Identification of tamaraw (Bubalus mindorensis) from natural habitat-derived fecal samples by PCR-RFLP analysis of cytochrome b gene

Abstract

Fecal DNA analysis is a useful tool for the investigation of endangered species. Tamaraw (Bubalus mindorensis) is endemic to the Philippine island of Mindoro but knowledge of its genetic and ecological information is limited. In this study, we developed a species identification method for tamaraw by fecal DNA analysis. Eighteen feces presumed to be from tamaraw were collected in Mount Iglit-Baco National Park and species-known feces from domestic buffaloes and cattle were obtained from a farm. Additionally, one species-unknown fecal sample was obtained in Mount Aruyan Preserve, where the sighting of tamaraw has not been reported in recent years. Based on DNA sequence data previously reported, the genus Bubalus- and tamaraw-specific primers for PCR of cytochrome b gene were newly designed. The Bubalus-specific primer yielded a 976 bp fragment of cytochrome b for all fecal samples from tamaraw and domestic buffaloes, but not for cattle, whereas the tamaraw-specific primer yielded a 582 bp fragment for all tamaraw fecal samples and for one of the four domestic buffalo samples. PCR-RFLP (restriction fragment length polymorphism) analysis of the 976 bp PCR fragment with AvrII or BsaXI provided distinct differences between tamaraw and domestic buffalo. PCR-RFLP analysis also showed that the species-unknown sample obtained in Mount Aruyan Preserve, originates from tamaraw.