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Three new species of the salamander genus *Hynobius* (Amphibia, Urodela, Hynobiidae) from Kyushu, Japan

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Abstract

Three new species of lotic breeding *Hynobius*, formerly assigned to *H. boulengeri*, are described from the Kyushu region, southwestern Japan. They differ from all the known congeners by a unique combination of body size, character ratios, coloration, mtDNA, and allozymic characteristics. Together with *H. stejnegeri* they form a clade, which is not a sister group of *H. boulengeri*, and their speciation in Kyushu is surmised to have occurred at the end of Miocene, accompanied by differentiations in larval period and metamorphosing size. Measures of conservation of these new species are discussed briefly.

Key words: Urodela, *Hynobius*, new species, Kyushu, Japan, taxonomy

Introduction

Hynobius boulengeri (Thompson 1912) is a small-sized Japanese salamander of pure lotic breeding behavior, like *H. hirosei*, *H. katoi*, *H. kimurae*, *H. naevius*, *H. stejnegeri*, and *H. yatsui* (after Nishikawa *et al.* 2007). Although *H. tsuensis* and *H. okiensis* also breed in lotic waters, they are thought to have acquired this habit secondarily (Matsui *et al.* 2007; Kim *et al.* 2007). *Hynobius boulengeri* was originally described under a distinct genus *Pachypalaminus* (Thompson 1912), and this name was long used (e.g. Sato 1943). After the species was moved to *Hynobius* (Nishio *et al.* 1987), intraspecific variation was little studied. In 2001, we first examined allozymic variation in populations of *H. boulengeri*, and found that they were separated into three groups, i.e., Kii Peninsula on Honshu mainland, Shikoku Island, and Kyushu Island groups, that differ from each other at distinct specific levels (Nishikawa *et al.* 2001). Later, we found that *H. stejnegeri*, endemic to the Kyushu Central Mountains, Kyushu Island, was genetically nested into the Kyushu populations of *H. boulengeri* (Nishikawa *et al.* 2005). On the basis of morphological and allozymic studies on *H. boulengeri* sensu lato and *H. stejnegeri* (hereafter, collectively called the *H. boulengeri* complex), we proposed to treat the population from the Kii Peninsula as *H. boulengeri* sensu stricto, that from Shikoku Island as *H. hirosei* Lantz 1931, and the population from the Kyushu Central Mountains as *H. stejnegeri* Dunn 1923. However, the name *H. boulengeri* sensu lato was retained for the remaining populations from Kyushu because of the paucity of comparable specimens (Nishikawa *et al.* 2007).

In order to determine the taxonomic status of the Kyushu populations of *H. boulengeri* sensu lato, we accumulated additional samples and compared them by use of mitochondrial DNA sequencing and external morphology. As a result, we confirmed independent specific status for Kyushu populations from the Sobokatamuki Mountains, Amakusa Islands, and Osumi Peninsula. Thus, in the present paper, we describe each of them as a new species.

Material and methods

Salamanders collected in the field were fully anesthetized by saturated acetone-chloroform solution and tissues

The larval life history variation would have promoted morphological differentiation between the ancestral populations in the Sobo-Katamuki Mountains and the Osumi Peninsula, and finally caused a speciation into *H. shinichisatoi* and *H. osumiensis*. For the remaining new species, *H. amakusaensis*, similar situation could be associated with its speciation, but data sufficient to evaluate such a hypothesis are lacking. In order to detect a factor that promoted speciation of *H. amakusaensis*, it is worth studying its life history variation to compare the remaining two new species. It is also necessary to compare *H. amakusaensis* with *H. stejnegeri* because they proved to be closely related phylogenetically.

The great difference in adult size among the three new species would affect the distribution pattern of the lotic breeding species in Kyushu. The large-sized *H. shinichisatoi* (and also large-sized *H. stejnegeri* and *H. naevius*) occurs with the small-sized *H. yatsui*, but the small-sized *H. osumiensis* and middle-sized *H. amakusaensis* do not occur with the small-sized *H. yatsui*. Body size is one of the factors to determine whether or not a species can occur sympatrically with the other species (Nishikawa *et al.* 2007). In order to discuss historical formation of their distribution pattern, it is necessary to know reliable time of their divergence. However, this is not easy: in the present mtDNA data, the differentiation of the three new species and *H. stejnegeri* was estimated to have started at 6.3 Mya, much older than 1.5 Mya previously estimated from the allozyme data (Nishikawa *et al.* 2005). Similar magnitude of discordance between mtDNA and allozyme data has been reported in other hynobiid species (Matsui *et al.* 2008), but in the present case, the estimate from mtDNA data seems more plausible, when geohistorical events in Kyushu are considered. The range of the species in question in central Kyushu is non-volcanic in nature and bordered in the north by the Beppu-Shimabara Graben, where extensive volcanic activity occurred approximately 6.0-5.0 and 2.0-0.5 Mya (Sakamoto *et al.* 2009). Furthermore, in southern Kyushu, the species are present in non-volcanic eastern area, but are absent in volcanic western area. The border of these two areas is the Kagoshima Graben, which was formed 1.8–1.0 Mya. Probably the divergence of the four species is deeply related to these geohistorical events, and the start of divergence would be more likely related to old volcanic activity around 6.0 Mya.

Further, the historical formation of the species range of the lotic breeding species in Kyushu must be more complex, related not only to divergence time and the body size, but also to breeding habit of the species and environmental factors (Tominaga *et al.* 2003, 2005, 2006; Sakamoto *et al.* 2005, 2009).

By the addition of three new species, the number of *Hynobius* species from Kyushu and its adjacent small islands is now increased to nine in total (lotic breeding *H. amakusaensis*, *H. naevius*, *H. osumiensis*, *H. shinichisatoi*, *H. stejnegeri*, *H. yatsui*, and *H. tsuensis*; lentic breeding *H. dunnii* and *H. nebulosus*). This number is much larger than that from similar-sized areas in Japan and other Asian regions where species of *Hynobius* occur. This fact is important for conservation. We must pay much attention to the conservation of these small salamanders because many original habitats in Kyushu area have been deteriorated. Especially, all the three new species described in this paper have very small distributional ranges, and although some parts of their habitats and the species themselves are protected at national and local governmental levels, their conservational status must be urgently updated based on the results of this study.

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APPENDIX 1. Specimens examined for morphological comparisons.

- Hynobius bouleengeri*: KUHE 2538, 5609, 7095, 8312, 8876, 8877, 16044, 22741–21744, 22772, 22775, 25647–25650, 25654–25658, and T 2694 from Kami-kitayama-mura, Nara Prefecture, Japan (n=23).
- H. Hirosei*: KUHE 24136 and 24467, and T 2162–2164, 2119, 2173–2178, 2180–2183, and 3002–3006 from Saijyo-shi, Ehime Prefecture, Japan (n=21).
- H. katoi*: KUHE 14664, 14665, 17946, 17949, 17950, 17952, 18391, 18392, 18404, 21686, 21689, and 26098 from Fujieda-shi, Shizuoka Prefecture, Japan (n=12).
- H. naevius*: KUHE 9671–9673 and 28637, and SK KM3 from Tara-cho, Nagasaki Prefecture, Japan (n=5).
- H. stejnegeri*: KUHE 12983, 14955, 14956, 22815, 22817–22819, 26065–26068, 27156–27158, 27559, 27560, and T 2537 and 2808, and ST 4 from Gokase-cho, Miyazaki Prefecture, Japan (n=19); KUHE 26073–26078, and 36083 from Nishimera-son, Miyazaki Prefecture, Japan (n=7).
- H. yatsui*: KUHE 25098, 27383, and 28846, and ST 5, 10mL, 10mS, and 13 from Saeki-shi, Oita Prefecture, Japan (n=7).

APPENDIX 2. Specimens examined for PSVN.

- Hynobius shinichisatoi* sp. nov.: KUHE 21619, 22813, 22889, 24507, 24878, 24967, 26141, 26142, 27125, 27159, 27185, 28748–28754, 28757, 35423, ST 1 and 2, and IABHU J518.
- Hynobius osumiensis* sp. nov.: KUHE 18923, 22892, 24961–24963, 27331–27333, 28539, 28747, 41263, 43161, 43673, 43681, 43869, 44623, 44624, 44752–44754, 45450, NSMT H03664 and H04534, OMNH Am261, and IABHU J617-1 and J617-2.
- Hynobius amakusaensis* sp. nov.: KUHE 30332–30341, 38446–38448, 42772–42779, 42783, 43870, 44622, 44749, 44750, 45426, 45427, 45443, 45445, 45447, 45449, SK KS1 and 2, and T 3117.
- Hynobius stejnegeri*: KUHE 12983, 14955, 14956, 22815, 22817–22819, 26065–26068, 27156–27560, ST 3 and 4, and T 2537 and 2808 from Gokase-cho, Miyazaki Prefecture, Japan; KUHE 26073–26078, and 26083 from Nishimera-son, Miyazaki Prefecture, Japan.