Amphibian Population Declines and Chytridiomycosis in South Korea

By Mi-Sook Min, Hang Lee, & Bruce Waldman

Korea has a diverse, but understudied, amphibian fauna comprising 18 species of which only two are considered to be of concern on the IUCN Red List. The systematics of the four species of hynobiid salamanders species have been well studied (Baek et al. 2011), but little is known about their ecology. Two endemic species, the Jeju salamander, *Hynobius quelpaertensis*, confined to Jeju Island and southern regions, and the Kori salamander, *H. yangi* , found in the southeast of the country, resemble the more widely distributed Korean salamander *H. leechii* and previously were classified as subspecies. Further work may reveal at least three additional species in this group. A lungless salamander, *Karsenia koreana*, was discovered recently and represents an enigma as the only known plethodontid in Asia, but it is not genetically close to North American *Plethodon* (Min et al. 2005).

Rana coreana. Photo: Jonathan Fong

Thirteen frog species, from five families, most of which are widely distributed in Asia, including the Asian toad, *Bufo gargarizans*, and the oriental fire-bellied toad, *Bombina orientalis*, appear not to be at risk although their populations in Korea have declined noticeably in recent years. Of special interest are the Korean water toad, *Bufo stejnegeri*, which spends much of the year in streams and rivers; the microhylid narrow-mouthed toad, *Kaloula borealis*, which although common across much of its range, is considered endangered in Korea; and the Suweon tree frog, *Hyla suweonensis*, which is restricted to small ranges and is reproductively isolated from the phenotypically similar tree frog *Hyla japonica*.

Of the 'true' frogs, the black-spotted pond frog *Pelophylax nigromaculatus* (*Rana nigromaculata*) and the gold-spotted pond frog *P. chosenicus* (*Rana chosenica*) are listed as 'near threatened' and 'vulnerable', respectively, on the IUCN Red List. *Pelophylax chosenicus* is known from fewer than 26 locations, and its remaining habitat is at risk from urban development. Other species, including the wrinkled or rough skinned frog, *Glandirana* (*Rana*) *rugosa*; the Korean brown frog, *Rana coreana*; Dybowski's brown frog, *Rana dybowskii*; and the Huanren brown frog, *Rana huanrenensis*, appear not to be at risk. The American bullfrog, *Lithobates catesbeianus (Rana catesbeiana),* now is common throughout South Korea and may be a vector for the spread of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*).

Prevalence of amphibian chytrid fungus in South Korea

We are studying how the amphibian chytrid fungus affects Korean species and its possible contribution to population declines. Although no mass mortality events have been reported, nor have any individuals from the wild been observed demonstrating clinical signs of chytridiomycosis among the Korean amphibians, population sizes have declined and ranges have contracted in several species. Habitat degradation and destruction may be primary causes, as well as harvesting for food or medicine especially in rural areas, but disease also may play a role. Since 2007, we have surveyed amphibians throughout the Korean peninsula and Jeju Island for *Bd* infection using a combination of histology, immunoperoxidase staining, and PCR (Yang et al. 2009a).

Based on samples of 1,110 individuals from 81 regions, we have found amphibians infected by *Bd* throughout the country. Incheon and Busan, near coastal ports, show the highest prevalence of infected animals (16% and 26%, respectively). Prevalence ranges between 3 and 9% elsewhere, lowest in western areas of the country. American bullfrogs, *L. catesbeianus*, show the highest prevalence (18%), consistent with the hypothesis that they may be the source and vector of *Bd* spread. Surprisingly, given its limited and restricted range, the Jeju salamander, *H. quelpaertensis*, also shows a prevalence of 18%. This is followed by *G. rugosa* (11%), *H. japonica* (10%), *R. dybowskii* (9%), *B. gargarizans* (7%), *R. coreana* (5%), *B. orientalis* (5%), *H. leechii* (2%), and *P. nigromaculatus* (1%). Sample sizes of other species are still too low to allow us to draw conclusions.

Has amphibian chytrid fungus arrived only recently in South Korea?

Analysis of the haplotypes of the *Bd* lineages observed infecting Korean amphibians reveals a diversity of strains including several unique lineages that have not been found elsewhere in the world but may be closely related to those in Japan (Goka et al. 2009). Among 13 *Bd* positive sequences, six were coincident with sequences previously entered into GenBank while 3 haplotypes from 7 sequences in South Korea were not. The possibility thus exists that although *Bd* may have been carried into Korea on introduced species such as *L. catesbeianus*, *Bd* may have been present in Korea prior to recent amphibian introductions. In that case, endemic *Bd* lineages may have evolved to be less virulent to their amphibian hosts, or conversely, amphibians may have evolved resistance to these lineages. We plan to further characterize the *Bd* lineages present in Korea and their relative virulence to amphibian populations.

Some anuran species which we investigated in our study. Top left: *Bufo gargarizans.* Photo: Jungbae Park. top right: *Hyla japonica*. Photo: Jungbae Park. bottom left: *Rana huanrenensis.* Photo: Jungbae Park. bottom right: *Pelophylax nigromaculatus*. Photo: Jonathan Fong.

Evolution of immunogenetic responses to amphibian chytrid fungus

The immunological response of amphibian hosts to *Bd* is a major focus of our research. We are studying the co-evolution of *Bd* lineages and the immunological responses of their Korean hosts, especially focusing on genetic variation in the major histocompatibility complex (MHC). By sequencing MHC class I and class II genes, we are examining whether particular MHC alleles confer resistance to *Bd* and thus spread in populations. Although this work still is in progress, we have compiled MHC sequences directly from Korean amphibians by the use of next-generation sequencing methodologies. This should allow us to examine the evolution of hosts' immunogenetic responses more rapidly, and in more extensive detail, than if we were to use traditional means of genetic analysis, which rely on sequence information obtained from model species such as *Xenopus laevis* (Bos and Waldman 2006). Should we find that particular MHC alleles confer resistance to *Bd*, we will develop plans for captive management of threatened species that may involve selective breeding for disease resistance (Barribeau et al. 2008).

While we have accumulated no evidence yet that *Bd* is significantly impacting Korean species, we cannot afford to be complacent. Our studies are still in the beginning stages, and although Koreans love nature and wildlife, remarkably little is known about the ecology or life history of Korean amphibians. South Korean government policy actively promotes nature restoration, for example, by releasing large number of amphibians into city reserves. But these reintroductions, even as they heighten awareness of Korean amphibians, may pose risks to population viability as genetic differentiation of source populations is not considered nor are introduced animals tested for *Bd* infection. We also are concerned that new, possibly more virulent lineages of *Bd* might be introduced into Korea through the continued unregulated importation of amphibians as pets (Yang et al. 2009b), which are not screened for *Bd* infection.

Acknowledgments

Our research is supported by grants from the National Research Foundation of Korea and the Seoul National University Brain Fusion Program.

Author details: Mi-Sook Min[1] (minbio@snu.ac.kr), Hang Lee[1] (hanglee@snu.ac.kr), and Bruce Waldman[2] (waldman@snu.ac.kr). [1]Conservation Genome Resource Bank for Korean Wildlife (CGRB), College of Veterinary Medicine, and [2]Laboratory of Behavioral and Population Ecology, School of Biological Sciences, College of Natural Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, South Korea.

Literature Cited

Baek, H.J., M.Y. Lee, H. Lee, and M.S. Min. 2011. Mitochondrial DNA data unveil highly divergent populations within the genus *Hynobius* (Caudata: Hynobiidae) in South Korea. Molecules and Cells 31: 105-112.

Barribeau, S.M., J. Villinger, and B. Waldman. 2008. Major histocompatibility complex based resistance to a common bacterial pathogen of amphibians. PLoS ONE 3(7): e2692.

Bos, D.B., and B. Waldman. 2006. Evolution by recombination and transspecies polymorphism in the MHC class I gene of *Xenopus laevis*. Molecular Biology and Evolution 23: 137-143.

Goka, K., J. Yokoyama, Y. Une, T. Kuroki, K. Suzuki, M. Nakahara, A. Kobayashi, S. Inaba, T. Mizutani, and A. Hyatt. 2009. Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Molecular Ecology 18: 4757–4774.

Min, M.S., S.Y. Yang, R.M. Bonett, D.R. Vieites, R.A. Brandon, and D.B. Wake. 2005. Discovery of the first Asian plethodontid salamander. Nature 435: 87–90.

Yang, H., H. Baek, R. Speare, R. Webb, S. Park, T. Kim, K.C. Lasater, S. Shin, S. Son, J. Park, M. Min, Y. Kim, K. Na, H. Lee, and S. Park. 2009a. First detection of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, in free-ranging populations of amphibians on mainland Asia: survey in South Korea. Diseases of Aquatic Organisms 86: 9-13.

Yang, H.J., S.C. Park, R. Speare, Y.J. Kim, H. Lee, and M.S. Min. 2009b. *Case report of chytridiomycosis in an exotic frog species, Litoria caerulea in South Korea.* Korean Journal of Herpetology 1: 71-78.