

## The origin of Amphibian chytridiomycosis: Did it come from Japan?

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A serious disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis* was first found in Japan in December 2006 in imported pet frogs. This was the first report of chytridiomycosis in Asia. To inspect the origin and expansion process of the chytrid fungus in Japan, we surveyed the distribution and genetic variation of the fungus among captive and wild frog populations. We established a nested PCR assay that uses two pairs of PCR primers to amplify the internal transcribed spacer (ITS) region of a ribosomal RNA cassette to detect mild fungal infections from as little as 0.001 pg (1 fg) of *B. dendrobatidis* DNA. We collected swab samples from 559 captive amphibians, and 5,565 wild collected at field sites from northern to southwestern Japan. We detected infections in native and exotic species, both in captivity and in the field. Sequencing of PCR products revealed 50 haplotypes of the *B. dendrobatidis* ITS region. Phylogenetic analysis for the haplotypes combined with haplotype sequences already detected in other countries showed that genetic diversity of *Bd* in Japan was higher than that in other countries. Furthermore, it was suggested that 3 of the haplotypes detected in Japan were specific to the Japanese giant salamander (*Andrias japonicus*) and appeared to have established a commensal relationship with this native amphibian. The highest genetic diversity of *B. dendrobatidis* was found in the sword-tail newt (*Cynops ensicauda*) endemic to Okinawa Islands and next in the alien American bullfrog (*Rana catesbeiana*). From these results, combined with no evidence of chytridiomycosis occurrence in the Japanese native species, we led to a new hypothesis for source of the fungus, “Asia or Japan origin hypothesis”. To improve chytridiomycosis risk management in the world, we must restrict the amphibian trade, especially from Japan.

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