPM2u+F+R2	HKY+G
16S	HKY+F+I+G4	HKY+G

TABLE 3. Transition of *Oncomelania hupensis iriomotensis* n. ssp. in experimental infection studies of *Schistosoma japonicum*.

Exp.	Exposure date	Locality of snail	No. of miracidia exposed	Number of snails at each postinfection period						
				Day 0	Week 4	Week 6	Week 7	Week 8	Week 10	Week 13
1	2022/07/20	Yuchin River	5	57	40	-	40	Dead all	-	-
		Yuchin River	1	20	16	-	16	Dead all	-	-
2	2022/09/08	Yuchin Falls	5	9	9	7	-	6	Dead all	-
3	2022/09/21	Yuchin Falls	5	33	32	32	-	30	29	Dead all
		Yuchin River	5	12	13	13	-	6	1	Dead all

S. japonicum-infected mice. Miracidia were then counted under a dissecting microscope, and either one or five miracidia were added to each well of a 96 well microplate. Subsequently, individual snails were placed into separate wells (one per well) and exposed to miracidia overnight. We conducted three experimental infections on July 20 (Exp. 1; $n = 77$), September 8 (Exp. 2; $n = 9$), and September 21 (Exp. 3; $n = 45$) 2022 (Table 3). After infection, snails were maintained in a dark box to prevent light-induced release of cercariae, the infective larvae capable of infecting mammalian hosts including humans. Beginning four weeks postinfection, the snails were examined to assess cercarial emergence once every two weeks. For assessment, infected snails were placed in the wells of a 24 well microplate with 2 ml of water, then exposed to fluorescent light for at least 3 h to induce cercarial release. Finally, the presence of cercariae in each well was confirmed using a dissecting microscope.

Detection of *Schistosoma japonicum* DNA in Water Samples and Snails

We collected river-water samples from three distinct habitat sites of the new subspecies on Iriomote Island. Collecting sites included an unnamed waterfall near a pathway towards Maryudō Falls (i.e., “Maryudō”), a site near Yuchin Falls, and the Yuchin River. At each site, we obtained two 500 mL water samples and stored them in plastic containers. Snail-rearing water samples were collected from Petri dishes

used for their maintenance in the laboratory, as described above. Descriptions of water samples are provided in Table 4. Water samples were then subjected to environmental DNA (eDNA) analysis (Sato et al., 2018, Fornillos et al., 2019). Briefly, to prevent DNA degradation, 0.1% (v/v) of a 10% (w/v) benzalkonium chloride solution was first added to water samples, which were then mixed thoroughly before being transported and stored under cool conditions until further use. Next, water samples were individually filtered using circular glass fiber filter paper (GF/F, 0.7 μ m; Whatman, Maidstone, UK) and a Büchi V-500 Vacuum Pump (Büchi Labortechnik AG, Switzerland). The resulting filter paper samples were preserved in 70% ethanol, then dried, wrapped in aluminum foil, and stored in a freezer at -20°C or below. DNA trapped on these filters was then extracted and purified using a DNeasy Blood and Tissue Kit (Qiagen) according to the method described by Sato et al. (2018).

Next, we gathered snails that died ~ 13 weeks after S_j infection in Exp. 3 and preserved them in 70% ethanol until use. In total, we obtained 27 snails and divided this sample into four experimental groups (i.e., A: 10 snails, B: 8, C: 6, D: 3). Due to tissue degeneration, which made separation from the shell difficult, the entire snail, including the shell, was used for DNA extraction. To obtain this DNA, snails from each group were placed in 2 mL tubes containing three 4 mm stainless steel beads to which 180 μ L of ATL Buffer was added. Samples were then homogenized using a bead crusher (μ T-

TABLE 4. Result of real-time quantitative PCR for *Schistosoma japonicum* (Sj).

Sample	Date of sample collection	Sample description	Volume (ml)	TaqMan qPCR Qty (CP) (ng/ml)
Water samples collected from snail localities	2022/04/21	Maryudō F-1	500	-
	2022/04/21	Maryudō F-2	500	-
	2022/04/20	Yuchin F-1	500	-
	2022/04/20	Yuchin F-2	500	-
	2022/04/20	Yuchin R-1	500	-
	2022/04/20	Yuchin R-2	500	-
Water in petri dish rearing snails	2022/04/27	Snails at Yuchin Falls	20	-
	2022/04/27	Snails at Yuchun River	20	-
	2022/06/24	Snails at Yuchin Falls	20	-
	2022/06/24	Snails at Yuchin River	20	-
Water in petri dish rearing Sj infected snails, in Exp. 3	2022/11/18	Exp. 3, 8 wpi, Yuchin River	8	-
	2022/11/18	Exp. 3, 8 wpi, Yuchin Falls	8	-
	2022/12/20	Exp. 3, 13 wpi, Yuchin River	8	-
	2022/12/20	Exp. 3, 13 wpi, Yuchin Falls	8	-
DNA extracted from dead snails in Exp. 3	2022/12/20	A: DNA extracted from 10 snails	-	67.76
	2022/12/20	B: DNA extracted from 8 snails	-	15.16
	2022/12/20	C: DNA extracted from 6 snails	-	12.28
	2022/12/20	D: DNA extracted from 3 snails	-	9.77

12, Taitec Corporation, Saitama, Japan). After incubation at 56°C for 1 h, samples were left to stand thereafter, and the supernatant, free of debris, was used for DNA purification using a DNeasy Blood and Tissue Kit.

To identify *Schistosoma japonicum* DNA present in water samples and snail samples, we performed real-time quantitative PCR (qPCR) following a method described by Sato et al. (2018). Briefly, the forward primer Sj_COI_F (5'-TTTGATAACTAATCACGGTATAGCAA-3') and the reverse primer Sj_COI_R (5'-CGAGGCAAAGCTAAATCACTC-3'), designed to amplify approximately 119 bp of the *cox1* gene, were used for all analyses. The qPCR reaction was then visualized using a TaqMan custom probe specific for *S. japonicum* (5'-FAM-TTTTGGTAAATATCTTCTCCG-MGB-NFQ-3'). qPCR was conducted using

a Thermal Cycler Dice® Real Time System II MRQ (TP960, Takara Bio Inc., Shiga, Japan). Finally, sample DNA quantity was determined using Multiplate RQ, a relative quantification analysis package; for this analysis DNA extracted from adult *S. japonicum* was used as the experimental standard.

SYSTEMATICS

Family Pomatiopsidae W. Stimpson, 1865
Subfamily Pomatiopsinae W. Stimpson, 1865
Tribe Pomatiopsini W. Stimpson, 1865

Genus *Oncomelania* Gredler, 1881: 120–122

Type species: *Oncomelania hupensis* Gredler, 1881, by monotypy.

Synonyms

Hemibia Heude, 1890: 168. Type species: *Oncomelania hupensis* Gredler, 1881, subsequent designation by Bouchet et al., 2017: 123.

Katayama Robson, 1915: 203. Type species: *Katayama nosophora* Robson, 1915, by original designation.

Schistosomophora Bartsch, 1936a: 29. Type species: *Tricula quadrasii* Möllendorff in Quadras & Möllendorff, 1895, by original designation.

Remarks

According to MolluscaBase (2021), five valid names of Recent species are recognised in *Oncomelania* apart from the type species: *O. cantoni* (Bartsch, 1925: 72, 73; ssp. of *Katayama fausti* Bartsch, 1925); *O. lii* (Bartsch, 1936a: 27, pl. 1, fig. 5; pl. 2, fig. 11; pl. 4, fig. 4; pl. 6, figs. 4, 7; in *Katayama*); *O. minima* (Bartsch, 1936b: 33, 34, pl. 1, fig. 9; pl. 2, fig. 7; pl. 3, fig. 3; in *Schistosomophora*); *O. moellendorffii* (Schmacker & O. Boettger, 1890: 126, 127, no. 36, pl. 2, fig. 7; in *Prososthenia*); *O. yaoi* Bartsch, 1939: 174, 175, figs. 1, 2. However, Davis et al. (1999b: 297) clearly mentioned that *Oncomelania* is comprised of “only two species” (*O. hupensis* and *O. minima*). For the other four “species,” the anatomy and relationships have been poorly examined. Therefore, some of them might be assigned to subspecies of *O. hupensis* in future studies.

Oncomelania hupensis Gredler, 1881,
sensu lato

Oncomelania hupensis Gredler, 1881:
120–122, pl. 6, fig. 5.

Type locality: “Von unserem Sammler P. Fuchs bei U-tschang-fu im März 1879 entdeckt, als ‘selten’ bezeichnet und in drei übereinstimmenden Exemplaren mitgeteilt.”

Remarks: According to classifications by Davis & Carney (1973), Davis (1980), and Davis et al. (1995, 1997, 1999a, b), *Oncomelania hupensis* includes the following eight subspecies: *O. h. chiui* (Habe & Miyazaki, 1962: 47–49, figs. 1–3; in *Tricula*); *O. h. formosana* (Pilsbry & Y. Hirase, 1906: 750, 751; in *Blanfordia*) タイワンミゾメタニシ(タイワンカタヤマガイ); *O. h. hupensis* Gredler, 1881ミゾヒダニナ (see above); *O. h. lindoensis* Davis & Carney, 1973: 4–33, figs. 5–20; *O. h. nosophora* (Robson, 1915: 203, figs. 7, 8, 11; in *Katayama*) ミヤイ

リガイ(カタヤマガイ, ヨシダミゾメタニシ); *O. h. quadrasii* (Möllendorff in Quadras & Möllendorff, 1895: 121, 138; in *Tricula*, *Prososthenia*); *O. h. robertsoni* (Bartsch, 1946: 4, 5, pl. 1, figs. 1, 7–10; in *Schistosomophora*); *O. h. tangi* (Bartsch, 1936b: 139–141; in *Katayama*). In contrast, Woodruff et al. (1999) elevated *Oncomelania lindoensis* and *O. quadrasii* to full species rank as well as *O. minima* and *O. hupensis sensu lato* because of the significant genetic distances, whereas *O. h. chiui*, *O. h. formosana*, *O. h. nosophora*, *O. h. robertsoni*, and *O. h. tangi* were maintained as subspecies of *O. hupensis*.

The shells of the *Oncomelania hupensis* complex are highly variable, from ovate-conical to elongate in shape, and with or without distinct axial ribs. However, they (except *O. h. robertsoni* and *O. h. tangi*) were reported to hybridise *in vitro*, producing viable and fertile F1 and F2 generations (Wagner et al., 1957; Komiya & Kojima, 1958; Wagner & Chi, 1959; Chi et al., 1971; Davis & Ruff, 1973). Also, the same chromosome number ($n = 17$, $2n = 34$) is shared in four of these subspecies (*O. h. hupensis*, *O. h. formosana*, *O. h. nosophora*, and *O. h. quadrasii*; Burch, 1960, 1964).

In our phylogenetic analysis (see below), the taxon from Iriomote Island was included within a clade comprising of *Oncomelania h. hupensis*, *O. h. chiui*, *O. h. formosana*, *O. h. tangi*, *O. h. nosophora*, *O. h. robertsoni*, and *O. h. quadrasii*. Thus, we here treat and describe the new taxon as a subspecies of *O. hupensis*.

Oncomelania hupensis iriomotensis Fukuda & Sawada, n. spp.

イリオモテミヤイリガイ (西表宮入貝
Iriomote-miyairi-gai, 福田・澤田 in 岩崎他
Iwasaki et al., 2025; 新亜種)
Figs. 1–50

Oncomelania hupensis subsp.—Iwasaki et al., 2025: 148, 149, fig. 9.

Type material

Holotype. Female. Yuchin (Yutsun) Falls, at the middle stream of the Yuchin River, Takana, Iriomote Island, Taketomi-chō, Yaeyama-gun, Okinawa Prefecture, Japan 沖縄県八重山郡竹富町西表島 高那 ユチン川中流域 ユチン(ユツン) 滝, 24°22'01"N, 123°53'03"E, April 3, 2021, collected by N. Sawada (NS) and Y. Morii (YM) (OMNH Mo 41176).

Paratypes. Same data as holotype. 50+ live individuals including five males and 19 females

(OMNH-Mo 41177–41184; OKCAB M31171; NSMT-Mo 79620–79621), same locality as holotype. 50+ live individuals including 10+ juveniles, April 20, 2022, collected by NS, J.U. Ōtani (JO), Y. Nakahara (YN), and H. Fukuda (HF) (NSMT-Mo 79616–79619); a small unnamed waterfall near a pathway toward Maryudō Falls along the middle stream of the Urauchi River, Uehara, Iriomote Island 西表島 上原 浦内川 中流域 マリュドウの滝へ向かう遊歩道沿いの小規模な無名滝, 24°21'37"N, 123°48'02"E. 20+ live individuals including three males and five females, April 7, 2021, collected by YM (NSMT-Mo 79615); April 21, 2022, collected by NS, JO, YN, and HF (OMNH-Mo 41185–41187; OKCAB M31172; NSMT-Mo 79612–79614).

Etymology: Named after Iriomote Island, the type location of the new subspecies.

Diagnosis: Shell thin, translucent, brownish in colour, ovate-conical with convex whorls and impressed suture. No distinct sculpture on teleoconch except for weak growth lines. Umbilicus narrow but distinctly open. Operculum pyriform, paucispiral, horny, simple. Dorsal surface of head-foot covered with black pigments. Testis and ovary both small. Penis large, wide, flat, very muscular with distinct terminal papilla. Sperm duct between oviduct and bursal duct extremely short.

Description

Shell (Figs. 1–13, 20, 21) ovate conical, thin, translucent, brownish in colour. Protoconch (usually eroded and lost in adults; observed in minute juveniles) with 2.5 convex whorls, surface almost smooth except for indistinct, discontinuous spiral sculpture along suture; demarcation point between protoconch and teleoconch (Figs. 20, 21, dp) distinct, with no varix. Teleoconch of 1.5–2.25 convex whorls with distinctly impressed suture; fine axial growth lines on surface of each whorl. Aperture wide, semicircular; outer lip slightly thickened in fully mature individuals, prosocline, with no varix; parietal lip rather long, nearly straight, covered with narrow, thin callus; columellar lip wide, distinctly curved, reflected to umbilicus, with simple, round anterior end. Umbilicus narrow but distinctly open. For measurements, see Table 5. Males usually smaller than females.

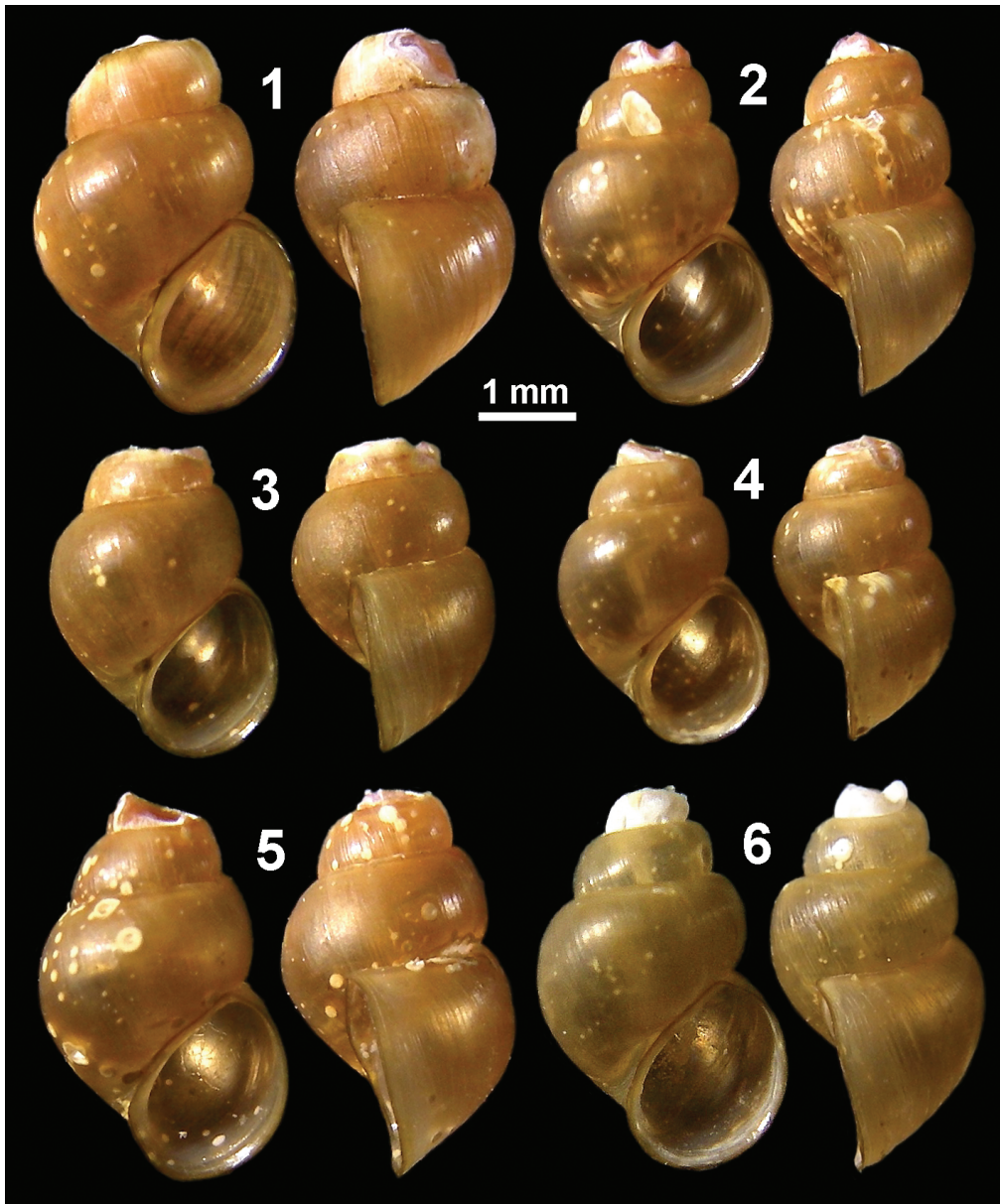
Operculum (Figs. 8, 13, 22–27, 33) pyriform, paucispiral, thin, horny, yellowish, transparent, slightly concave; nucleus situated near middle part. Outer surface with fine growth lines

regularly found on outer surface. Inner surface glossy; muscle scar long, elongate-rectangular, occupying about four-fifths of opercular length, one-third in width; prominent branching and anastomosing system present around nucleus (Figs. 24, 27).

Head-Foot (Figs. 7–13, 28–33). Majority of head-foot dark grey to black except for colourless sole (Figs. 9, 32, s) and inside of omniphoric grooves (Figs. 28, 30, 31, rog, log). Cephalic tentacles (Figs. 7–11, ct) moderately long, tapering with blunt tip; spreading out when crawling in water (Figs. 7, 8, 11–13, 28, 30–32) but shrinking and facing forwards in air (Figs. 10, 29). Distinct black eyes (e) in lateral bulges at outer bases of cephalic tentacles; mass of tiny white or pale yellowish granules at proximal portions of cephalic tentacles and eye bulges. Snout (sn) long, cylindrical in water, rectangular in air, distally bilobed, with approximately eight constrictions on dorsal surface at even intervals. Foot wide, rectangular; anterior edge simple, slightly expanded on both sides; lateral edge almost parallel; posterior end simple, rounded. Opening of anterior pedal mucous gland wide, transverse slit at anterior edge of foot (Fig. 32, ms); anterior pedal mucous gland (amg) composed of many small, white cells arranged in transverse row just behind slit. Narrow omniphoric grooves (rog, log), running anteroventrally on both sides of head (Figs. 28, 30, 31); right groove (rog) twice as wide as left groove (log); short protuberances at anteroventral edges of grooves; right groove carrying faecal pellets from inside mantle cavity to outside; both grooves carrying mucous to foot (observed in living animals).

Suprapedal fold (Figs. 28, 30, 31, spf) distinct, nearly straight, arising from posterior edge of omniphoric grooves, running parallel to lateral edge of foot, then connecting to edge of opercular lobe. White granules visible through anterior region of translucent operculum (Fig. 33). Sole (Fig. 32, s) broad, flat. Mode of locomotion “step-like” (see Davis, 1967: 11; Ponder, 1988: 157, 158; Fukuda, 2023: fig. 4); animal active.

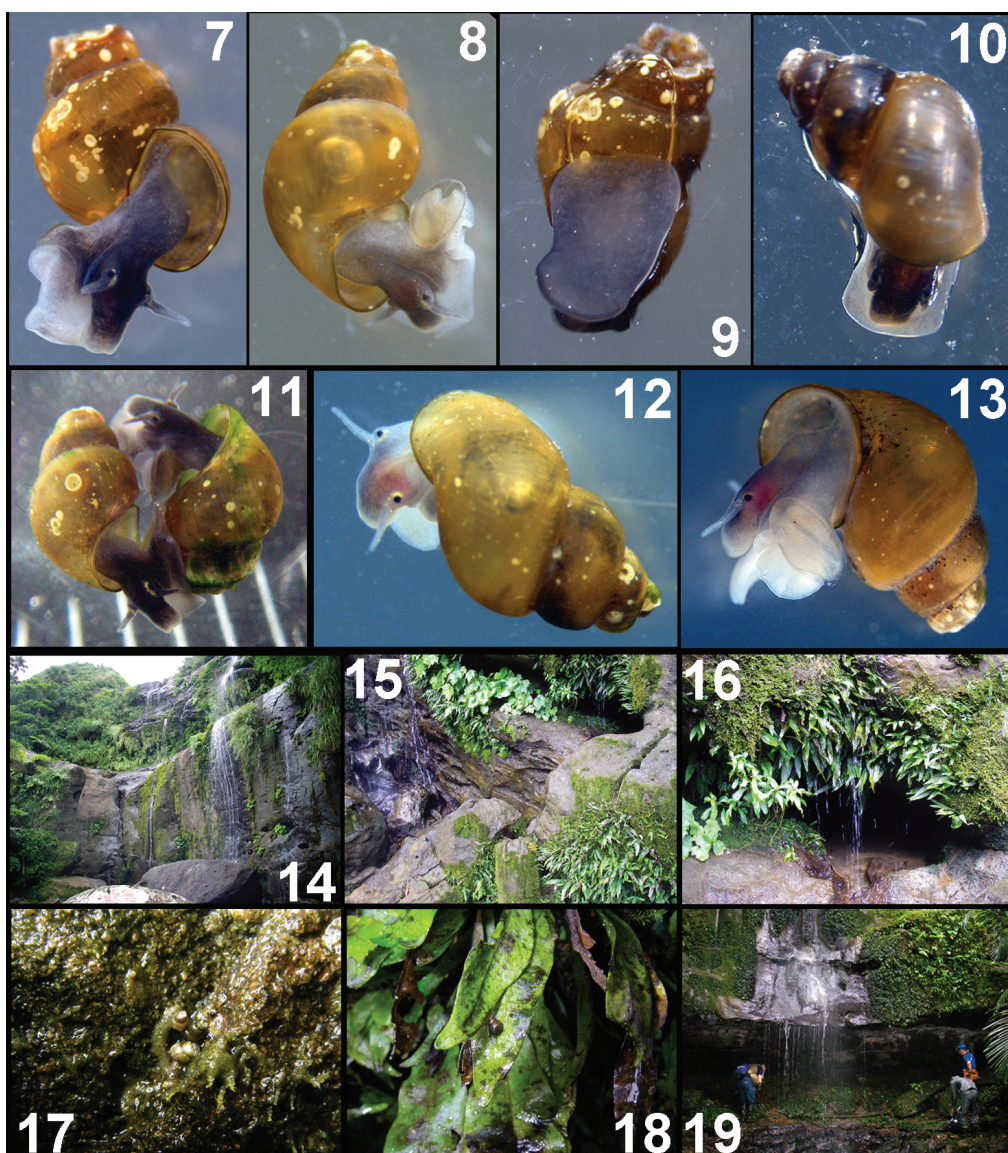
Mantle Cavity (Fig. 34) rather long (depth of cavity ~ 3.5 mm), occupying about half of last whorl of shell. Whole dorsal mantle roof translucent with thin black pigmentation in some individuals. Kidney compact, consisting of mass of colourless, translucent cells, situated entirely behind mantle cavity with indistinct opening at posteriormost corner of cavity. Gill (ctenidium) well developed, comprising ~ 30–35 conspicu-



FIGS. 1–6. *Oncomelania hupensis iriomotensis* n. ssp., shells. Iriomote Island, Okinawa, Japan. (1) Holotype, OMNH Mo 41176. (2–6): Paratypes, OKCAB M 31171, 31172. (1–5) Yuchin Falls, Yuchin River, Takana (type locality); (6) unnamed waterfall, near Urauchi River, Uehara.

ous translucent filaments of gf1 and gf2 (*sensu* Davis et al., 1984: 170, fig. 6A); located on posterior portion of efferent vein near heart (auricle, au + ventricle, ve) on left ventral side of cavity; gf1 short, rectangular, abutting each

other; gf2 arising from mid-dorsal portion of each gf1, long, narrow, with sharp tip, queueing with regular intervals. Osphradium (os) long, elongate-fusiform with undulating dorsal edge, containing short, elliptical osphradial ganglion



FIGS. 7–19. *Oncomelania hupensis irimotensis* n. ssp. (7–13) Living individuals; (14–19) habitats: (7–11, 14–18) Yuchin Falls, Yuchin River; (12, 13, 19) unnamed waterfall, near Urauchi River.

(osg) at anterior portion (see also Central nervous system, below). Hypobranchial gland not examined.

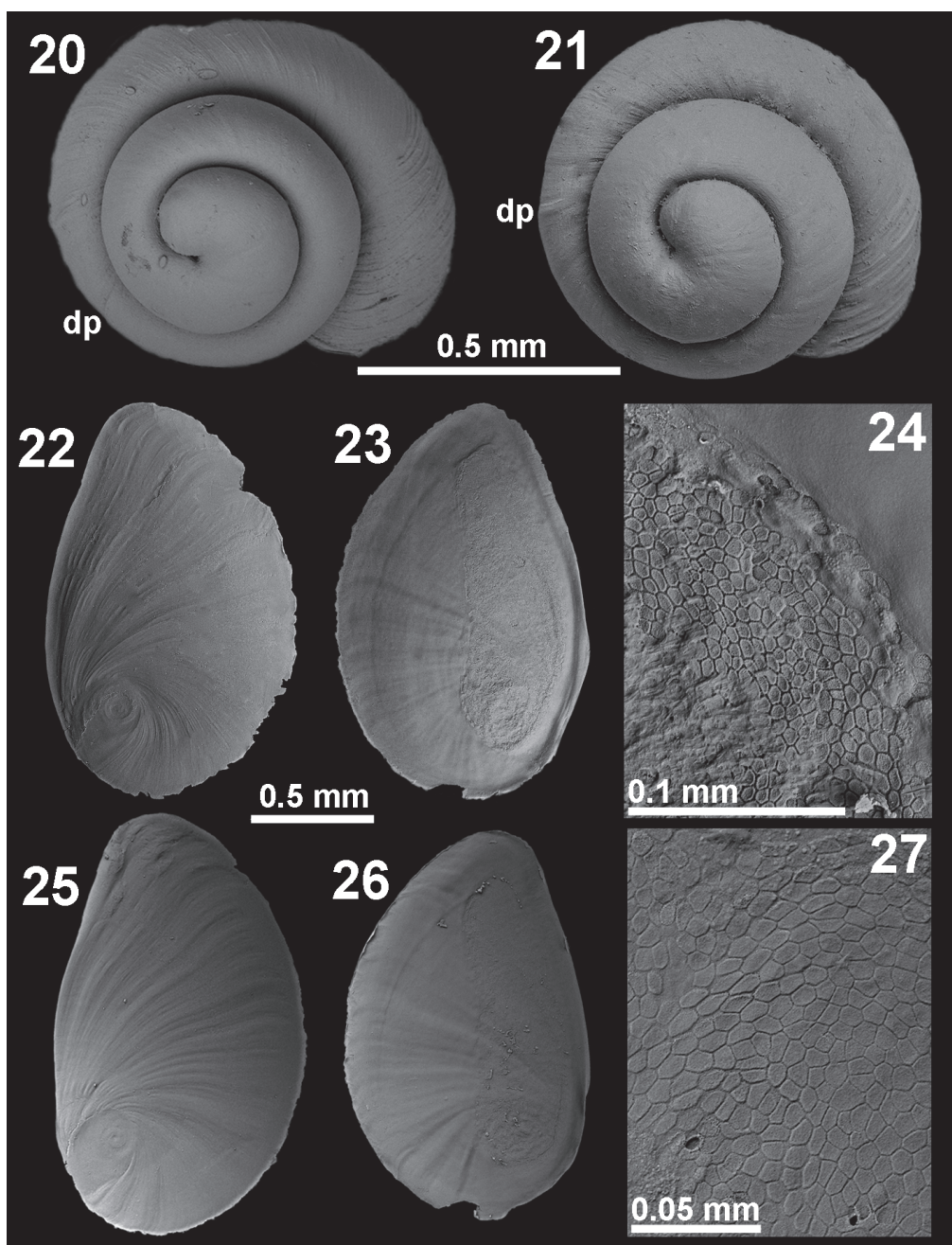
Digestive System (Figs. 32, 35–39, 40, 48). Mouth (Figs. 32, 48, m) opening between pair of muscular lips into buccal cavity. Buccal mass (Fig. 48, bm) rather large, wide, occupying most of snout. Radula (rad) visible through translucent dorsal surface. Pair of salivary

glands (sg) at posterior corner of dorsolateral sides of buccal mass, not passing through nerve ring; salivary glands colourless, short, simple, finger-like, sometimes bent. Buccal pouches probably weakly developed, not observed in gross dissection. Radular sac very short, not visible laterally.

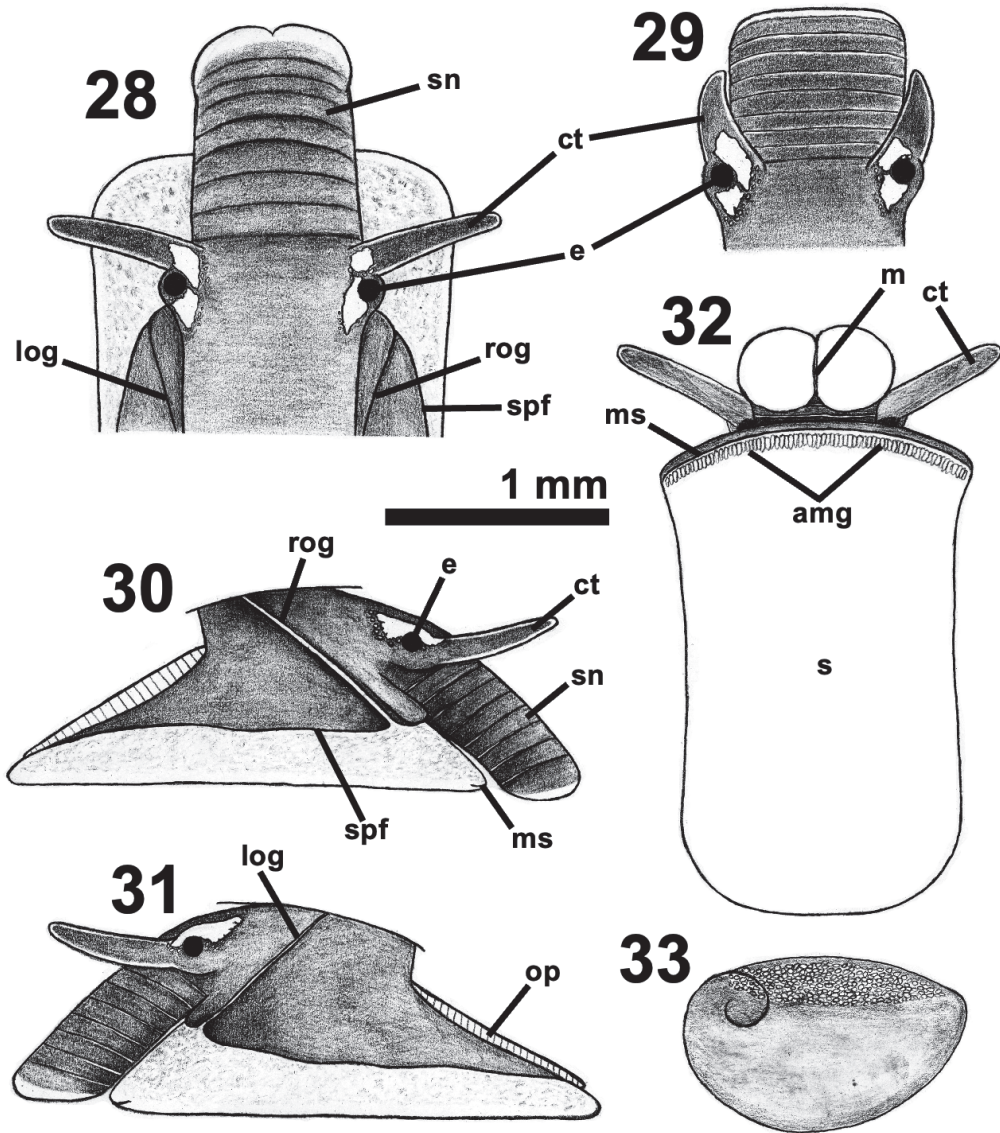
Radula (Figs. 35–39) taenioglossate, whole length ~ 0.6 mm (Fig. 35). Central teeth trap-

TABLE 5. Shell measurements (mm) for *Oncomelania hupensis iriomotensis* n. ssp.

		Shell length	Shell width	Body whorl length	Aperture length	Aperture width	Total number of whorls	
Yuchin Falls, Yuchin River, Takana	Holotype	♀	3.9	2.6	3.2	2.0	1.5	4.0
	Paratypes	♂ (n = 5)	3.0 ± 0.1 (2.9–3.1)	2.1 ± 0.1 (2.0–2.2)	2.4 ± 0.1 (2.3–2.6)	1.6 ± 0.1 (1.5–1.7)	1.3 ± 0.1 (1.2–1.4)	3.8 ± 0.3 (3.5–4.0)
		♀ (n = 13)	3.6 ± 0.3 (3.0–4.0)	2.4 ± 0.2 (2.0–2.6)	2.9 ± 0.2 (2.4–3.1)	1.8 ± 0.1 (1.6–2.0)	1.4 ± 0.1 (1.1–1.6)	4.0 ± 0.4 (3.3–4.8)
		Total (n = 19)	3.5 ± 0.4 (2.9–4.0)	2.3 ± 0.2 (2.0–2.6)	2.8 ± 0.3 (2.3–3.2)	1.8 ± 0.2 (1.5–2.0)	1.4 ± 0.1 (1.1–1.6)	3.9 ± 0.4 (3.3–4.8)
nameless waterfall, along Urauchi River, Uehara	Paratypes	♂ (n = 3)	2.9 ± 0.2 (2.8–3.1)	2.1 ± 0.1 (2.0–2.3)	2.4 ± 0.2 (2.3–2.6)	1.6 ± 0.2 (1.5–1.8)	1.3 ± 0.0 (1.3–1.3)	3.4 ± 0.4 (3.0–3.8)
		♀ (n = 5)	3.5 ± 0.3 (3.1–4.0)	2.3 ± 0.2 (2.1–2.5)	2.7 ± 0.3 (2.4–3.1)	1.8 ± 0.1 (1.6–2.0)	1.4 ± 0.2 (1.2–1.6)	4.0 ± 0.3 (3.5–4.3)
	Sex unknown (n = 1)		3.3	2.6	1.8	2.3	1.4	4.0
	Total (n = 9)		3.3 ± 0.4 (2.8–4.0)	2.3 ± 0.2 (2.0–2.5)	2.6 ± 0.2 (2.3–3.1)	1.7 ± 0.2 (1.5–2.0)	1.4 ± 0.1 (1.2–1.6)	3.8 ± 0.4 (3.0–4.3)
Total (n = 28)		3.4 ± 0.4 (2.8–4.0)	2.3 ± 0.2 (2.0–2.6)	2.7 ± 0.3 (2.3–3.2)	1.8 ± 0.2 (1.5–2.0)	1.4 ± 0.1 (1.1–1.6)	3.9 ± 0.4 (3.0–4.8)	



FIGS. 20–27. *Oncomelania hupensis iriomotensis* n. ssp. (20, 21) Protoconchs. (22–27) Opercula. (20–24) Yuchin Falls; (25–27) unnamed waterfall, near Urauchi River. dp, demarcation point between protoconch and teleoconch.



FIGS. 28–33. *Oncomelania hupensis iriomotensis* n. ssp., head-foot, Yuchin Falls. (28) Dorsal view of head in water; (29) dorsal view of head in air; (30) from right lateral side; (31) from left lateral side; (32) ventral view of foot; (33) dorsal view of operculum showing white spots on opercular lobe (visible through translucent operculum). amg, anterior pedal mucous gland; ct, cephalic tentacle; e, eye; log, left omniphoric groove; m, mouth; ms, mucous slit; op, operculum; rog, right omniphoric groove; s, sole; sn, snout; spf, suprapedal fold.

ezoid; cutting edge approximately two-thirds of total width, with five large cusps; median cusp longest and widest, up to two to three times as wide as adjacent cusps; lateral edges

wide, spread at $\sim 30^\circ$ to cutting edge; three pairs of prominent basal cusps laterally; short, broad basal process arising from middle part of ventral edge. Lateral teeth with elongate

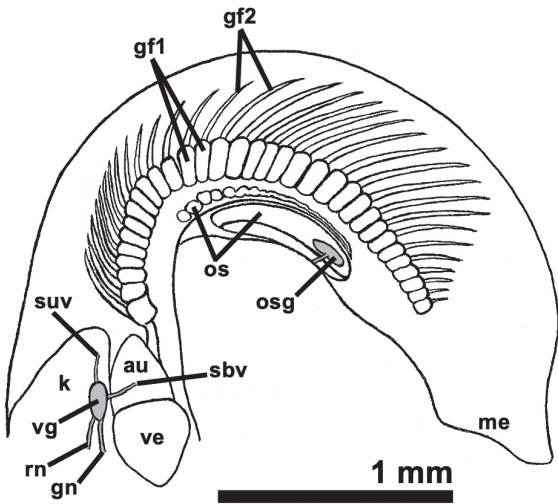
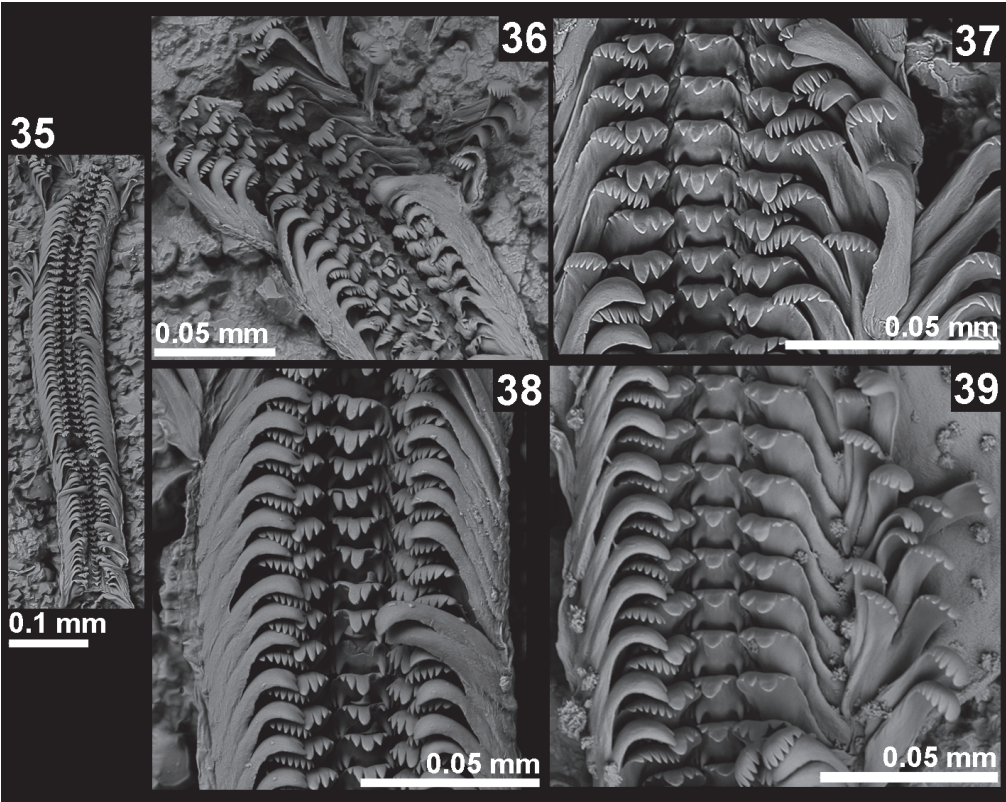


FIG. 34. *Oncomelania hupensis iriomotensis* n. ssp., inside of mantle cavity, right lateral view, after pallial genital duct and rectum removed, Yuchin Falls. au, auricle; gf1, gill filament 1; gf2, gill filament 2; gn, gonadal nerve; k, kidney; me, mantle edge; os, osphradium; osg, osphradial ganglion; rn, renal nerve; sbv, subvisceral connective; suv, supravisceral connective; ve, ventricle; vg, visceral ganglion.



FIGS. 35–39. *Oncomelania hupensis iriomotensis* n. ssp., radula. (35–37) Yuchin Falls; (38, 39) unnamed waterfall, near Urauchi River.

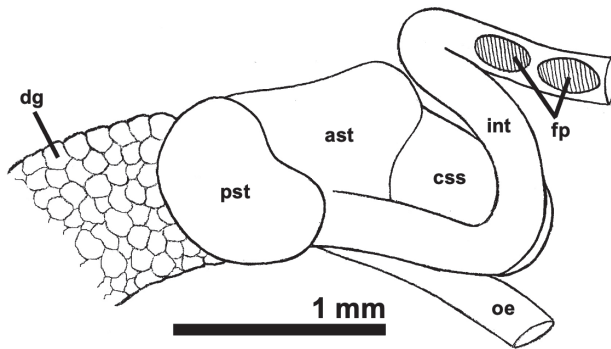


FIG. 40. *Oncomelania hupensis iriomotensis* n. ssp., stomach, Yuchin Falls. ast, anterior chamber; css, crystalline style sac; dg, digestive gland; fp, faecal pellet; int, intestine; oe, oesophagus; pst, posterior chamber.

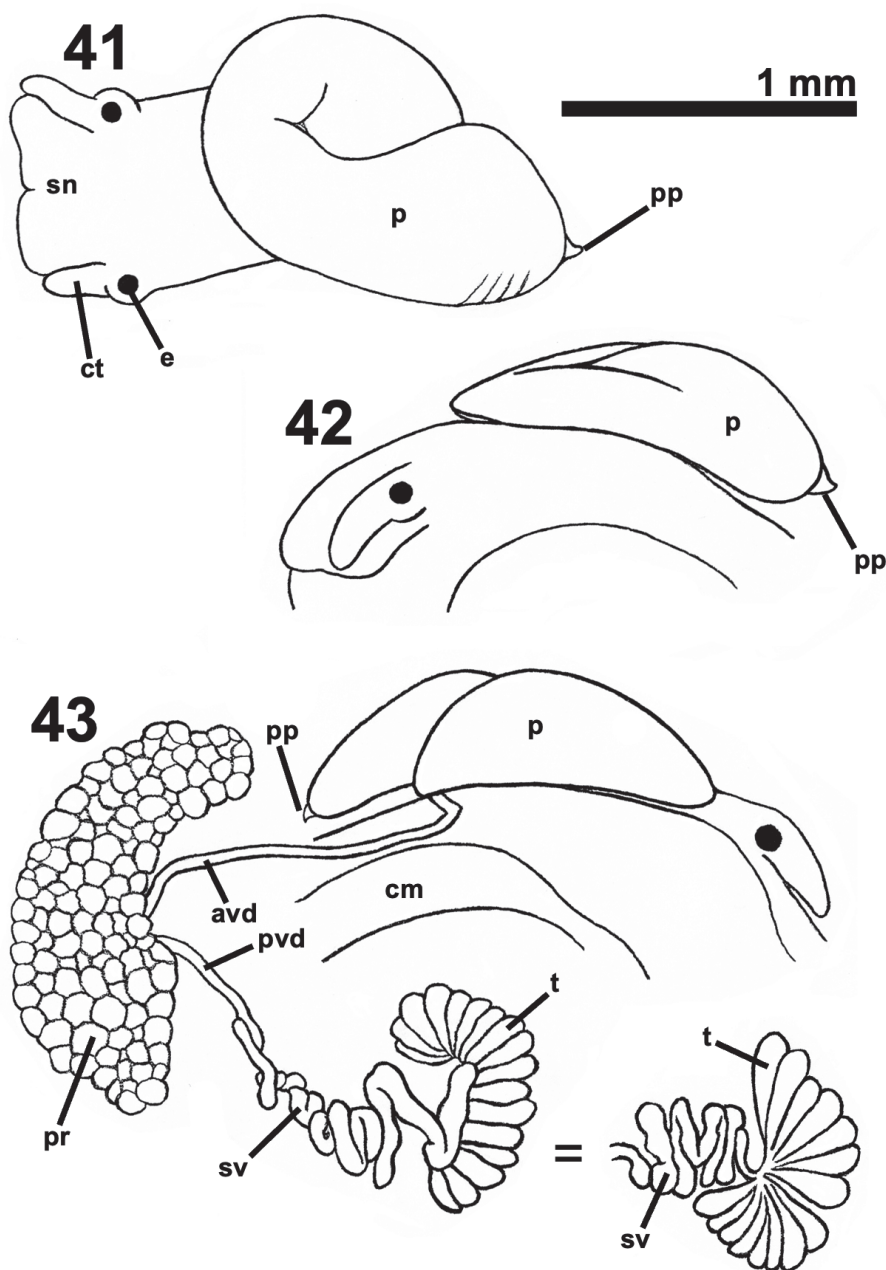
shaft, wide cutting edge, with six large, wide cusps on inner side and five small, narrow cusps on outer side. Inner marginal teeth long, slender, curved in distal portion, with parallel sides; cutting edge with eight uniform cusps subequal in size. Outer marginal teeth similar to inner marginal teeth in shape and size, with six uniform cusps.

Oesophagus (Figs. 40, 48, oe) opening widely to buccal cavity; running nearly straight at posterior part of nerve ring; entering stomach on left side at junction of anterior and posterior chambers. Stomach (Fig. 40) consisting of two large chambers as anterior and posterior parts (ast, pst), with single opening to digestive gland (dg) at ventral side of posterior chamber slightly behind oesophageal opening. Digestive gland large (~ 2 mm in length), pale lemon yellow, composed of simple mass of globular cells with no distinct arms or rows. Crystalline style sac (css) large, elongate bean-shaped, pale olive in colour with tiny black spots. Origin of intestine at left anterior end of anterior chamber of stomach. Intestine (int) tightly looped over middle of style sac before continuing to rectum; rectum running nearly straight with no distinct curve on mantle roof. Oval faecal pellets (fp) queued in single file in intestine and rectum. Anus simple, situated slightly posterior to anterior mantle edge.

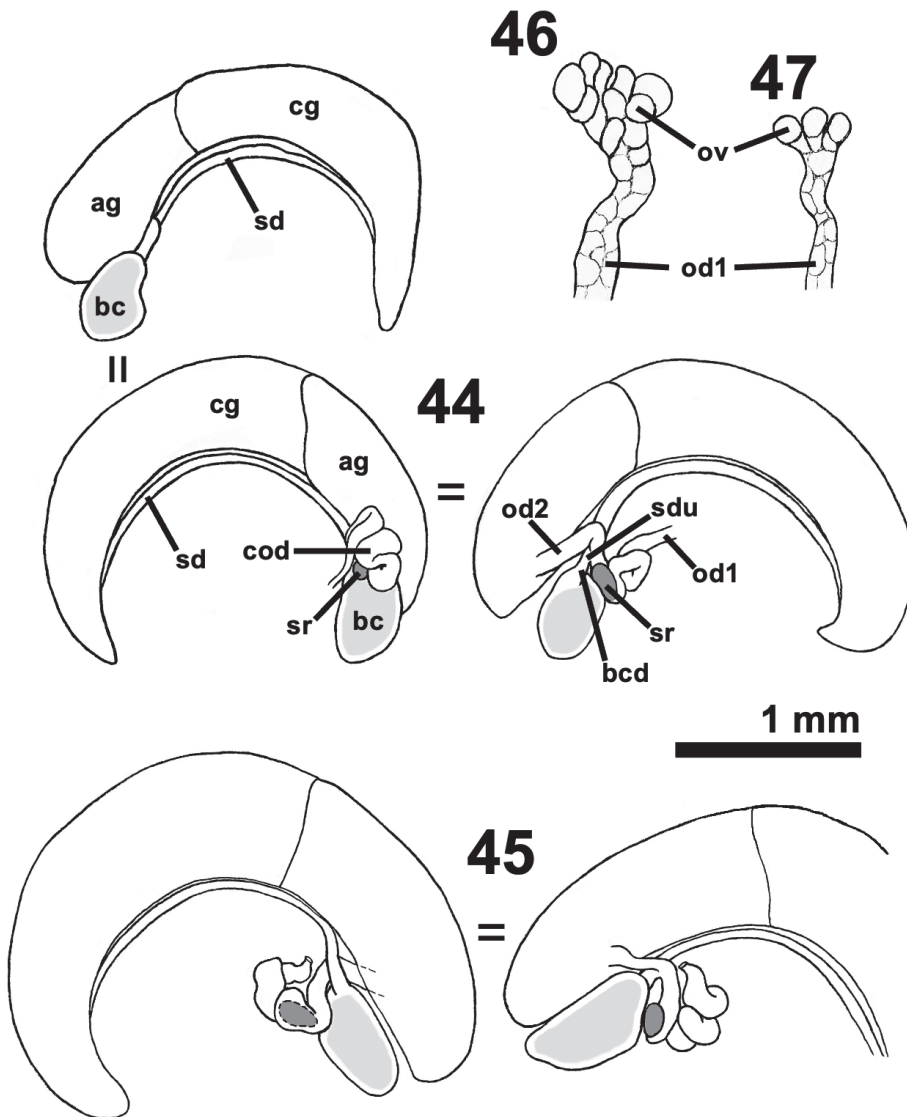
Male Reproductive System (Figs. 41–43). Testis (Fig. 43, t) small (~ 0.7 mm in length), pale yellowish, consisting of two groups of bundles with several elongate lobes; each group connected with wide ventral duct. Coiled seminal vesicle (sv) arising from vas deferens, situated on ventral side of testis; tube highly convoluted. Posterior vas deferens (pvd) running from seminal vesicle as simple narrow tube running to middle part of prostate gland (pr) just within mantle cavity. Prostate

gland moderately long (~ 1.3 mm in length), approximately half within posterior mantle cavity, elongate bean-shaped with many globular colourless cells. Anterior vas deferens (avd) arising from middle part of prostate, passing nearly straight between ventral edge of prostate and dorsal edge of columellar muscle, then turning 90° to left before reaching proximal end of penis (Figs. 41–43, p). Penis large, wide beyond head width, usually showing left-handed coil, expanding in distal region, highly muscular, attached to head ~ 0.5 mm behind eye (e); distal end with sharply pointed terminal papilla (pp); no distinct accessory glandular swellings found.

Female Reproductive System (Figs. 44–47). Ovary (Figs. 46, 47, ov) very short (~ 0.4–0.7 mm in length) at midventral area of digestive gland, with some short lobes containing large pale yellowish oocytes. Posterior oviduct (od1) wide, arising from anterior region of ovary. Renal (coiled) oviduct (Figs. 44, 45, cod) narrow, with two or three conspicuous loops proximal to seminal receptacle (sr). Bursa copulatrix (bc) small, elongate oval, variable in size (~ 0.5–0.7 mm in length, 0.3–0.4 mm in width), posterior to albumen gland (ag); bursal contents pale reddish brown in colour. Bursal duct (bcd) short, narrow, straight, arising from left side of anteroventral portion of bursa; bursal duct and oviduct united by very short sperm duct (sdu) near right side of posteroventral portion of albumen gland; then oviduct (ov2) distinctly bending backwards and merging with lumen inside albumen gland; bursal duct continuing to spermathecal duct (sd) after junction with sperm duct; spermathecal duct narrow, running along ventral edge of albumen gland and capsule gland (cg) towards end of oviduct. Seminal receptacle (sr) a short (~ 0.15 mm in length), oval sac, colourless except for iridescent



FIGS. 41–43. *Oncomelania hupensis iriomotensis* n. ssp., male reproductive system, Yuchin Falls. (41) Dorsal view of penis; (42) left lateral view of penis; (43) whole reproductive system with right lateral view of penis. avd, anterior vas deferens; cm, columellar muscle; ct, cephalic tentacle; e, eye; p, penis; pp, penial papilla; pr, prostate; pvd, posterior vas deferens; sn, snout; sv, seminal vesicle; t, testis.



FIGS. 44–47. *Oncomelania hupensis iriomotensis* n. ssp., female reproductive system, Yuchin Falls. (44, 45) Pallial oviduct; (46, 47) ovary. ag, albumen gland; bc, bursa copulatrix; bcd, bursal duct; cg, capsule gland; cod, coiled (renal) oviduct; od1, oviduct 1; od2, oviduct 2; ov, ovary; sd, spermathecal duct; sdu, sperm duct; sr, seminal receptacle.

sperm, lying anterior to coiled oviduct; seminal receptacle duct very short. Pallial oviduct (ag, cg) rather long (~ 3–3.3 mm in length), narrow (~ 0.7 mm in maximum width), gently curved on mantle roof; in dissection, clearly divided into albumen gland posteriorly and capsule gland anteriorly; albumen gland opaque white, capsule gland translucent. Genital opening at

anterior end of albumen gland with no distinct vestibule.

Central Nervous System (Figs. 34, 48–50). Circumoesophageal nerve ring streptoneurous, epiathroid, with ganglia weakly concentrated. Cerebral ganglia (Figs. 48–50, rcg, lcg) ~ 0.3 mm in length, 0.2 mm in width, between posterior end of buccal mass (bm) and anterior por-

tion of oesophagus (oe); cerebral commissure (cc) short, wide (0.1 mm in length, 0.25 mm in width). Tentacular nerves (rtn, ltn) thicker than optic nerves (ropn, lopn), with weak swellings at their bases, each divided into two nerves distally, reaching cephalic tentacles. Optic nerves arising from midventral portion of cerebral ganglia. Cerebropedal and pleuropedal connectives (rcpc, lcpc, rppc, lppc) rather long (0.22 mm in length), right connectives slightly shorter than left ones in length, showing asymmetry. Buccal ganglia (rbg) small, globular, joined by long, narrow commissure at posterolateral sides of buccal mass. Right pleural ganglion (rpl) smaller than cerebral ganglia (0.2 mm in length, 0.14 mm in width), oval, partially fused with right cerebral ganglion, separated by distinct constriction. Supraoesophageal ganglion (spg) distinct, oval (0.16 mm in length, 0.1 mm in width); right pleural and supraoesophageal ganglia separated by long (0.3 mm in length) pleurosupraoesophageal connective (spc). Left pleural ganglion (lpl) slightly smaller than right pleural ganglion, separated by short connective from left cerebral ganglion.

Suboesophageal ganglion (sbg) as large as left pleural ganglion (0.15 mm in length, 0.1 mm in width); connective between these ganglia not visible. Pedal ganglia (rpg, lpg) connected posteriorly to cerebral ganglia. Left and right pedal ganglia of equal size (0.3 mm in length, 0.2 mm in width). Pedal commissure (pc) very short but clearly visible. Metapodial (rmg, lmg) and propodial (rpr, lpr) ganglia small but distinct, fusiform to globular, connected with pedal ganglia by rather long, wide connectives; some heavy nerves arising from these ganglia to foot. No metapodial commissure observed. Small statocysts (rst, lst) dorsal at posterior ends of pedal ganglia. Osphradial ganglion (Fig. 34, osg) small, slender, fusiform (0.15 mm in length, 0.05 mm in width), on osphradium (os). Visceral ganglion (vg) oval, as large as osphradial ganglion; supravisceral connective (suv), subvisceral connective (sbv), and gonadal and renal nerves (gn, rn) arising from visceral ganglion.

Distribution: Known only from two waterfalls on Iriomote Island. We surveyed several other waterfalls on Iriomote Island and the neighbouring Ishigaki and Yonaguni islands, but the new subspecies has not been found.

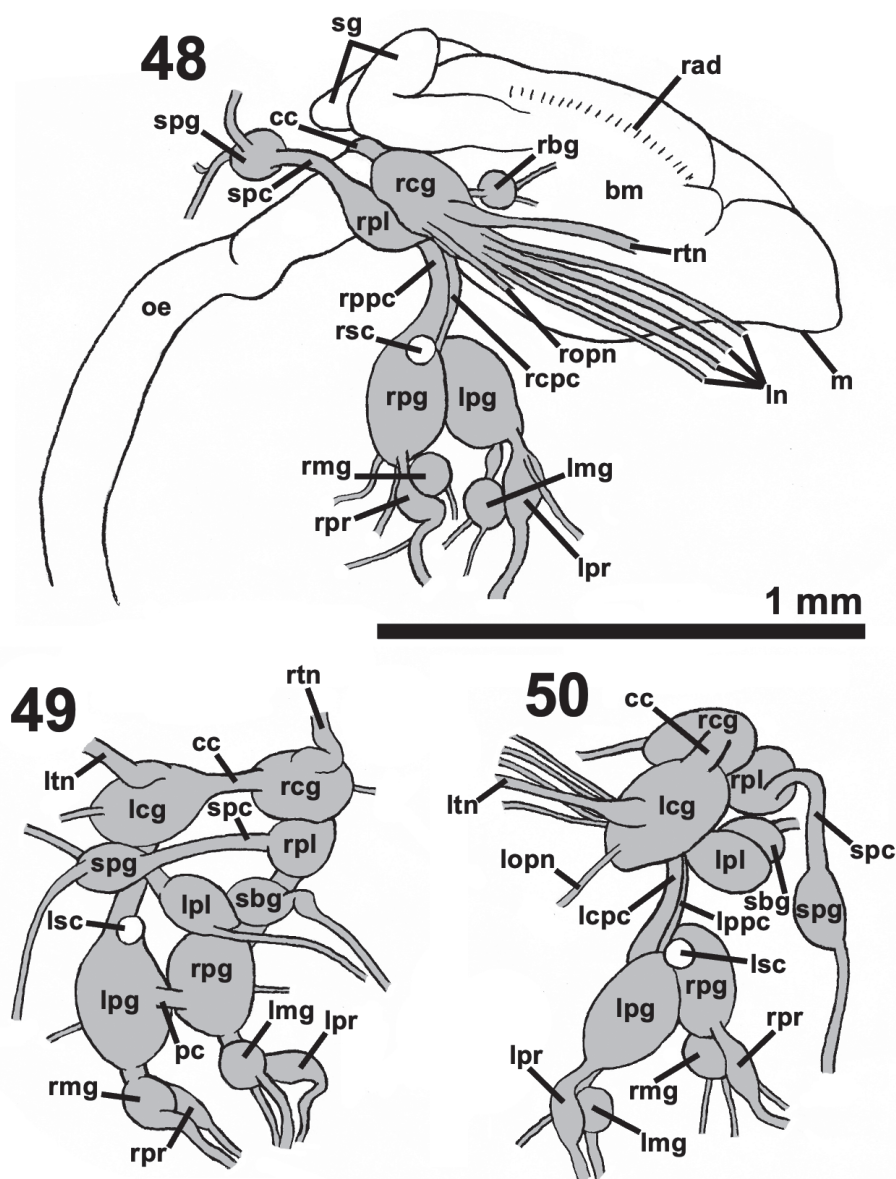
Habitat: Living individuals were found crawling among ferns (*Crepidomanes thysanostomum* (Makino) Ebihara & K. Iwats, 2006 カンシノ

ブホラゴケ; Hymenophyllaceae コケシノ ブ科) on rock walls around bottoms of waterfalls in mountainous areas (Figs. 14–19). Fern leaves and rock surfaces are usually wet from splash water from the falls. No other molluscs were observed at the two known localities of the new subspecies.

Remarks

***Oncomelania hupensis iriomotensis* n. ssp.** is somewhat similar to *O. h. chiui* and *O. minima* in shell shape and size, but the whorl outlines are more convex and the suture is more deeply impressed. Although some of other subspecies of *O. hupensis* have a strong longitudinal varix on the outer lip of the aperture, this is absent in the new subspecies, even in fully mature individuals.

The anatomical characters are generally similar to those of the other subspecies of *Oncomelania hupensis* (e.g., *O. h. chiui*, *O. h. formosana*, *O. h. lindoensis*) details of which were reported by Davis (1967, 1969a, 1979, 1980, 1981; Davis & Carney, 1973). However, a major difference is found in the very short sperm duct that connects the oviduct and the bursal duct (Figs. 44, 45, sdu). The only known taxon with such an extremely short (almost invisible in gross anatomy) sperm duct is the Japanese *O. h. nosophora*, as reported by Itagaki (1955: 165, pl. 1, figs. 3, 5). On the other hand, Nakamoto (1923: fig. 5) illustrated the sperm duct together with an additional tiny duct based on an examination using serial sections of *O. h. nosophora*, although those ducts were not drawn in his diagrammatic figure 4. Therefore, *O. h. nosophora* and ***O. h. iriomotensis* n. ssp.** could have similar minute ducts that are not detectable in gross anatomy alone. Either way, this character condition of the sperm duct is unique to the new subspecies and *O. h. nosophora*, based on Davis's illustrations for all other non-Japanese subspecies. As well as *Oncomelania hupensis sensu stricto*, most pomatiopsis taxa (e.g., species of *Pomatiopsis* Tryon, 1862, *Cecina* A. Adams, 1861) have long, conspicuous sperm ducts (Davis, 1979, 1980). The third author (in preparation) confirmed that the sperm duct is also long and easily recognisable in seven species of *Blanfordia* A. Adams, 1863 [*B. japonica* (A. Adams, 1861), *B. bensoni* (A. Adams, 1861), *B. simplex* Pilsbry, 1902] and *Fukuia* Abbott & Hunter, 1949 [*F. kurodai* Abbott & Hunter, 1949, *F. multistriata* Abbott & Hunter, 1949, *F. integra* (Pilsbry, 1924), "*F.*" *ooyagii* Minato, 1982].



FIGS. 48–50. *Oncomelania hupensis iriomotensis* n. ssp., supraoesophageal nerve ring and anterior alimentary canal, Yuchin Falls. (48) Right lateral view; (49) rear view of nerve ring after buccal mass and oesophagus removed; (50) left lateral view of nerve ring. bm, buccal mass; cc, cerebral commissure; lcg, left cerebral ganglion; lcpc, left cerebro-pedal connective; lmg, left metapodial ganglion; ln, labial nerve; lopn, left optic nerve; lpg, left pedal ganglion; lpl, left pleural ganglion; lppc, ganglion; lsc, left statocyst; ltn, left tentacular nerve; m, mouth; oe, oesophagus; pc, pedal commissure; rad, radula visible through skin of buccal mass; rbg, right buccal ganglion; rcg, right cerebral ganglion; rcpc, right cerebro-pedal connective; rmg, right metapodial ganglion; ropn, right optic nerve; rpg, right pedal ganglion; rpl, right pleural ganglion; rppc, right pleuropedal connective; rpr, right propodial ganglion; rsc, right statocyst; rtn, right tentacular nerve; sbg, suboesophageal ganglion; sg, salivary gland; spc, pleurosupraoesophageal connective; spg, supraoesophageal ganglion.

In the present anatomical examination, the osphradium was observed to have an elongate-fusiform ventral part and an undulating outer ridge (Fig. 34, os). The latter ridge is found in several taxa of the Truncatelloidea and Rissooidea (e.g., some genera of Assimineidae; Fukuda & Ponder, 2005: 359) and corresponds to one of the “Si1-Si2-Si4-type” *sensu* Haszprunar (1985: 477, fig. 7k, as “Rissoa”). However, the ridge did not show in the illustrations of *Oncomelania hupensis sensu lato* by Davis (1967, 1969a, b) and Davis & Carney (1973). This should be re-examined in each subspecies.

The new subspecies is the first discovery of *Oncomelania* in the subtropical Ryūkyū Archipelago. Its distribution is very distant and isolated from those of the other conspecific subspecies. The nearest taxon is *O. h. chiui* of northern Taiwan, which is located ~ 250 km west and separated by the East China Sea. The other Japanese subspecies, *O. h. nosophora*, is known in Honshū and northern Kyūshū. The southernmost populations of Fukuoka and Saga prefectures (now extinct in the wild) were ~ 1,250 km northeast of Iriomote Island. Further, *O. h. iriomotensis n. ssp.* was not included in a clade comprising *O. h. chiui* and *O. h. nosophora* in our molecular phylogenetic analysis and the new subspecies exhibited > 10% COI genetic distances against the other two subspecies (Table 6; Fig. 51; see below). Although further investigation is required for the phylogenetic relationships among the subspecies of *O. hupensis*, the significant genetic variation between *O. h. iriomotensis n. ssp.* and other geographically related subspecies suggest that the present new subspecies might be a relic. A similar case in Pomatiopsidae is the species of *Gammatricula* Davis & Liu, 1990, three of which are known from China and one from Yonaguni Island, westernmost of the Ryūkyū Archipelago (Davis et al., 1990).

The habitat of the new subspecies is also unique. All other subspecies of *Oncomelania hupensis sensu lato* are known to live in marshes around shallow-water streams in the plains. In contrast, *O. h. iriomotensis n. ssp.* is restricted to waterfalls in mountain areas. This is somewhat similar to the habitats of *O. minima* and species of *Fukuia* that live in seepages at the foot of cliffs (Davis, 1969b; Kameda & Kato, 2011).

Genetic analysis

The ML (Fig. 51) and BI (not shown) trees had almost identical topologies. The recon-

structed trees strongly supported the monophyly of *Oncomelania hupensis quadrasi* [BS = 97, Bayesian posterior probabilities (BPP) = 0.99], *O. h. iriomotensis n. ssp.* (BS = 100, BPP = 1.00), and the clade of *O. h. chiui*, *O. h. formosana*, *O. h. hupensis*, *O. h. nosophora*, and *O. h. tangi* (BS = 89, BPP = 1.00). Monophyly of the clade formed by these six species (BS = 98, BPP = 1.00) and by all subspecies analysed (BS = 100, BPP = 1.00) was also strongly supported.

The examined *Oncomelania* species showed 0–5.5% intraspecific and 1.8–18.3% interspecific COI genetic distances (Table 6). Between the subspecies of *O. hupensis*, *O. h. formosana*, *O. h. hupensis*, and *O. h. tangi* represented considerably small interspecific distances (< 3%). Each of the three subspecies *O. h. robertsoni*, *O. h. quadrasi*, and *O. h. iriomotensis n. ssp.* was ~ 10% or more genetically distant from any other subspecies.

EPIDEMIOLOGICAL IMPLICATIONS

Examination of the Potential Intermediate Host for *Schistosoma japonicum*

Three experimental infections (i.e., Exps. 1, 2, and 3) of *Oncomelania hupensis iriomotensis n. subsp.* with *Schistosoma japonicum* were conducted (Table 3). Cercariae were then monitored using the shedding method, although we detected no cercaria throughout the approximately three-month observation period. Although direct observation by dissecting the tissue of snail hosts is the most reliable method for confirming the presence of cercariae, this approach was avoided due to the unknown developmental period if *S. japonicum* was to use *O. h. iriomotensis n. ssp.* as a host. Instead, we waited for natural shedding under lighting to act as an indicator of cercarial maturation. However, in Exp. 1, an accidental event led to snail mortality eight weeks postinfection, while in Exps. 2 and 3, the remaining snails died by unknown causes at 13 weeks postinfection, respectively, in both cases without any detectable cercarial emergence. The soft tissues of the deceased snails were then degenerated, and no *S. japonicum* larvae were observed upon inspection by crushing. However, for all snails that died in Exp. 3, we conducted a DNA detection assay as per the method described in the following section.

TABLE 6. Mean intraspecific (along the diagonal) and interspecific (below the diagonal) pairwise genetic distances (%) of *Oncomelania* sp.

	OHT	OHH	OHF	OHC	OHN	OHQ	OHI	OHR	OM
<i>O. hupensis tangi</i> (Bartsch, 1936b) (OHT)	-	-	-	-	-	-	-	-	-
<i>O. hupensis hupensis</i> Gredler, 1881 (OHH)	0.018	0.021	-	-	-	-	-	-	-
<i>O. hupensis formosana</i> (Pilsbry & Y. Hirase, 1906) (OHF)	0.019	0.020	0.000	-	-	-	-	-	-
<i>O. hupensis chiui</i> (Habe & Miyazaki, 1962) (OHC)	0.026	0.026	0.024	0.018	-	-	-	-	-
<i>O. hupensis nosophora</i> (Robson, 1915) (OHN)	0.044	0.039	0.040	0.037	0.029	-	-	-	-
<i>O. hupensis quadrasii</i> (Möllerndorff, 1895) (OHQ)	0.138	0.133	0.139	0.132	0.126	0.026	-	-	-
<i>O. hupensis iriomotensis</i> n. ssp. (OHI)	0.098	0.101	0.108	0.106	0.106	0.109	0.016	-	-
<i>O. hupensis robertsoni</i> (Bartsch, 1946) (OHR)	0.119	0.119	0.121	0.122	0.119	0.127	0.110	0.055	-
<i>O. minima</i> (Bartsch, 1936b) (OM)	0.183	0.177	0.183	0.177	0.177	0.178	0.167	0.167	0.037

Detection of *Schistosoma japonicum* DNA from Water Samples and Snails

We attempted to determine whether *Schistosoma japonicum* DNA was present in river water samples from the habitats of *Oncomelania hupensis iriomotensis* n. ssp. and in laboratory-reared water. However, no *S. japonicum* DNA was detected in any of the samples. In contrast, *S. japonicum* DNA was detected in all four groups of snails in Exp. 3, including in several (i.e., 3–10) snails that died 13 weeks postinfection (Table 4).

DISCUSSION

We surveyed the molluscan fauna in other waterfalls on Iriomote Island and the neighbouring Ishigaki and Yonaguni islands, and the new subspecies has been found only from the above-mentioned two localities. Therefore, it is likely to be restricted to the extremely narrow distributional range and can be evaluated as critically endangered (CR) according to the criteria of the Red List by the Japanese government.

The monophyly of the new subspecies was robustly supported within the clade of *Oncomelania hupensis* in the phylogenetic analysis based on the mitochondrial COI and 16S (Fig. 51). These results strongly support that *O. h. iriomotensis* n. ssp. is an independent subspecies of *O. hupensis*. On the other hand, mitochondrial and nuclear phylogenies can be inconsistent in freshwater molluscs because of rapid evolution (David et al., 2022) and doubly uniparental inheritance (Doucet-Beaupré et al., 2012; Zouros, 2012) of mitochon-

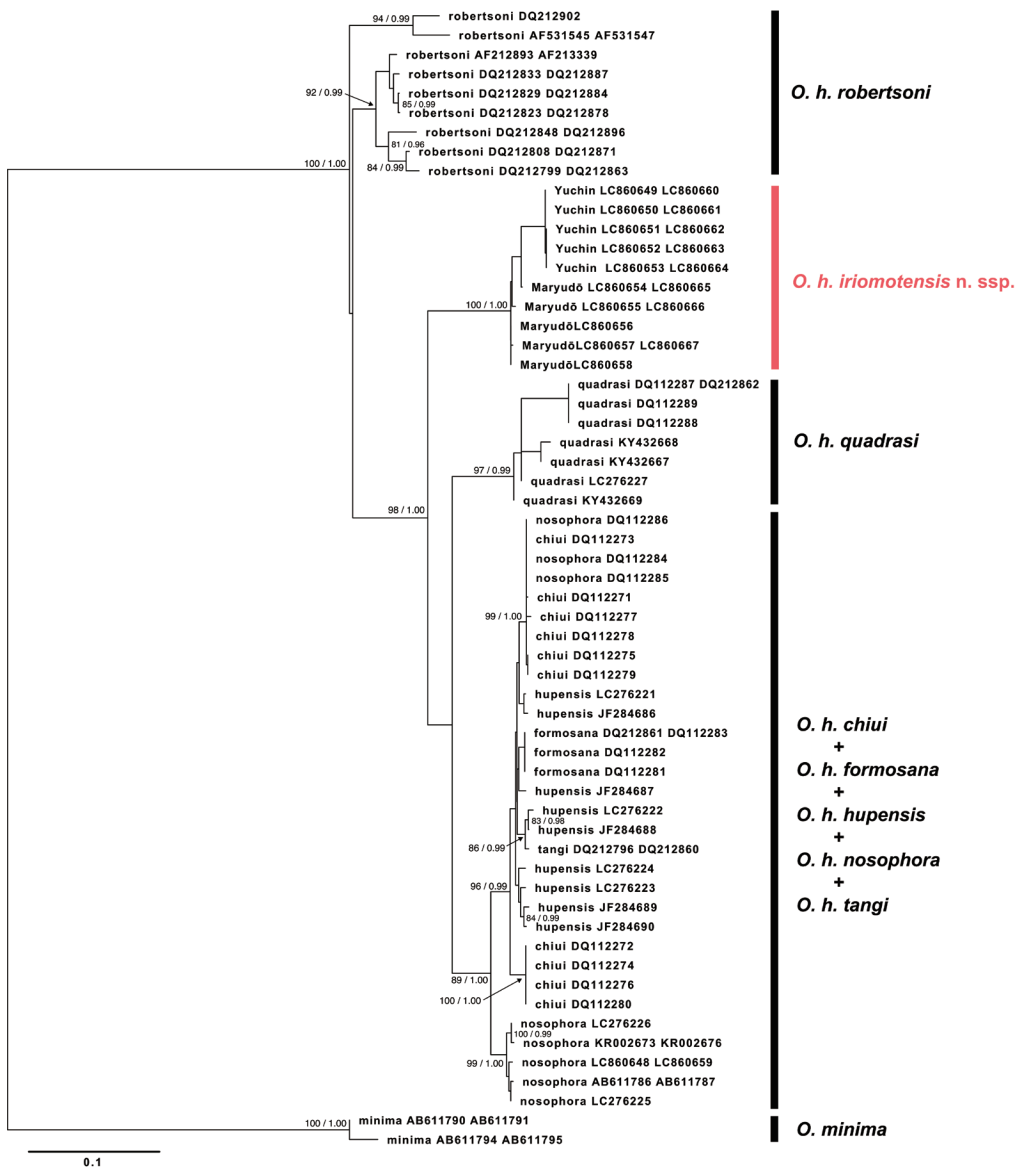


FIG. 51. Maximum likelihood tree for 1162 bp-aligned positions of the COI and 16S sequences. The numbers associated with the nodes represent the nonparametric bootstrap (BS) values for ML/and Bayesian posterior probabilities (BPP). BS > 80% and BPP > 0.95 are indicated.

drial DNA, and introgressive hybridization (Hirano et al., 2019; Miura et al., 2020). Such incongruences might also be present in other *Oncomelania* species, and therefore, further investigations of their nuclear phylogeny are also required.

Several subspecies of *Oncomelania hupensis* are known to be intermediate hosts of the parasitic trematode *Schistosoma japonicum*. Moreover, this study investigated the overall relationship between *O. h. iriomotensis n. ssp.* and *S. japonicum*. First, we conducted

eDNA analyses on river-water samples from the habitats of *O. h. iriomotensis* n. ssp. as well as in the water used for snail rearing in the laboratory. As a result, *S. japonicum* DNA was not detected in any of the water samples (Table 4).

Previous studies reported that *Oncomelania* could be susceptible to *Schistosoma japonicum* strains that are distributed in geographically distant regions and use different subspecies as their natural hosts (Kirinoki et al., 2005). To evaluate the susceptibility of *O. h. iriomotensis* n. ssp. for *S. japonicum*, we next conducted an infection experiment using a Japanese strain of *S. japonicum*. The results of this experiment showed that no cercarial shedding was observed during the study period, either from naturally collected intact snails or from those snails exposed to *S. japonicum* miracidia. Based on these results and the absence of any reported cases of schistosomiasis on Iriomote Island, we speculate that there is currently no evidence supporting an infection risk. However, because this study was limited in both duration and sample size, further studies are necessary to confirm these results. Moreover, no definitive conclusion has been reached regarding *S. japonicum* susceptibility, and further experiments are highly warranted.

In this study, we did not observe cercarial emergence under lighting, although PCR analysis of DNA extracted from dead snails (i.e., at 13 weeks postinfection) tested positive for *Schistosoma japonicum*. However, a major limitation of this study was the inability to confirm parasite developmental status within the snail host. In general, the most reliable method for this involves dissecting live specimens for direct observation. However, priority was instead given to infecting mice with any obtained cercariae, thus leading to the decision to avoid destroying live snails. In addition, the assessment was postponed until the snails were clearly dead. At that point, a postmortem microscopic examination revealed degeneration of soft tissue, thereby preventing confirmation of the developmental status of any parasite that might have been present.

The end of a schistosomiasis epidemic was declared in 1996 in Yamanashi Prefecture, one of the regions of Japan in which *Schistosoma japonicum* was most abundant. Although *Oncomelania* still inhabits this region, no new domestically acquired cases have been reported for > 40 years, that is, since the late 1970s (Ohmae et al., 2003, Nihei et al., 2009). This

suggests that even if the snails remain susceptible to *S. japonicum*, this does not necessarily pose an immediate epidemiological threat.

To date, no evidence of schistosomiasis on Iriomote Island has been recorded in the scientific literature, a finding that is consistent with those of this study. However, further investigations are required, including additional environmental DNA analyses, faecal examinations of wild animals in surrounding habitats, and further investigations of the susceptibility of *O. h. iriomotensis* n. ssp. to schistosomes.

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