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NOTE

# Isolation of sporocyst broodsacs of the Genus *Leucochloridium* (Leucochloridiidae: Trematoda) from the intermediate host, *Succunea lauta*, in Japan

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**Abstract**

**Green- or brown-striped trematode sporocyst broodsacs typical of *Leucochloridium* infecting the ocular tentacles of a land snail, *Succinea lauta*, were collected in Abashiri, Hokkaido in northern Japan (N43° 59′, E144° 14′) in June of 2000 and 2001. The metacercariae isolated from the sporocyst broodsac were morphologically identified as *Leucochloridium* spp. (Leucoclhoridiidae Poche). This report is the first to describe evidential specimens of the sporocyst broodsac of the genus *Leucochloridium* Carus, 1835, infecting the intermediate host in Japan, suggesting that *Leucochloridium* spp. completes their life cycle in Hokkaido, Japan.**

Keywords: Japan, *Leucochloridium*, sporocyst broodsac

The genus *Leucochloridium* Carus, 1835 (Leucochloridiidae: Brachylaimoidea: Trematoda) distributes widely in the northern hemisphere and parasitizes the avian final hosts in the cloaca and Bursa fabricii[2]. The name *Leucochloridium* is relatively well known to curious public because of the genus' remarkable mimicry. The distinctly striped sporocyst broodsac parasitizes in the ocular tentacles of the intermediate hosts, Succineidae land snails, and moves in a pulsating manner. The appearance and movement of the broodsac closely resemble those of caterpillars, and this mimicry is believed to increase the parasite's chance of being preyed upon by a wide variety of bird species, the definitive hosts of these parasites[6].

At present, four species of *Leucochloridium*, *L. cardis*[12], *L. japonicum*[4], *L. sime*[11] and *L. turdi*[12], have been reported in their adult stages from final avian hosts collected in Japan. However, information is limited about the larval stage of *Leucochloridium* in the intermediate hosts in Japan. Minato and Takeda[7] accidentally found a *Succinea lauta* snail parasitized in its ocular tentacle by *Leucochloridium* sp. broodsacs in Teuri Island, Hokkaido, and reported their

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photographs. Yet, no evidential specimens of *Leucochloridium* sporocysts or broodsacs infecting intermediate hosts in Japan have been described in literature or registered at any research institute. In this report, we describe detection of the sporocyst broodsacs of *Leucochloridium* spp. in *Succinea lauta* snails collected in Hokkaido, Japan and their morphologically established species identity. This report is the first to describe the geological distribution and intermediate host infection of the genus *Leucochloridium* spp. in Japan.

*Succinea lauta*, the intermediate host snail of *Leucochloridium*, was collected on five separate occasions in Mt. Tento, Abashiri, Hokkaido in northern Japan (N43° 59′, E144° 14′) (Fig. 1). Snails infected with *Leucochloridium* were identified by the presence of characteristically moving striped broodsacs in the ocular tentacles. Among *Succinea lauta* snails collected in Abashiri, Hokkaido, two snails had large maggot-like broodsacs in the ocular tentacles (Table 1). Present sporocyst infected land snails were identified as *Sussinea lauta* by shape and size of shell, and geological distribution in Japan[1]. They were both captured in June. The broodsacs were moving in a pulsating manner while alive, and the one found in 2000 was brown-banded (Fig. 2A) and that found in 2001 was green-banded. The specimen collected in 2000 was morphologically observed and dissected (Fig. 2B). The broodsac was 29.0 mm long and 4.9 mm wide. The colored stripes lost their colors while preserved with formalin. About 300 metacercae were contained within the broodsac. The body of metacercaria isolated from the broodsac was almost oval and, on average, 1.00 mm long and 0.48 mm wide (n = 10). The oral sucker, pharynx and ventral sucker were well developed and located close to each other. The oral sucker was 0.32 mm by 0.25 mm in size, apparently larger than the ventral sucker, 0.22 mm by 0.25 mm in size. The pharynx was almost round and muscular, 0.12 mm by 0.10 mm in diameter. The caeca was located close to the posterior extremity (Fig. 2C). The cyst wall of metacercaria was not clearly recognized.

Three genera, *Urotocus* Looss, 1899, *Urogonimus* Monticelli, 1888, and *Leucochloridium* Carus, 1835, are recognized in the family Leucochloridiidae Poche, 1907[8]. These trematodes parasitize in the cloaca and Bursa fabricii of the avian final hosts. Bakke[2] revised the genera in Leucochloridiidae on the basis of the morphological and ecological characteristics of both the adult forms in the final hosts and the larval forms in the intermediate hosts.

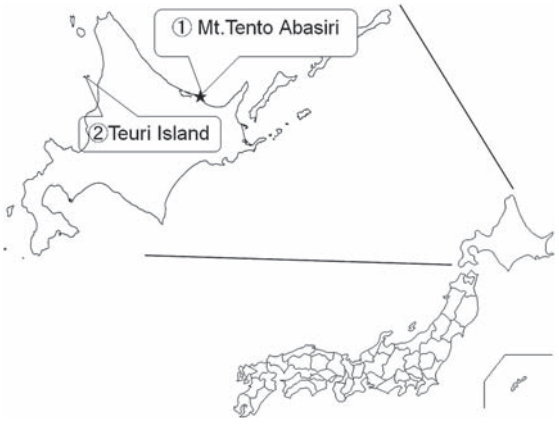


**Fig. 1. Locations where the snails infected with the *Leucochloridirum* spp. sporocysts were found.** (1) Mt. Tento, Abashiri city. Sporocyst-infected snails were collected in 2000 and 2001 (the present report). (2) Teuri island. Sporocyst-infected snails were photographed in 1993[6].

**Table 1. Infection of *Leucochloridium* spp. sporocysts in a land snail, *Succinea laura* Gould, 1859, collected in Mt. Tento, Abashiri, Hokkaido.**

| Date | 2000 June 18 | 2001 June 24 | 2006 October 9 | 2007 June 15 | 2008 July 21 |
|---|---|---|---|---|---|
| Number of snails collected | 15 | 6 | 52 | 94 | 48 |
| Number of sporocyst-infected snails | 1 | 1 | 0 | 0 | 0 |

According to the revised criteria of the genus *Leucochloridium*, all intermediate host snails belong to Succineidae, the sporocyst infection sites include the hepatopancreas, haemocoel and ocular tentacle, and the sporocyst broodsac has bright green or brown stripes and is able to move in a pulsating manner, enlarging the ocular tentacle. All these characteristics were evident in our present specimens. The larvae of the other two genera in the family Leucochloridiidae were excluded because both *Urogonimus* Monticelli, 1888, and *Urotocus* Looss, 1899, are characterized by lack of distinct colored band and broodsac[9,10]. Thus, the present specimens were diagnosed as belonging to the genus *Leucochloridium*.

However, species could not be identified for the present specimens solely on the grounds of the morphological characteristics. A recent study showed that the sequence of internal transcribed spacers of the 5.8S rDNA gene differs by 6.8% between the green-banded broodsac of *L. paradoxum* Carus, 1835, and brown-banded broodsac of *L. variae* McIntosh, 1932; since there were no intraspecies differences within each color morph, this finding suggests that the broodsac color can be used to diagnose the species[3]. In the light of these findings, the present specimens of both green- and brown-banded broodsacs indicate the presence of at least two species of *Leucochloridium* in Hokkaido.

Both of the present infected snails were captured in Abashiri area in June when the climate in the area still was that of spring. It takes two months or more for the cercaria of Leucochloridiiae to develop into metacercaria within the intermediate host[5]. *Succinea lauta* snails infected with *Leucochloridium* larvae hibernate through the winter until the following spring and are seen primarily from April to August in European countries while hardly seen in other seasons[5]. Thus, the infection of the present specimens likely had occurred in the preceding year.

At present, adult parasites have been reported for four species of *Leucochloridium* from birds collected in Japan: *L. cardis* from *Turdus cardis* collected in various locations in Japan[12], *L. japonicum* from *Phasiaus sommeringii* in Ibaraki[4], *L. sime* from *Coccothraustes japonicus* in Shizuoka[11] and *L. turdi* from *Turdus cardis* in Kyoto[12]. Except for *P. sommeringii*, all these avian hosts come from outside of Hokkaido to Abashiri area as well as Teuri Island in western Hokkaido (Fig. 1) where snails infected with the

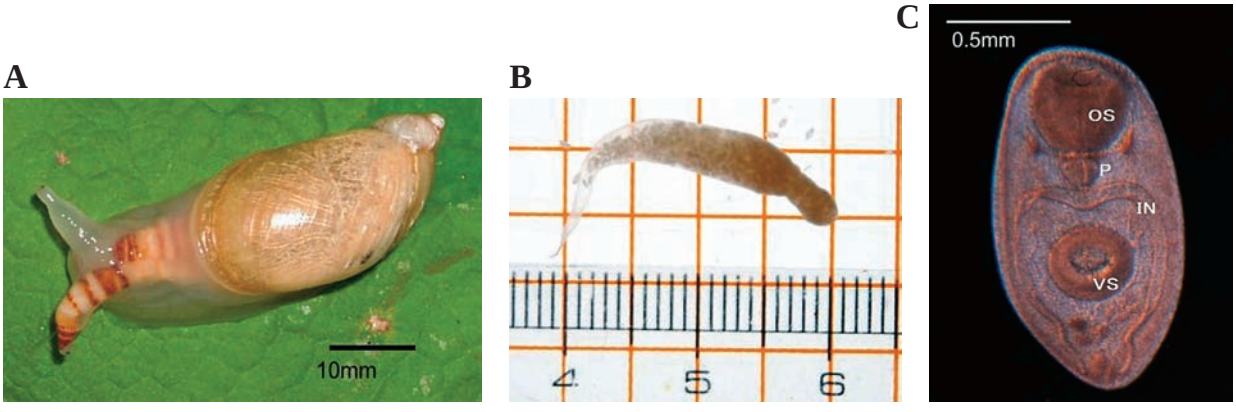


**Fig. 2. Sporocysts and metacercariae of the *Leucochloridium* sp. from the intermediate host.** A. Two brown-banded broodsacs of *Leucochloridirum* sp. in the ocular tentacle of a land snail, *Succinea laura* Gould, 1859, captured in Abashiri, Hokkaido in 2000. B. A broodsac of *Leucochloridirum* sp. isolated from a *Succinea laura* land snail collected in Abashiri, Hokkaido (the specimens shown in panel A). The grid is 10 × 10 mm, and the scale of the ruler is in mm. C. A metacercaria isolated from a broodsac of *Leucochloridium* sp.. OS: oral suker, P: pharynx, IN: intestine, VS: ventral sucker. These specimens were photographed, and the broodsacs were preserved in 10% formalin for morphological examination. After that, these specimens were observed under a dissecting microscope and measured, photographed under a light microscope.

broodsacs of *Leucochloridium* were photographed[7]. Because both the intermediate and final hosts share the time and location, it is possible that *Leucochloridium* spp. have a complete life cycle at least in Abashiri area and, possibly, in western Hokkaido.

Our present findings demonstrated the presence of *Leucochloridium* sporocysts in Abashiri area, Hokkaido, and suggest that *Leucochloridium* spp. have a complete life cycle in the area. Further studies are needed to identify the species of *Leucochloridium* spp. currently present in Hokkaido, including experimental infection of laboratory birds with living sporocyst broodsacs to collect adult worms and larvae for morphological and genetic analyses.

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