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SHORT NOTE
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Interdigital membrane of the Hokkaido salamander *Hynobius retardatus*

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INTRODUCTION

Salamanders stand out among vertebrate animals in the following characteristics: their ability to regenerate body parts, their large and variable genome size, and larval phenotype variation (Kohmatsu, 2001; Kishida et al., 2009; Sessions & Wake, 2020).

The Hokkaido salamander, *Hynonius retardatus* Dunn (1923) (Urodela: Hynobiidae), is a pond-type salamander indigenous to Hokkaido and at the northern limit of the genus of *Hynobius* in Japan (Fig. 1A, B) (Sato & Matsui, 2013; Tokuda, 2015). Interestingly, this species was discovered in the neotenic zone in Lake Kuttarush (Fig. 1A), that is located in the eastern region of Noboribetsu Onsen as type locality (Dunn, 1923; Sasaki & Nakamura, 1937). Only in this population has sustained neoteny been observed (Jia & Gao, 2016; Jiang et al., 2018). In May 2018, I obtained two capsules of fertile eggs from a private garden in Kayabe-gun (Fig. 1A, C, D). This was a new record for a natural population from 100 km south of the Noboribetsu area (Fig. 1A, C, D). It seems to be the southernmost population of *H. retardatus* in Hokkaido. Over the Tsugaru Strait another pond-type species, *Hynobius lichenatus*, is distributed in the Shimokita peninsula in northern Honshu.

The stages of larval development in the family Hynobiidae have been investigated and enumerated (Iwasawa & Kera, 1980; Iwasawa & Yamashita, 1991). Recently there have been several studies describing the development

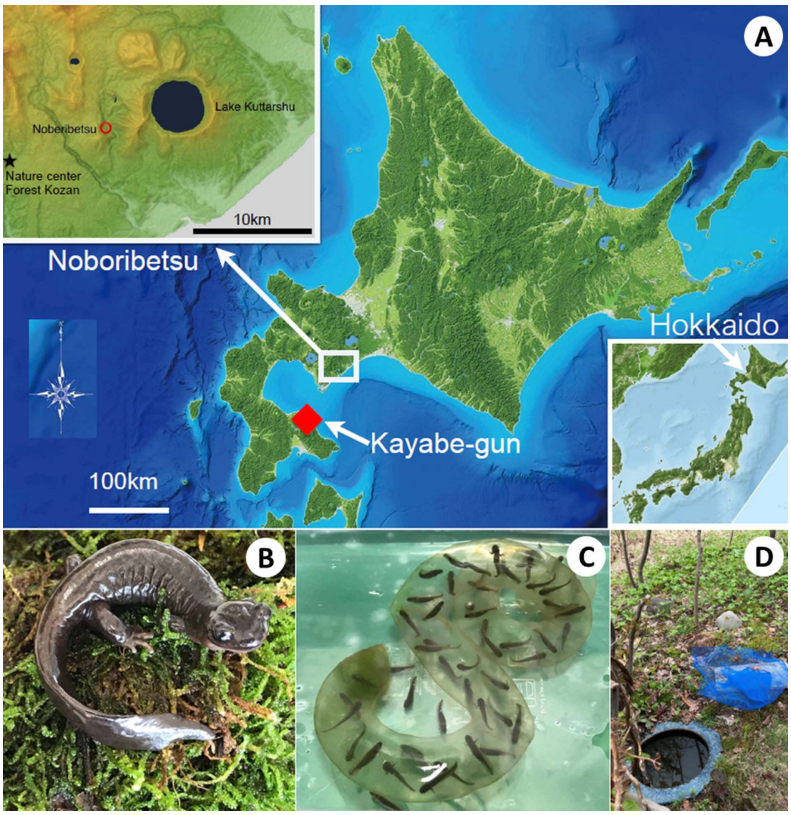

Figure 1. A. Maps showing the type locality of *Hynobius retardatus*- white rectangle indicates Noboribetsu region, red circle is the type locality, black star is the location of the Nature Center, Forest Kozan, Noboribetsu-shi, red diamond is the collection site of fertile eggs in Kayabe-gun, Lake Kuttarush is the crater lake where neotenous specimens were observed about 80 years ago but are now extinct, **B.** Adult specimen from Forest Kozan, Noboribetsu-shi, **C.** Two egg capsules (lab photo), **D.** Collection site of a private garden in Kayabe-gun

of interdigital membranes (IMs) or vestigial membranes during limb development in hynobiid salamanders from the islands of Honshu - *Hynobius lichenatus*, *Hynobius nigresens*, *Hynobius tokyoensis*, and Kyushu - *Hynobius dunni* (Table 1) (Iizuka et al., 2005; Iizuka, 2009; Iizuka et al., 2010; Iizuka & Akiyama, 2018). In this paper I present observations on the development of the IM in limbs of *H. retardatus* from the island of Hokkaido.

MATERIALS AND METHODS

This study was included as part of an investigation of salamander chromosomes in accordance with the direction and guidance of the Nanae-cho Ramsar Convention in Ohnuma quasi-National Park and the Ministry of the Environment of the Hokkaido Government in Hakodate and Sapporo, Hokkaido. Two egg sacs were collected in Honbetsu, Shikabe-cho, Kayabe-gun (42° 01'92" N; 140° 78'65" E) (Fig. 1A, C, D). The embryos and larvae from the two egg sacs (Fig. 1C) were placed in an aquarium kept in a wine cellar at 8 ˚C and their developmental stages identified according to those already described for *H. nigrescens* (Iwasawa & Yamashita, 1991). At selected intervals two or three larvae of each stage were fixed in buffered 75 % ethanol and photographed.

RESULTS AND DISCUSSION

All specimens examined in this study had interdigital membranes (IM, Fig. 2). These pond-type larvae also had well-developed balancers (Fig. 2A-C). The early forelimb buds show a spear-like morphology that, by the 2-digit stage, resolves itself as a symmetrical structure consisting of a pointed IM, with digits 1 and 2 developing on either side (Fig. 2A-E). As the forelimb develops, the IM regresses and is nearly gone by the 3-digit stage (Fig. 2D). As in most other salamanders with aquatic larvae, the development of the hindlimbs is delayed relative to the forelimbs (Fig. 2F). It is noteworthy that in this species the hindlimbs also develop a distinct IM that regresses as the limbs develop (Fig. 2F).

The patterns of forelimb and hindlimb development of *H. retardatus* appear to be identical to that of other pond-type species (Table 1) (Iizuka et al., 2005; Iizuka et al., 2009; Iizuka & Akiyama, 2018), having a fin like IM that forms between digits 1 and 2 during early limb development which then disappears as limb development proceeds (Fig. 2). Among hynobiid salamanders only the pond-adapted species have well-developed IMs. Systematic analyses of hynobiid and non-hynobiid salamanders, including the present one, indicate the presence of vestigial IM during limb development of the forelimbs on stage 51 of larva (Table 1; Fig. 2) (Iizuka et al., 2005; Iizuka, 2009; Iizuka et al., 2010; Iizuka & Akiyama, 2018). This reinforces our previous conclusions that 1) an IM is probably a character specific to cryptobranchoid salamanders, 2) the IM seems to have some functional significance in pond-larva habitat, since IM is absent in larvae living in fast-stream habitats, and 3) the IM has evolved or disappeared two or three times independently in cryptobranchoid lineages (Table 1) (Iizuka et al., 2005; Iizuka, 2009).

Table 1. A list of larval habitat and interdigital structures* among salamanders

	Species	Larval habitat	Interdigital structure
Hynobiidae			
Salamandrella	*S. keyserlingii*	pond	IM
Hynobius	*H. retardatus*	pond	IM
	H. abei	pond	IM
	H. lichenatus	pond	IM
	H. nigrescens	pond	IM
	H. tokyoensis	pond	IM
	H. dunni	pond	IM
	H. kimurae	stream	vIM
	H. formosanus	stream	vIM
	H. sonani	stream	vIM
Onychodactylus	*0. japonicus*	stream	IW
Cryptobranchidae			
Andrias	*A. japonicus*	stream	vIM
Cryptobranchus	*C. alleganiensis*	stream	vIM

*IM - interdigital membrane, vIM – vestigial interdigtial membrane, IW – interdigital web

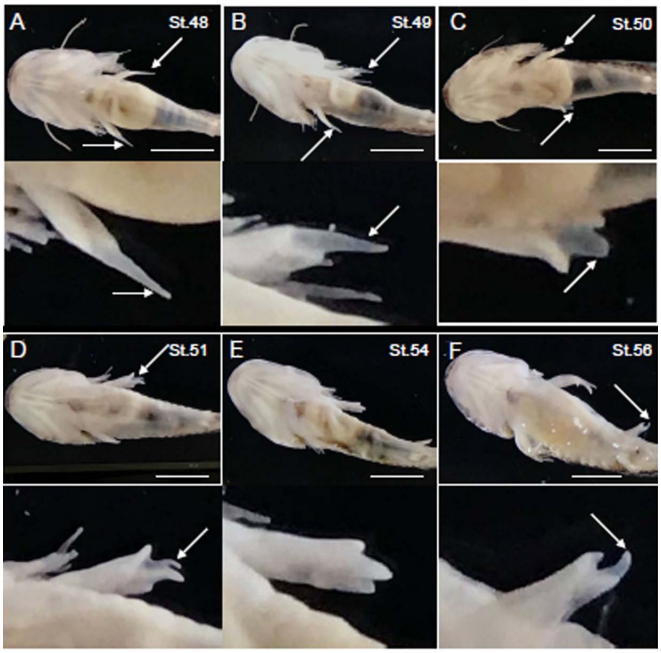

Figure 2. A-F. Ventral views of larval *Hynobius retardatus* at different stages (stage number shown upper right of each image) with the anterior balancers projecting laterally in A-C. Lower associated images are close ups of the interdigital membrane (arrows) of the forelimb, except in F. where the hindlimb is shown. Interdigital membranes disappear soon after stages 51-52 so that in E. (stage 54) a prominent 3rd digit has developed after apoptosis of the IM. The scale denotes 5 mm.

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