Application of CHD1 Gene and EE0.6 Sequences to Identify Sexes of Several Protected Bird Species in Taiwan


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ABSTRACT: Many bird species, for example Crested Serpent Eagle (Spilornis cheela hoya), Collared Scops Owl (Otus bakkamanoena), Tawny Fish Owl (Ketupa flavipes), Crested Goshawk (Accipiter trivirgatus), Grass Owl (Tyto longimembris), etc., are monomorphic, which is difficult to identify their sex simply by their outward appearance. Especially for those monomorphic endangered species, finding an effective tool to identify their sex beside outward appearance is needed for further captive breeding programs or other conservation plans. In this study, we collected samples of Crested Serpent Eagle, Collared Scops Owl, Tawny Fish Owl, Crested Goshawk, and Grass Owl, five protected monomorphic species in Taiwan, as well as Black Swan (Cygnus atratus) and Nicobar Pigeon (Caloenas nicobarica), two aviary introduced monomorphic species served as a control group. We used sex-specific primers of avian CHD1 (chromo-helicase-DNA-binding) gene and EE0.6 (EcoRI 0.6-kb fragment) sequences to identify the sex of these birds. The results showed that CHD1 gene primers could be used to correctly identify the sex of Black Swans, Nicobar Pigeons and Crested Serpent Eagles, but it could not be used to correctly identify sex in Collared Scops Owls, Tawny Fish Owls, and Crested Goshawks. In the sex identification using EE0.6-sequence fragments, A, C, D and E primer sets could be used for sexing Black Swans; A, B, C, and D primer sets could be used for sexing Crested Serpent Eagles; and E primer set could be used for sexing Nicobar Pigeons and the two owl species. Correct determination of sex is the first step if a captive breeding measure is required. We have demonstrated that several of the existing primer sets can be used for sex determination of several captive breeding and indigenous bird species.

KEY WORDS: Collared Scops Owl, Crested Serpent Eagle, Crested Goshawk, sex identification of bird, Tawny Fish Owl.

INTRODUCTION

The Crested Serpent Eagle (Spilornis cheela hoya), Crested Goshawk (Accipiter trivirgatus), Tawny Fish Owl (Ketupa flavipes), and Collared Scops Owl (Otus bakkamanoena) are native bird species in Taiwan and all are enlisted as “Rare and Valuable” wildlife species and legally protected by Taiwan government. Breeding programs in captivity are important for their conservation. However, all the species are sexually monomorphic which makes it difficult to discriminate between sexes via outward appearance. Therefore, it is important to develop accurate methods to enhance accuracy of sex determination (Duan and Fuerst, 2001; Dubiec and Zagalska-Neubauer, 2006; Cerit and Avanus, 2007) and therefore, improve captive breeding success.

Sex chromosomes in birds are Z and W; ZZ andZW chromosome combination are male and female, respectively. Chromo-helicase-DNA-binding gene (CHD1 gene) is located on sex chromosomes and CHD-W on W (Griffiths and Tiwari, 1995), and CHD-Z on Z (Griffiths and Korn, 1997). Because there are possible differences in intron length of the CHD1 gene between Z and W, primer sets of P2/P8 (Griffiths et al., 1998), 1237L/1272H (Kahn et al., 1998) and 2550F/2718R (Fridolfsson and Ellegren, 1999) were designed for PCR to determine the sex of birds. Due to the genetic differences, some birds have similar intron length in CHD-W genes in both sex chromosomes that make these primers inapplicable to detect sex differences (Kahn et al., 1998; Griffiths et al., 1998; Fridolfsson and Ellegren, 1999). Therefore, the applicability of each set of primer needs to be tested for the accuracy of detection in sex determination. The different EE0.6 (EcoRI 0.6-kb fragment) sequences located on the avian Z and W sex chromosomes are also available for sex determination (Ogawa et al., 1997, 1998; Itoh et al., 2001).

We previously reported several reliable sex-specific genetic markers for sex determination in the Crested Serpent Eagle and Crested Goshawk (Hsu et al., 2009). In this study, we tested the primer sets and report their applicability in determining the sex of four native Taiwanese bird species.