Fecal DNA-based discrimination between indigenous *Martes zibellina* and non-indigenous *Martes melampus* in Hokkaido, Japan

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The sable *Martes zibellina* is distributed in northeastern Asia including Siberia, Sakhalin, northern Mongolia, northern China, and northernmost Japan (Hokkaido) (Bakeyev and Sinitsyn 1994). The Japanese sable *M. zibellina brachyura*, a subspecies indigenous to Hokkaido, was previously distributed throughout this region, but extensive hunting led to a reduction in population size, resulting in prohibition of hunting since 1920 (Inukai 1938). Only a few ecological and genetic studies have been conducted on the Japanese sable (e.g., Hosoda et al. 1999; Kurose et al. 1999; Miyoshi and Higashi 2005), and it is currently designated as data deficient (DD) on the IUCN Red List.

The Japanese marten *Martes melampus* was originally distributed in Honshu, the largest island of Japan, and was reportedly introduced to southern Hokkaido from Honshu in the 1940s for the fur industry (Kadosaki 1996). Some of the individuals escaped from farms and spread naturally. *Martes melampus* is more similar to *M. zibellina* in terms of body size, habitat preference (Anderson 1970), and phylogenetic relationships (Sato et al. 2003), than other non-indigenous species in Hokkaido, such as the American mink *Neovison vison*, the Japanese weasel *Mustela itatsi*, and the raccoon *Procyon lotor*, and therefore, habitat expansion of *M. melampus* in Hokkaido could cause a significant impact on *M. zibellina*, and possible hybridization between the two is an issue of concern.

The distribution of *M. zibellina* and *M. melampus* in Hokkaido was investigated by Murakami and Ohtaishi (2000) using photographs, videotapes, carcasses, and live observations. They showed that *M. melampus* occupied southern Hokkaido and *M. zibellina* occupied other areas but not the southern areas. This clear parapatric distribution occurred along the Ishikari lowland, located in central Hokkaido (Fig. 1). Their results, however, were based on only 43 samples, and thus, further observations are needed to confirm whether *M. zibellina* is still distributed in southern Hokkaido and whether *M. melampus* is distributed in the area across the Ishikari lowland.

To explore the distribution of such elusive species, feces are useful materials, since they can be collected in larger quantities from diverse locations compared to carcasses or live samples. DNA extracted from feces can provide accurate species identification. Fecal DNA-based species identification has been conducted for mustelid species (see Gomez-Moliner et al. 2004; Kurose et al. 2005; Namba et al. 2007; Shimatani et al. 2008) and is recognized as an effective approach for identifying the presence of such elusive species.

Currently, four other mustelid species, the ermine *Mustela erminea*, the least weasel *Mustela nivalis*, the Japanese weasel *Mustela itatsi*, and the American mink *Neovison vison*, are distributed in Hokkaido, and they deposit feces similar to those of *M. zibellina* and *M. melampus*. Murakami (2002) attempted species identification for mustelid feces collected in Hokkaido by sequencing a part of mitochondrial DNA extracted from feces, but the success rate was extremely low (2.6%; 5/189), indicating that this method is impractical for studying *M. zibellina* and *M. melampus*. Murakami (2002) attempted species identification for mustelid feces collected in Hokkaido by sequencing a part of mitochondrial DNA extracted from feces, but the success rate was extremely low (2.6%; 5/189), indicating that this method is impractical for studying *M. zibellina* and *M. melampus*. A novel fecal genetic study by Shimatani et al. (2008) developed species specific primers for ten species of carnivores in Hokkaido including *Martes* species. Since they did not apply the primers for discriminating between the fecal samples of *M. zibellina* and *M. melampus*, its use for this purpose is still uncertain. Therefore, the present study aimed to develop a fecal DNA-based discrimination method with high practicality for feces of *M. zibellina* and *M. melampus*, to contribute to the understanding of their distribution in Hokkaido.

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