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Validation of *Spilornis cheela hoya* TaqMan probes for potential gender identification of many Accipitridae species

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Abstract

The objective of this study was to test the hypothesis that genders of Accipitridae species, with the same or similar sequences to our previously proposed *Spilornis cheela hoya* (*S. c. hoya*) chromo-helicase-DNA binding protein (*CHD*)-*W*-specific and *CHD-ZW*-common TaqMan probes, can be successfully determined. Eight species of Accipitridae with known genders were collected. After PCR, TA cloning, sequencing, and alignment analyses, sequence length differences of Griffiths P2/P8 PCR amplicons between *CHD-Z* and *CHD-W* genes ranged from 2 to 19 bp for these Accipitridae species, and they were unsolved in 3% agarose gel. Using our previous proposed *S. c. hoya* TaqMan probes, the genders of *Circaetus gallicus*, completely homologous to the sequences for these *CHD* probes, were successfully identified. With one nucleotide difference to *S. c. hoya CHD-W*-specific probe, gender identification of *Accipiter gularis*, *Accipiter soloensis*, *Accipiter trivirgatus*, *Accipiter virgatus*, and *Butastur indicus* were validated. With two nucleotide differences in the *CHD-W*-specific probe and one nucleotide difference in the *CHD-ZW*-common probe, *Pernis ptilorhyncus* also performed well for gender identification. In conclusion, the *S. c. hoya CHD* probes, coupled with the Griffiths P2/P8 primers, were validated to provide accurate and high-throughput gender identification for many Accipitridae species.

Keywords: Accipitridae species; *CHD* gene; Eagle; Gender identification; Sex-specific probe; TaqMan

1. 1. Introduction

Accipitridae is the family of hawks, eagles, kites, harriers, and Old World vultures [1,2]. All Accipitridae species are protected under the Convention on International Trade in Endangered Species (CITES) [3]. As

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top predators, birds of prey are sensitive indicators of environmental quality [2]. Monitoring of the sex ratio of the Accipitridae species became an attractive method to evaluate their population stability. However, the length difference of intron for *CHD-Z* and *CHD-W* genes in many Accipitridae species is extremely short (approximately 3 to 9 bp) [4–10], making it difficult to determine gender of these species by the traditional Griffiths procedure [11]. This problem is resolved by methods of redesigned primers [7,9], PCR-restriction fragment length polymorphism (RFLP) [5,6], microsatellite DNA markers [12,13], random amplified polymorphic DNA (RAPD) [14,15], single-strand conformation polymorphism (SSCP) analysis [16], amplified fragment length polymorphism (AFLP) [17], melting curve assay (MCA) [10], and TaqMan probe [8]. Except for the electrophoresis-free methods for MCA and TaqMan probe (96 or 384 wells available), these methods were currently unsuitable for high-throughput avian gender identification because they required extra time for multiple sample loadings and for electrophoresis after PCR amplification.

In our previous study [8], we reported that *CHD-W*-specific and *CHD-ZW*-common TaqMan probes provided high-throughput gender identification for Accipitridae species such as *Spilornis cheela hoya* (*S. c. hoya*). Most likely, our method [8] may be implemented for gender identification of other Accipitridae species based on the sequence alignments of *CHD* gene. For example, the sequence of the *CHD-W*-specific probe designed for *S. c. hoya* was completely conserved in *Circaetus gallicus* (*C. gallicus*), *Gyps indicus* (*G. indicus*), and *Gyps bengalensis* (*G. bengalensis*), and it was only one nucleotide different from those of *Accipiter nisus* (*A. nisus*), *Spizaetus nipalensis* (*S. nipalensis*), *Aquila chrysaetos* (*A. chrysaetos*), *Circus spilonotus* (*C. spilonotus*), and *Milvus migrans* (*M. migrans*) as described [8]. For the *CHD-ZW*-common probe, all these species were completely conserved. However, this method has been developed for and tested only in the *S. c. hoya* species [8]. The potential use of these *CHD* probes [8] with regard to other species of Accipitridae still requires proof.

The objective of the current study was to test the hypothesis that our proposed *CHD-W*-specific and

Table 1
Summary of gender identification of eight species of Accipitridae using *S. c. hoya* TaqMan probes.

Wells	Species	ID No.[a]	Gender[b]	RFU 1[c]	RFU 2[c]	Call[d]	References
				(*CHD-W*)	(*CHD-ZW*)		
A3	*A. virgatus*	Bd-2225	♂	−6.99	127.82	Allele 2	This study
A5		Bd-3108	♀	732.47	159.00	Heterozygote	
B3	*A. gularis*	Bd-1505	♂	−9.80	110.82	Allele 2	
B5		Bd-1506	♀	678.64	144.12	Heterozygote	
C3	*B. indicus*	Bd-3264	♂	−12.72	87.95	Allele 2	
C5		Bd-4857	♀	662.09	156.80	Heterozygote	
D3	*A. soloensis*	Bd-2466	♂	−13.04	113.51	Allele 2	
D5		Bd-3088	♀	725.54	110.18	Heterozygote	
E3	*P. ptilorhyncus*	Bd-3861	♂	−9.49	126.30	Allele 2	
E5		Bd-434	♀	363.23	98.46	Heterozygote	
G3	*A. trivirgatus*	Bd-6223	♂	−14.93	66.10	Allele 2	
G5		Bd-6224	♀	537.09	75.03	Heterozygote	
F3	*C. gallicus*	Bd-cgB4	♂	−17.51	75.75	Allele 2	[5]
F5		Bd-cgB2	♀	1299.66	172.43	Heterozygote	
B7	*S. c. hoya*	Bd-498	♂	−29.10	93.39	Allele 2	[8]
C7		Bd-475	♀	267.99	59.44	Heterozygote	
G8		Blank		−7.56	−9.04	None	
H8		Blank		−4.19	−7.17	None	
B9		Blank		−4.87	−11.92	None	
D9		Blank		−5.37	−2.25	None	
E9		Blank		−8.13	−12.16	None	
F9		Blank		−7.73	−10.05	None	
G9		Blank		−10.27	−15.24	None	
H9		Blank		−44.35	−13.23	None	

[a] The ID No. is the original banded number for each sample.
[b] The female and male controls were confirmed by anatomic inspection.
[c] RFU, relative fluorescence unit; RFU 1, RFU of Allele 1 (*CHD-ZW*-common probe); RFU 2, RFU of Allele 2 (*CHD-W*-specific probe).
[d] "Heterozygote" indicates female, that is, RFU 1 (+) and RFU 2 (+). "None" indicates there is no calling for gender.