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TECHNICAL NOTE

# A preliminary study of PCR-RFLP for species identification among the Falconiformes of Japan

**ORNITHOLOGICAL SCIENCE**



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The Falconiformes includes predator, which, ecologically, are at the peak of food webs and thus are important indicators of habitat quality. The fallen feathers and rotten carcasses of such species provide important data with regards their distribution, however identification of some species by their appearance alone may be difficult. In such cases, DNA analysis of such samples may prove a useful aid to species identification (Ishida 1996, Teletchea et al. 2005). In addition to species identification, additional valuable information such as sex, individual and genetic variation may be obtained using DNA from samples (Rudnick et al. 2005, Asai et al. 2006).

Species identification by DNA analysis is carried out by sequencing, polymerase chain reaction (PCR) and restriction enzymes (Teletchea et al. 2005). In particular, the PCR-Restriction Fragment Length Polymorphism (RFLP) is often used (Pfeiffer et al. 2004, Fajardo et al. 2006), because it is less time consuming and less expensive than DNA sequencing, although the PCR-RFLP technique detects base substitution only in specialized regions of DNA. For the Falconiformes, DNA sequencing has been described by Riesing et al. (2003) and Lerner and Mindell (2005), contributing to not only phylogenetic analysis but also species identification. DNA barcodes have been recently utilized for species identification of birds (Hebert et al. 2004). However, despite the above mentioned advantages of PCR-RFLP, there have been no reports on species identification within the Falconiformes using this method.

Database analysis of the Falconifomes suggested appropriate interspecific variation, among genes in mitochondrial DNA (mtDNA), and lower intraspecific variation in the 12S rRNA region. In this study, PCR-RFLP for 12S rRNA was applied to the identification of 14 species of diurnal raptors as a preliminary study of species identification of Falconiformes in Japan.

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## MATERIALS AND METHODS

1) Collection of samples and DNA extraction

Blood, feathers and livers were obtained from 14 species of Falconiformes from Japan: Northern Goshawk *Accipiter gentilis*, Golden Eagle *Aquila chrysaetos*, Gray-faced Buzzard-eagle *Butastur indicus*, Common Buzzard *Buteo buteo*, White-tailed Eagle *Haliaeetus albicilla*, Steller's Sea Eagle *Haliaeetus pelagicus*, Black Kite *Milvus migrans*, Osprey *Pandion haliaetus*, Honey Buzzard *Pernis apivorus*, Crested Serpent Eagle *Spilornis cheela*, Hodgson's Hawk-eagle *Spizaetus nipalensis*, Merlin *Falco columbarius*, Peregrine Falcon *F. peregrinus* and Kestrel *F. tinnunculus* (Table 1). All samples were kept at −80°C until DNA extraction. Five feathers were collected from cadavers in the wild, and the remaining 20 were from cages in zoos. Total DNA was extracted from blood and liver using a DNeasy kit or a QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's instructions. A single feather shaft was cut from a feather to a length of 3 to 8 cm, dissected to less than 5 mm, and digested with proteinase K (Wako) according to the procedure described by Trefil et al. (1999). After removal of the undigested debris by centrifugation, the supernatant was subjected to DNA extraction with a phenol: chloroform: isoamylalcohol (25 : 24 : 1) mixture. The DNA was purified by precipitation with 2-propanol, and finally dissolved in 30 $\mu$l of distilled water. Ten microliters of the DNA suspension was subjected to