Observation of limb development in the Oita salamander, *Hynobius dunni*

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The Oita salamander, *Hynobius dunni* is endemic to eastern Kyushu, and distributed mainly in the central area of Kyushu island (Sugawara et al., 2018). The species has a number of interesting characteristics that have attracted the attention of biologists, including cannibalism and development (Michaels, 2015, 2017). The breeding season commences in December until the end of April, with peak activity in February (Sparreboom, 2014). We had an opportunity to observe embryos from Kunisaki Peninsula, Kyushu on February 15, 2018 (Fig. 1A) in an artificial environment for comparative study with the northern species of *Hynobius*, Hokkaido salamander, *H. retardatus* and southern species, Oita salamander, *H. dunni*. An aquarium for the developing embryos was deposited inside a wine cellar used as a cool environment (Foresta Japan, ST-SV140G), which maintained water temperature at 13° C. We observed and analyzed several developmental stages from both embryos and larvae of *H. dunni* to after metamorphosis according to normal development of *H. nigrescens* (Fig. 1 B, D) (Iwasawa & Yamashita, 1991).

Pond-type (larval adaptation to quiet stream), hynobiid salamanders have an interdigital membrane (IM), which is a fin-like structure that forms between digits 1 and 2 during early limb development and disappears as limb development proceeds (Iizuka et al., 2005; Iizuka, 2009). Only the pond adapted species of hynobiid salamanders stand out among hynobiid salamanders in having IM as vestigial organ. A phylogenetic analysis, including using our observations along with that available for other hynobiid and non-hynobiid salamanders indicated the presence of vestigial IM during limb development of the forelimbs on 51 stages of larva (Fig. 1C). This leads us to conclude that 1) an IM is probably characteristic of cryptobranchoid salamanders, 2) the IM has some functional significance in pond larva habitat, a function that is absent in fast stream larva habitat, and 3) the IM has been recognised or reduced two or three times independently in cryptobranchoid lineages (Iizuka et al., 2005; Iizuka, 2009). However, the evolutionary implication of IM require discussion within the genera *Hynobius* and *Salamandrella* as sister taxa. We previously

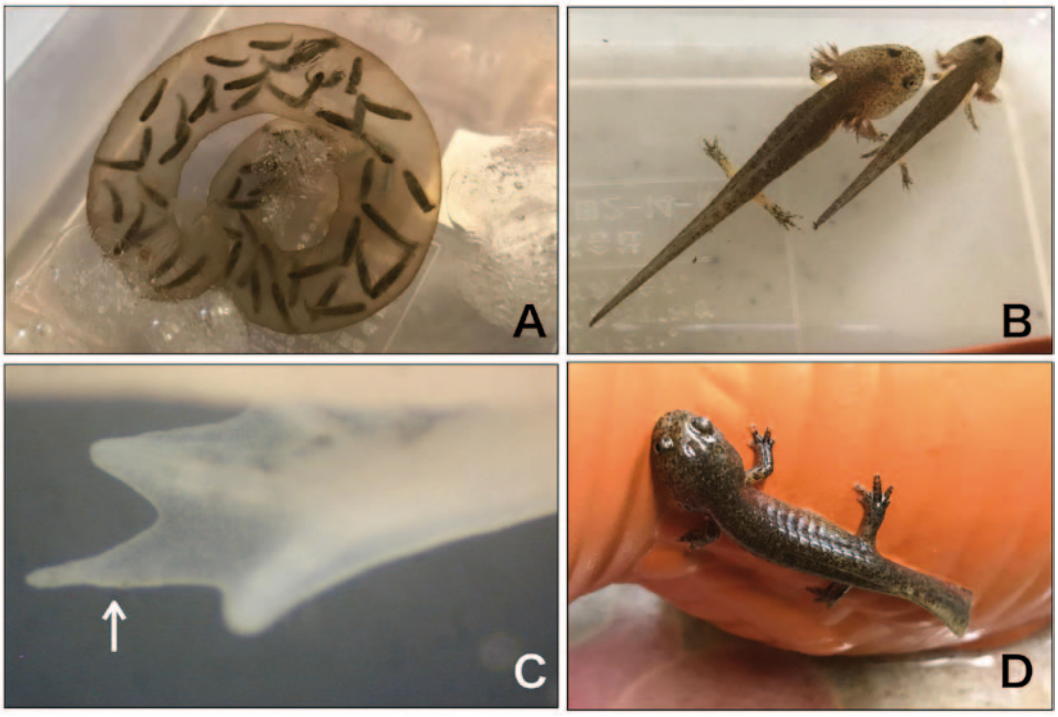

Figure 1. **(A)** eggs, **(B)** two different sized larvae, **(C)** white arrow indicates vestigial organ, interdigital membrane of left forelimb, **(D)** specimen just after metamorphosis but with lost tail tip.

published an integrative analysis of hynobiid salamanders with IM for adaptation of larvae to an aquatic environment (Iizuka et al., 2016). However there is currently no information about IM in hynobiids endemic to Kyushu and this paper presents the first observation of vestigial IM based on limb development in *Hynobius* from this area (Iwasawa & Yamashita, 1991).

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