

A Method Combining Geographic Information Systems and Environmental DNA Reveals Hidden Populations of the Endangered Japanese Clouded Salamander, *Hynobius nebulosus*

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The loss of biodiversity is a serious environmental problem, and factors such as global warming and deforestation due to human activities are considered to be primarily responsible for the marked decrease in animal populations around the world.

Amphibian numbers, in particular, have decreased significantly in recent decades. One such example is the Japanese clouded salamander (*Hynobius nebulosus*), an endemic species listed as endangered in the Japanese Red List, which has a highly restricted distribution in Gifu Prefecture. Before 2016, only two viable populations were known to exist; however, as a result of 30-year investigation, a third population of the species was discovered by an expert. The discovery implies that some populations requiring conservation may have been overlooked. In this study, we used a combination of geographic information system (GIS) and environmental DNA analysis to develop a rapid and effective method for identifying the optimal populations of endangered species for conservation. We selected five sites with similar topography and vegetation characteristics to known *H. nebulosus* habitats using GIS. We collected water samples at the sites and analyzed environmental DNA for the presence of *H. nebulosus*. As a result, *H. nebulosus*-specific DNA was detected from one of the sites. After conducting field investigations for finding *H. nebulosus* eggs at the site, we succeeded in discovering a new population of this species. To the best of our knowledge, this is the first study to employ this methodology to discover previously unknown populations of rare species in about one year. In addition, the developed method can easily be applied to other rare species.

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