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Morphometric Sexing of Little Spiderhunter (*Arachnothera longirostra*) in Peninsular Malaysia

Chong Leong Puan[1,2,3]*, Wei Lun Ng[4], Christina S.Y. Yong[5] and Abdl Jalil Norehan[1]

[1]*Faculty of Forestry, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*
[2]*Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*
[3]*Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*
[4]*School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275 Guangdong, China*
[5]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

ABSTRACT

Sexual dimorphism is often directly linked to sexual selection, mating systems and resource partitioning, which are crucial in species conservation and management. Many avian species, including pollinator birds, are sexually dimorphic with respect to size and colour, yet, such differences may be subtle for some species. In this study, molecular sexing was performed in addition to determining morphological parameters that can aid in future sex determination of a common forest pollinator, the little spiderhunter (*Arachnothera longirostra*), in Peninsular Malaysia. Based on 23 captures made in four forests, two out of seven body measurements (i.e. wing and tail lengths) were useful in predicting the sexes of the bird with 100% accuracy. In addition, significant differences were found in the head, bill, and total body lengths. Such findings will facilitate more effective sex identification in future field studies, particularly in the case of juveniles.

Keywords: *Arachnothera longirostra*, Discriminant function analysis, Morphometric sexing, Pollinators, Sexual dimorphism

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E-mail addresses:
chongleong@upm.edu.my;
clpuan@yahoo.com (Chong Leong Puan),
ng.wl85@gmail.com (Wei Lun Ng),
chrisyong@upm.edu.my (Christina S.Y. Yong),
raehan_jalil@yahoo.com.my (Abdl Jalil Norehan)
* Corresponding author

INTRODUCTION

Globally, three bird families, namely Trochilidae, Meliphagidae and Nectariniidae, are known as pollinators (Cronk & Ojeda, 2008). These families maintain key ecological functions and services whether in the natural or agricultural ecosystems (Ollerton, et al.,

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2011). Spiderhunters (Nectariniidae) are morphologically distinctive with their long decurved bill. Besides small arthropods, many spiderhunter species consume nectar and hence, also serve as pollinators (Yumoto et al., 1997; Momose et al., 1998; Sakai, et al., 1999; Phillipps & Phillipps 2011; Sakai, et al., 2013), which often exhibit trap-lining behaviour. Unlike the confamilial sunbirds that are often sexually dimorphic with males being relatively more colourful than females, it may be difficult to differentiate the sexes of spiderhunters, unless through careful examination of their body size and pectoral tufts (Cheke et al., 2001).

Malaysia has a total of 10 spiderhunter species with the majority of species living in wooded habitats (MNS-BCC 2015). This includes two Bornean endemics (Whitehead's spiderhunter *Arachnothera juliae* and Bornean spiderhunter *A. everetti*) as well as the recently included of the purple-naped spiderhunter (*A. hypogrammicum*; Moyle et al. 2011) which was once treated as a sunbird. The presence of a relatively high diversity of spiderhunter species, some of which are sympatric, poses more intricate questions with regard to interspecific segregation of niches (Collins, 2008), intersexual partitioning of resources (Paton & Collins, 1989; Temeles & Roberts, 1993) as well as mate choice of these species that are confined to the Oriental region. In addition, knowing the sexes of these pollinating birds may have implications on the understanding of key ecological functions (Ollerton, et al., 2011).

The little spiderhunter (*A. longirostra*) is abundant in disturbed and regenerating forests (Rahman et al., 2010; Wells 2010) and is frequently encountered during mist-netting surveys (Wells, 2010; Phillipps & Phillipps, 2011) compared with other Malaysian spiderhunter species. Male little spiderhunters can be differentiated from females by their larger sizes and the presence of pectoral tufts (Cheke, et al., 2001; Cheke & Mann, 2008; Wells, 2010, but see Jeyarajasingam, 2012), although Wells (2010) noted that the latter feature may develop later than other adult characteristics.

In this study, sexes of randomly caught little spiderhunters from Peninsular Malaysia were determined using molecular methods, before conducting discriminant function analysis on the morphometric measurements obtained from the field. The intention was to develop a way for morphometric sexing of the species for future field studies, eliminating the need for invasive sex determination.

MATERIALS AND METHODS

Sampling

Using mist-nets, we sampled little spiderhunters from three forest reserves located in Peninsular Malaysia, i.e. the Bintang Hijau (5°26'20"N, 100°55'27"E; Perak state), Sungai Lalang (3°1'29"N, 101°54'49"E; Selangor state), and Panti (1°51'38"N, 103°54'26"E; Johor state) Forest Reserves, as well as an isolated forest patch within the Shah Alam National

Botanical Garden (3°5'57"N, 101°30'12"E; Selangor), from January to August 2016.

Using a calliper and a ruler, morphological measurements were taken for the total body, head, wing chord, tarsus, tail, and bill (culmen) lengths, in mm (Figure 1). Weight was measured using a spring balance, in gram. Blood samples were collected by pricking the brachial vein with a sterile 27-gauge needle (Davis 2005), mixed with 100% ethanol and stored at 4°C. All the birds were released after their blood samples were taken.

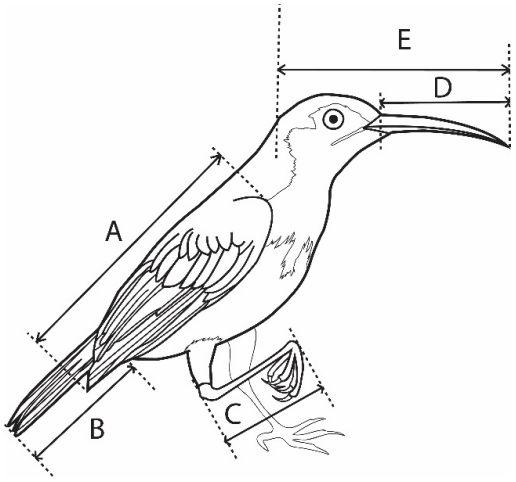

Figure 1. Measurement of (A) wing, (B) tail, (C) tarsus, (D) bill, and (E) head lengths

Molecular sexing

Total genomic DNA from blood samples was extracted from 20 µl of blood using the DNeasy Blood and Tissue Kit (Qiagen) based on the manufacturer's protocol. The extracted DNA samples were stored at -20°C until further analyses.

In birds, sex is determined using the ZW sex determination system, in which males are the homogametic sex (having two Z-chromosomes) and females are the heterogametic sex (having one each of the Z- and W-chromosomes). A primer pair (CHD1F: 5'-TATCGTCAGTTTCCTTTTCAGGT-3' and CHD1R: 5'-CCTTTTATTGATCCATCAAGCCT-3'; Lee et al. 2010) was used to amplify a section of the sex chromosome CHD gene that is present on both the avian W- and Z-chromosomes in differing fragment lengths. The PCR amplifications were performed in 10 µL reactions, each containing approximately 30 ng of genomic DNA as template, 1 µM of each primer, and 5 µL of NEXproTM ePCR 2× master mix (NEX Diagnostics). The PCR reaction profile comprised an initial denaturation of 3 minutes at 95°C; followed by 30 cycles of 30 sec at 95°C, 30 sec at 50°C, and 2 min at 72°C; and finally, an extension step at 72°C for 7 min. The PCR amplicons

were analysed by electrophoresis on 1.0% (weight/volume) agarose gel, stained with ethidium bromide and viewed under UV illumination. Males would present one DNA band (at ~500bp), while females would present two DNA bands (at ~300bp and ~500bp), on the gels.

Morphological data analyses

A Mann-Whitney U test was performed to compare differences in the measurements between male and female birds. All body measurements were reported in means and standard errors. Discriminant Function Analysis (DFA) with a stepwise procedure was applied on the measurements. All statistical tests were performed using SPSS Version 16.0 (SPSS, Inc., Chicago, Illinois).

RESULTS AND DISCUSSION

A total of 23 little spiderhunters were caught, including 14 from Sungai Lalang Forest Reserve, four from Bintang Hijau Forest Reserve, two from Panti Forest Reserve and three from Shah Alam National Botanical Garden. Eleven (47.8%) were males, as determined through DNA analysis (Figure 2). Visually, we successfully identified eight males (72.7% of the total males caught) through the presence of pectoral tufts. Of the three males which sex was difficult to identify in the field, there was only one juvenile with noticeable orange-yellow gape flange.

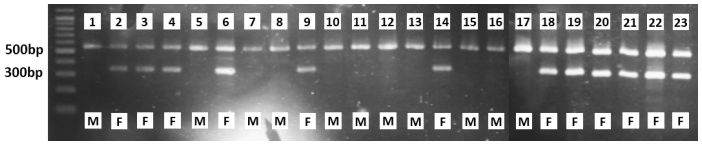

Figure 2. Molecular sexing results. Males show 1 band (ZZ) at ~500bp, and females show 2 bands (ZW) at ~300bp and 500bp

Significant differences were found in the head, bill, wing, tail, and total body lengths ($p \leq 0.01$; Table 1), but not in the tarsus length and weight. Based on DFA, a parsimonious model comprising wing and tail lengths as predictors provided the best possible prediction of the sex of a little spiderhunter with 100% accuracy (Figure 3). The discriminant function (D; Wilk's Lambda = 0.087, $\chi 2 = 48.785$, $P < 0.001$) was:

[D = 0.852*Wing length + 0.531*Tail length]

Based on centroids derived from DFA, a bird with a score on the DF closer to 3.580 would be a male whereas it was -2.983 for females.

Table 1
Morphometric measurements from little spiderhunter and results of Mann-Whitney U-test

Measurements	Male (n = 11)		Female (n = 12)		*Z*	*p*
	$\bar{X}$ ± SE	95% CI	$\bar{X}$ ± SE	95% CI		
Head length (mm)	60.09 ± 3.62	51.51-69.49	52.75 ± 0.54	51.57-53.93	-3.721	<0.001
Bill length (mm)	38.27 ± 0.88	35.92-40.08	34.25 ± 0.54	33.07-35.43	-3.382	0.001
Wing length (mm)	68.18 ± 0.33	67.33-68.67	60.17 ± 0.49	59.09-61.25	-4.097	<0.001
Tail length (mm)	45.18 ± 0.55	43.80-45.80	38.71 ± 0.59	37.42-40.00	-4.101	<0.001
Tarsus length (mm)	20.20 ± 0.13	19.90-20.50	19.25 ± 0.46	18.23-20.27	-2.458	0.014
Total body length (mm)	152.27 ± 2.49	145.80-158.20	142.50 ± 1.98	138.14-146.86	-2.994	0.003
Weight (g)	13.18 ± 0.52	11.90-14.50	12.75 ± 1.72	8.97-16.53	-1.686	0.092

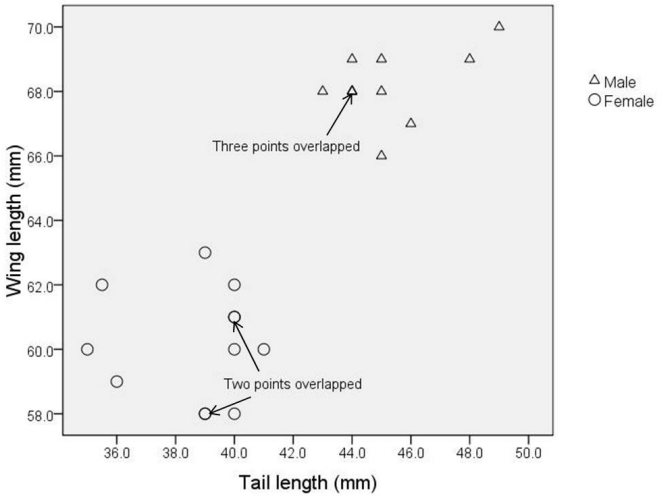

Figure 3. Scatterplot of wing length against tail length measured from the little spiderhunter showing a clear separation according to sexes (n = 23)

In this study, we obtained almost equal sample sizes for both sexes and all body measurements were similar to those reported in literature (Cheke et al., 2001; Cheke & Mann, 2008; Wells, 2010; Jeyarajasingam, 2012). Our results indicated that the little spiderhunter can be sexed based on five morphological parameters. Albeit not significantly heavier, male birds have longer bodies, wings, tails, bills, and heads. Despite the small sample size, the study has also demonstrated that pectoral tufts may be a good indication of sex i.e. adult male. However, such characteristics may not be observed in juveniles and subadults that do not have visible orange-yellow gape flange (Wells, 2010). Hence, for subadults and juveniles, wing and tail lengths may be useful to discriminate the sexes.

Being frequently encountered during mist-netting sessions, and being the most widespread among its congeneric counterparts, with specialised feeding structure, the little spiderhunter has great potential as a focal species for the study of complex ecological interactions in the tropics (Olsen et al., 2013). For example, since male little spiderhunters have longer bills than the females, it would be interesting to examine if differential foraging is present between the sexes, i.e. possible interactions between the birds, as pollinators, and floral anatomy (Paton & Collins, 1989; Sakai et al., 1999; Cronk & Ojeda, 2008; Sakai et. al., 2013). Such research is especially important in view of the increasing risk of losing avian pollinators around the world (Regan et al., 2015).

CONCLUSION

Using morphometric data collected in the field, and supported by molecular sex determination, we were able to evaluate the feasibility of morphometric sex determination in the little spiderhunter in Peninsular Malaysia. Since the sexing of birds is important in behavioural and ecological studies, this study provided an inexpensive, rapid, and accurate way for future sex determination of the little spiderhunter.

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