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Limb malformations in *Bombina orientalis* (Anura: Bombinatoridae) in the Republic of Korea based on museum specimens

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Amphibians are important bioindicators because their life history traits are highly sensitive to environmental alterations, with results often visible through developmental abnormalities (Taylor et al., 2005). A broad scope of morphological anomalies in amphibians has been documented over the years, including incomplete or missing digits and limbs, additional digits and limbs, and body deformations (Blaustein and Johnson, 2003). Among the different types of deformities documented, limb malformation seems to be the most prevalent (Gardiner and Hoppe, 1999; Blaustein and Johnson, 2003). In wild amphibian populations, limb malformations have been associated with environmental degradation, diseases, parasitism, chemical pollution, UV radiation, mutation and trauma (Stopper et al., 2002; Blaustein and Johnson, 2003; Taylor et al., 2005; Johnson et al., 2006). Although knowledge on such malformations is important to understand amphibian biology and local environmental quality, detailed and comparative studies are hindered by the general paucity of historical data on deformed amphibians (Johnson et al., 2003; Medina et al., 2013). In this context, specimens preserved in museum collections can aid in revealing the historical presence and prevalence of deformed amphibians.

The oriental fire-bellied toad, *Bombina orientalis*, is a common species in the Republic of Korea. It is commonly found in creeks and streams and also around human settlements (Kang et al., 2016). There are approximately 435 preserved and labelled *B. orientalis* specimens in the collection of Ewha Womans University Natural History Museum (EWNHM; Sabaj, 2016) in Seoul, Republic of Korea. The specimens were collected from various locations throughout the country, with the oldest sample collected in 1951 and the most recent collected in 2007. While examining these specimens we found eight animals from four localities with different degree of limb malformations (Fig. 1). One specimen collected in the Gyeryong Mountain (EWNHM.5677) had shortened left tarsal and digits reduced both in numbers and length (Fig. 2). Two other specimens collected in the same location (EWNHM.5624 and 5663) had deformed right tarsals. In these two specimens, the digits were completely missing, with the deformed tarsal bones tapering off to pointy tips (Fig. 2). The last examined

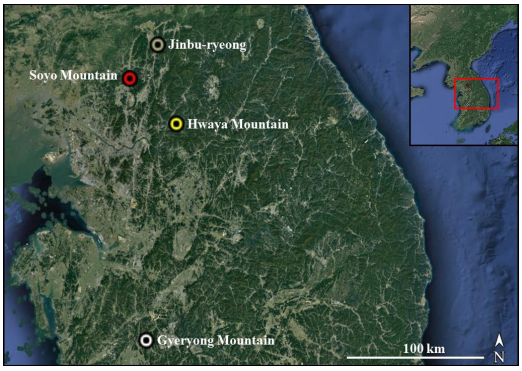

Figure 1. Collection sites of the eight deformed *Bombina orientalis* specimens in the Republic of Korea. Map generated using Google Earth Pro (Google Inc., California, USA), with data credits to SIO, NOAA, U.S. Navy, NGA, GEBCC. Image Landsat/Copernicus.

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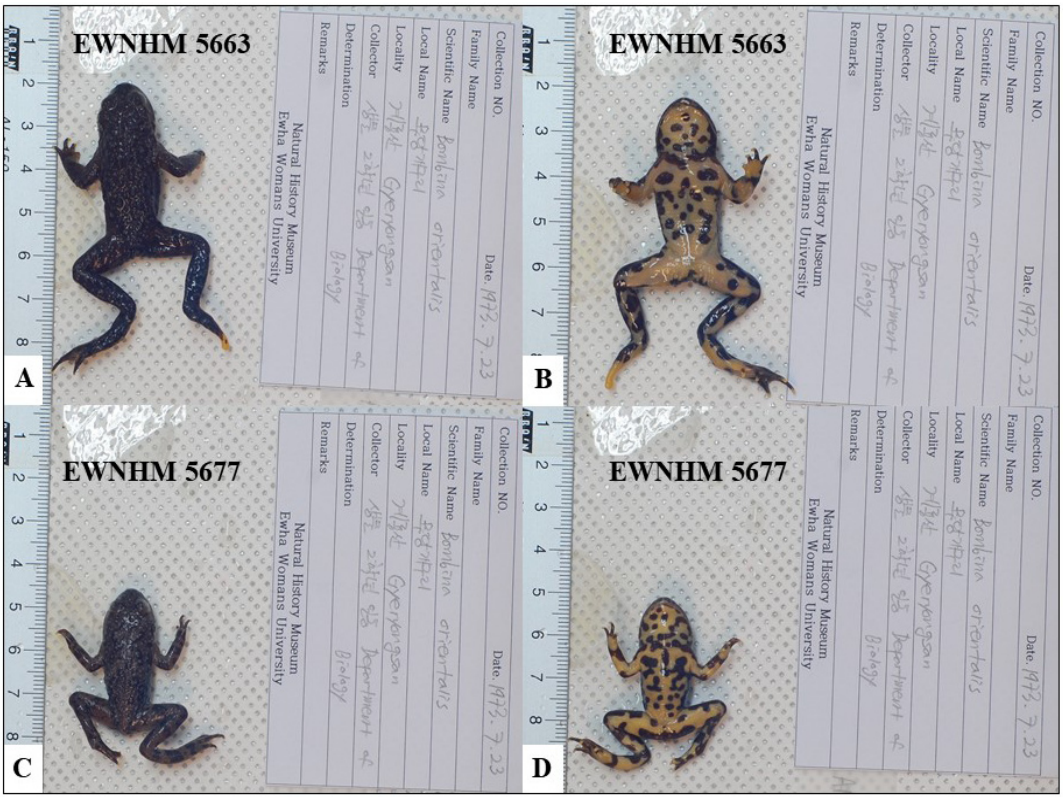

Figure 2. Two individual *Bombina orientalis* collected from Gyeryongsan on 23 July 1973 with malformed hindlimbs. (A): specimen voucher ANIMAL 5663, dorsal side. (B): specimen voucher ANIMAL 5663, ventral side. (C): specimen voucher ANIMAL 5677, dorsal side. (D): specimen voucher ANIMAL 5677, ventral side. All specimens are housed in the collection of Ewha Womans University Natural History Museum (EWNHM).

specimen from the same location (EWNHM.5642) had a reduced number of digits on its right hindlimb, with the number of digits reduced from the usual five to three. Two specimens from Jinbu-ryeong (EWNHM.5437 and EWNHM.5463) showed digits reduced in length on the right hindlimbs. One specimen from the Hwaya Mountain (EWNHM.5449) had a reduced left tarsal with missing digits as well as reduced left tibio-fibula. The specimen from the Soyo Mountain (EWNHM.5733) had a malformed left forelimb, with its radio-ulna and digits completely missing.

The four deformed specimens from the Gyeryong Mountain are part of a series of 150 specimens collected between 23 and 24 July 1973 (prevalence = 2.7%). The two specimens from Jinbu-ryeong are part of 77 specimens collected on 12 August 1972 (2.6%), and the specimen from the Hwaya Mountain is from a series of 15 specimens collected on 22 April 1985 (6.7%). Finally, the specimen from the Soyo Mountain is one of 50 specimens collected on 11 June 1972 (2.0%). The exact origin of the specimens within a locality could not be determined from the information provided by the labels.

The causes of malformations are unclear as the surrounding environments of the sampling sites at this time period were not documented. Weeding and other agricultural practices can cause injuries and deformations in amphibians, though these are unlikely causes here as the collection sites are mountains, in habitats more likely to be valleys and creeks than agricultural lands. Nevertheless, estimates for the cause can be made for the Gyeryong Mountain specimens, the 1970s coincide with the development of Gyeryongsan National Park, thus implying increased human activity (e.g. road construction) in the region (Korea National Parks, 2008). Previous study on deformities in wild

B. orientalis in Republic of Korea also showed that although the frequency of abnormal individuals in each population is low, the malformations are generally associated with human activity (Kang et al., 2016). In addition, the cause of malformations in these specimens are likely to be multifactorial (Blaustein and Johnson, 2003; Haas et al., 2018). These specimens add to valuable historical references for future research into amphibian malformations, and call for further analyses on the morphology of amphibian vouchers from museums.

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