

## Research Article

## The third introduced species of amber snail (Mollusca: Heterobranchia: Succineidae) from Japan

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### Abstract

The Succineidae Beck, 1837 are a family of terrestrial mollusks that are increasingly transported worldwide in horticultural trade. Succineids are extremely difficult to identify morphologically, especially if collected from locations beyond their native range and in the absence of knowledge of their native provenance. In this study, we report an unidentified species of the Succineidae in a flower bed of *Petunia* sp., Ariake, Tokyo, Japan. A fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was sequenced, and the shell and genital morphologies were compared with those of other similar species. This is the third succineid species introduced in Japan, as a species found in the field.

**Key words:** terrestrial mollusk, land snail, Gastropoda, flower bed, Tokyo, COI, East Asia

### Introduction

Succineidae Beck, 1837 is a family of terrestrial mollusks (Rundell et al. 2004) that shows a global presence (Patterson 1971; Rundell et al. 2004; Schileyko 2007; Holland and Cowie 2009). Members of this family is known as hosts of parasites such as trematodes (Yamada and Fukumoto 2011). Sometimes succineid snails inhabit human-related habitats, such as gardens and fields, and are recorded as introduced species (Holyoak et al. 2013; Benocci et al. 2014). They are increasingly being transported worldwide in the horticultural trade (Cowie et al. 2008). Many species of the family have been described worldwide, in most cases, based only on their shell characteristics (Benocci et al. 2014). However, it is extremely difficult to identify succineids morphologically, especially if collected from locations beyond their native range and in the absence of knowledge of their native provenance (Robinson 1999; Benocci et al. 2014).

Japan is a hotspot of terrestrial mollusks (Azuma 1995; Hirano et al. 2014, 2019a). In the case of succineids, eight native species including undescribed

**Table 1.** List of succineid species recorded in the fields of Japan.

Taxa	Shell width (mm)	Shell height (mm)	Distributional status in Japan	Reference
<i>Succinea lauta</i>	14.5	22	Native (widely distributed)	Azuma (1995)
<i>Succinea hystrix</i>	5–5.3	8	Native (widely distributed)	Azuma (1995)
<i>Succinea kofui</i>	5.8	8	Native (Honshu Island)	Patterson (1971)
<i>Succinea</i> sp.1	3.5–6.6	5.5–11.5	Introduced (Ryukyu Islands, Aichi Prefecture, Shizuoka Prefecture, and Osaka Prefecture of Honshu Island)	Hayase and Kimura (2011), Hayase (2018), Uechi et al. (2023)
<i>Succinea</i> sp.2	5.7–6.6	10.1–11.5	Introduced (Ryukyu Islands, Osaka Prefecture of Honshu Island)	Hayase and Kimura (2011), Fukuda et al. (2017), Uechi et al. (2023)
“ <i>Succinea</i> ” sp.	5.5	8	Native (Daito Islands)	Kubo et al. (2017)
<i>Succinea</i> sp.	–	15	Native (Hokkaido Island)	Kuwahara and Taru (2011)
<i>Oxyloma hirasei</i>	6.5	12.5	Native (widely distributed)	Azuma (1995)
<i>Boninosuccinea ogasawarae</i>	9.6	13.3	Native (Ogasawara Islands)	Azuma (1995)
<i>Boninosuccinea punctulispira</i>	9.2	14.2	Native (Ogasawara Islands)	Azuma (1995)


**Figure 1.** Photographs of the locality of *Succinea* sp. in Ariake, Koto, Tokyo, Japan. A. garden areas. B. a flower bed of *Petunia*. C. an individual of *Succinea* sp. in the flower bed. Photographs by F. Takahashi.

species (Azuma 1995; Ueshima 1995; Kuwahara and Taru 2011; Kubo et al. 2017) and two introduced species have been recorded (Hayase and Kimura 2011; Uechi et al. 2023) in Japan (Table 1). In October 2020, an unknown succineid species was found in the flower bed of a small garden in Tokyo for the first time (Figure 1). This unknown species has distinctive morphological traits compared to other succineid species in Japan, including a colorful mantle and comparatively wider shell width. We also obtained sequence information of this species for comparison with other species. Considering the habitat, morphological traits, and genetic information, this succineid is an introduced species. Therefore, we have documented the morphological and habitat information of the third introduced species of this family in Japan, as a species found in the field.

**Table 2.** Shell measurements of *Succinea* sp. from Ariake, Koto, Tokyo.

Specimen ID in Tohoku University	Shell width (mm)	Shell height (mm)
TUMo-HCtokyo1	9.67	13.69
TUMo-HCtokyo2	8.52	12.48
TUMo-HCtokyo3	9.1	13.2
TUMo-HCtokyo4	8.13	12.14
TUMo-HCtokyo5	7.33	11.13
TUMo-HCtokyo6	8.03	12.41
TUMo-HCtokyo7	8.65	12.57
TUMo-HCtokyo8	8.35	12.91
Mean	8.47	12.57
SD	0.71	0.76

## Materials and methods

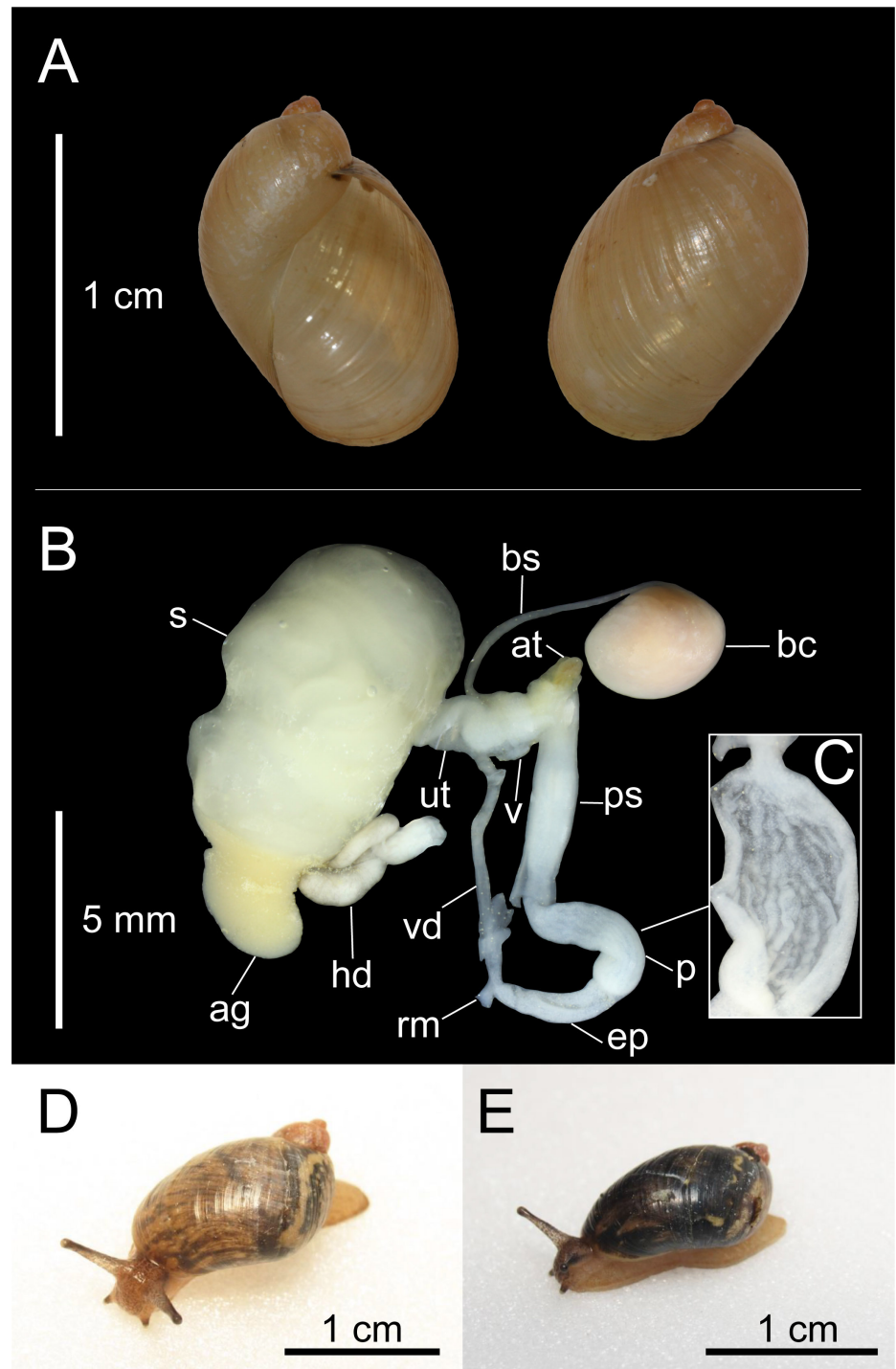
We collected eight individuals of this succineid species from a flower bed of *Petunia* sp. in a garden area in Ariake, Koto, Tokyo (November 5, 2020; N35.633620; E139.793466). The foot muscle of each sample was stored in 99.5% ethanol for DNA extraction, and the remaining soft bodies were stored in 70% ethanol for dissection. We deposited the specimens at Tohoku University (Table 2).

For obtaining DNA, we used the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) with the manufacturer's standard protocol. To estimate phylogenetic position of the population that we found in Tokyo, a fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) gene was amplified using the primers LCO1490 (5'-GGTCAACAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATC-3') (Folmer et al. 1994). The PCR was conducted under the following conditions: an initial denaturation for 3 min at 94 °C, 34 cycles of 30 s at 94 °C, 30 s at 40 °C and 1 min at 72 °C, with a final extension for 5 min at 72°C. The PCR product was purified using Exo-SAP-IT (Amersham Biosciences, Little Chalfont, UK). Sequencing was performed using Eurofins Genomics (Ohta, Tokyo, Japan). The two newly generated sequences were deposited in the GenBank database (GenBank accession numbers; pigmented form: LC702441, less pigmented form: LC702442). We downloaded 1,003 COI sequences for Succineidae from GenBank. We also used COI sequences of two slugs (*Arion rufus* and *Meghimatium bilineatum*) from GenBank as outgroups (Guzmán et al. 2021). In addition to these sequences, we used two sequences of the snails we collected (both pigmented and less pigmented forms; see results). In total, the phylogenetic analysis was conducted using 1,007 sequences. Unique haplotypes were identified using Phylogears2 2.2.2012.02.13 (Tanabe 2012). We then performed phylogenetic analysis using a maximum likelihood (ML) method. We used MEGA7 (Kumar et al. 2016) to select appropriate models of sequence evolution. For ML analysis, we used T92+G+I model. ML analysis was conducted using the selected model with MEGA7 (Kumar et al. 2016). We assessed nodal support for ML analysis using bootstrap analyses with 1,000 replications.

## Results and discussion

The shell measurements are presented in Table 2. The morphological description is as follows: the shell is thin, the shell is translucent, the external shell shape is ovoid, the number of whorls is three, the growth lines are visible on the body whorl, the aperture is large and tear drop-shape, and the lip is not reflected (Figure 2A). The shell is capable of fully housing the retracted animal. The periostracum is brownish. The base color of the animal is yellowish-brown and the mantle is variably pigmented dark brown (pigmented with yellow-zigzag lines to less pigmented with yellow-zigzag lines) (Figure 2D, E). The tips of the tentacles and snout are also pigmented. The hermaphroditic duct (hd) is a long and bulky tube (Figure 2B). The albumen gland (ag) is of moderate size, and yellow in color. The spermoviduct (s) is broad, whitish transparent in color. The uterus (ut) is slender, rather muscular, and weakly coiled. The vagina (v) is thick and muscular. The genitalia lack the vaginal appendix. The bursa copulatrix (bc) is spheroidal and the bursa stalk (bs) is long and narrow. The bursa copulatrix is located beside the albumen gland. The epiphallus (ep) is slender and a rather long muscular tube. The penis (p) is also a long muscular tube with broader diameter than the epiphallus. The penis sheath (ps) of rather thick muscular wall is present and encloses the basal part of penis. The major portion of the penial internal surface is covered by crenulated longitudinal pilasters (Figure 2C). The penial retractor muscle (rm) attaches the epiphallus. The vas deferens (vd) is a thin muscular tube. The vas deferens runs down along the uterus, and then, turns up along the penis sheath to open into the epiphallus. The atrium (at) is located between the vagina and the penis, but not prominent. In the Succineidae, there are three subfamilies (Succineinae, Catinellinae and Oxylomatinae; Schileyko 2007). Catinellines lack the penial sheath, whereas succineines have an external sheath around the penis (Patterson 1971; Cowie 2006; Schileyko 2007). Oxylomatinae have a blind appendix (“flagellum”) on the border between penis and epiphallus (Schileyko 2007). The species we found in Tokyo has an external sheath around the penis and the blind appendix is absent (Figure 2B), suggesting that this species is Succineinae.

Japanese succineid species can be identified based on external morphology (Azuma 1995; Hayase and Kimura 2011; Kubo et al. 2017). The present species is not similar to any other Japanese succineid (Tables 1, 2), supporting the present species represents a newly discovered introduction in Japan. Two native species (*S. kofui* and *S. lyrata*) having similar with indistinguishable shell morphology each other (Patterson 1971; Ueshima 1995) showed relatively are similar entire shell in shape to this introduced species. *Succinea lyrata* cannot be distinguished from *S. kofui*, but the shell size and shape of the introduced species is larger and rounder



**Figure 2.** Shell and genital morphologies of *Succinea* sp. from Ariake, Koto, Tokyo, Japan. A. Shell views, shown from left to right: ventral and dorsal (photographs by T. Hirano). B. external genital morphology (photographs by T. Hirano): ag, albumen gland; at, atrium; bc, bursa copulatrix; bs, bursa stalk; ep, epiphallus; hd, hermaphroditic duct; p, penis; ps, penial sheath; rm, penial retractor muscle; s, spermoviduct; ut, uterus; v, vagina; vd, vas deferens. C. internal penial ornamentation. D and E. morphological variations of the species (photographs by F. Takahashi): D. less pigmented form; E. pigmented form.

than the two species (Tables 1, 2). In addition, *S. kofui* has a vaginal appendix in its genitalia, which is lacking in *S. lyrata* (Ueshima 1995) and the introduced species (Figure 2B).

The phylogenetic analysis showed that many nodes have low support values, but the individuals we collected composed a clade with 99% bootstrap

support (Supplementary material Figure S1 and Table S1). Therefore, morphological variation of the mantle color in the present population (Figure 2D, E) does not reflect difference of species. *Succinea caduca* from Puu Kohola of Hawaii also composed a clade, and considering the tree topology, this clade of *S. caduca* might be closely related to the population we found, but the support value on the node between them is 30%. Holland and Cowie (2009) showed a phylogenetic tree for the Succineidae of the world, including two lineages of Japanese succineids from the Ogasawara Islands and Sendai (Honshu Island). These Japanese succineids are not shown their species names, but there may be *Boninosuccinea* spp. and *Succinea lauta*, respectively (Table 1). These species are not closely related to each other, and other succineids (Holland and Cowie 2009). However, the species names of these Japanese succineids were not shown and these sequences data are not available in GenBank.

The COI sequences of three Japanese succineids (*S. lyrata*, *S. kofui* and *Oxyloma hirasei*) have been deposited in GenBank (Rundell et al. 2004). Considering the results of the previous studies (Rundell et al. 2004; Holland and Cowie 2009) and the results of the genetic analyses and morphological evaluation (Figures 2 and S1; Tables 1, 2 and S1), the present introduced species is succineine and not closely related to any other Japanese succineids. However, the morphology of the introduced species we found was not similar to that of *S. caduca* and *S. konaensis*, which are Hawaiian endemic snails, or other Hawaiian endemic succineids (Cowie 2006; Rundell et al. 2004; Holland and Cowie 2009). Considering morphological similarity of other succineids, to our best knowledge, *Succinea* sp. (Image Number: 5433677, Center for Invasive Species and Ecosystem Health 2017), which was found with *Ocimum basilicum* from Colombia at Customs and Border Protection of Atlanta International Airport, and *Succinea (Calcisuccinea)* sp. (Holyoak et al. 2013), which was found in Portugal and Spain, have similar external morphologies, such as a mantle color pattern, to that of the introduced species. However, it is unclear whether they belong to the same species. As our genetic results are based on a single mitochondrial gene, our phylogenetic tree could accurately reflect incomplete lineage sorting or/and hybridization between species (i.e., Hirano et al. 2019b, c). In addition, the specimens of succineids might have been misidentified in previous studies. However, our findings will contribute to a much-needed baseline for tracking the spread of the readily introduced species.

According to an interview with the management company of the small garden, the flower bed was completed in March 2020. They planted perennials in a small area of the flowerbed and replaced most of the other areas with seasonal flowers multiple times per year. We confirmed the replanting of the *Petunia* planting area to *Viola × wittrockiana* on December 3, 2020. They were preparing for replanting (removing *Viola × wittrockiana* and clearing the land) on May 20, 2021. In November 2020, a number of individuals with a shell length of approximately 1.3 cm were found.

Nonetheless, only 10 individuals with a shell length of less than 1 cm were found on May 20, 2021. They spawned under captivity conditions in November 2020 (F. Takahashi *unpublished*). Considering these situations, these snails might have been first introduced with *Petunia*. Large individuals (approximately 1.3 cm) died in winter, and only those individuals that escaped disturbance during replanting may have been able to overwinter as eggs or juveniles. In fact, multiple species from this family were found associated with the plants during airport quarantine in Japan, even though they had not been previously recorded in the field (Matsukuma and Takeda 2009).

Approximately 100 species of terrestrial mollusks have been proven to be highly adaptive to environmental changes brought about by human activity, and they often become highly abundant in modified habitats (Barker 2001; Richling and von Proschwitz 2021). Japan has a large number of populated cities, and some introduced species of terrestrial mollusks have been recorded in Tokyo (Ueshima et al. 2014). Further surveys of terrestrial malaco-fauna are needed to clarify how and which species establish populations in the non-native regions.

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### Authors contribution

TH: research conceptualization; data analysis and interpretation; roles/writing – original draft; writing – review and editing. FT: investigation and data collection; roles/writing – original draft; writing – review and editing.

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## Supplementary material

The following supplementary material is available for this article:

**Figure S1.** Maximum likelihood (ML) phylogenetic tree of the succineid snails based on 612 bp of the COI gene.

**Table S1.** Each OTU information of the phylogenetic tree (Figure S1).

This material is available as part of online article from:

[http://www.reabic.net/journals/bir/2023/Supplements/BIR\\_2023\\_Hirano\\_Takahashi\\_SupplementaryTables.xlsx](http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Hirano_Takahashi_SupplementaryTables.xlsx)

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