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# Morphological Sex Determination of East Asian Barn Swallows (*Hirundo rustica*) in Tropical Wintering Region

Nor Adibah Ismail<sup>1</sup>, Ummi Nur Syafiqah Daud<sup>1</sup>, Noor Fatihah Najihah Arazmi<sup>2</sup>, Nurfatin Batrisyia Md Ali<sup>2</sup>, Shukor Md Nor<sup>3</sup> and Mohammad Saiful Mansor<sup>2\*</sup>

<sup>1</sup>Department of Earth Science and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia <sup>2</sup>Department of Biological Sciences and Biotechnology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia <sup>3</sup>50 Jalan 7, Taman Tenaga, Batu 9, Jalan Puchong, 47100 Puchong, Selangor, Malaysia

## ABSTRACT

The Barn Swallow *Hirundo rustica* is a non-breeding, sexually dimorphic, and diurnal migrant that overwinters worldwide, including in Peninsular Malaysia. While numerous studies on Barn Swallows have been conducted, their ecology in wintering sites, particularly tropical regions, remains poorly understood. Notably, little information is available on the morphological sex determination of Barn Swallows, especially for the East Asian Barn Swallow *H. r. gutturalis* population. This population migrates through or winters in the Thai-Malay Peninsula, while breeding occurs from the eastern Himalayas to northeast Russia (Siberia), China, the Korean Peninsula, and Japan. This study aims to determine the best parameters for the morphological sexing of East Asian Barn Swallows and was conducted in Bentong, Pahang, central Peninsular Malaysia. Swallows were captured using a modified scoop net attached to a telescopic pole, and their morphological data were recorded. A total of 46 individual East Asian Barn Swallows (19 females and 27 males) were captured for sex determination. We confirmed the sex and subspecies of sampled individuals

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E-mail addresses:

nadibahismail@gmail.com (Nor Adibah Ismail) ummisyafiqah96@gmail.com (Ummi Nur Syafiqah Daud) n.fatihahnajihah@gmail.com (Noor Fatihah Najihah Arazmi) fatinbatrisyia5@gmail.com (Nurfatin Batrisyia Md Ali) shukor63@live.com (Shukor Md Nor) msaifulmansor@gmail.com (Mohammad Saiful Mansor) \*Corresponding author using a molecular approach. We observed that two of the seven measured variables—tail fork depth (the length difference between the outermost and innermost tail feathers; T6-T1) and the length of the outermost tail feather (6th rectrix; T6) were chosen as the best predictors for sex determination. According to the quadratic discriminant functions constructed, approximately 89.47% of females and 96.3% of males were correctly classified using a combination of both chosen predictors. These morphological determination findings represent

ISSN: 1511-3701 e-ISSN: 2231-8542 baseline knowledge that can help to provide more accurate and convenient Barn Swallow sex determination in the field.

Keywords: Discriminant function analysis, molecular sexing, swallows, sexual dimorphism, wintering region

# INTRODUCTION

The Barn Swallow *H. rustica* is a widespread species that breeds extensively in human settlements. It is one of 83 species belonging to Hirundinidae, a 39 family of birds composed of swallows and martins (communally recognized as hirundines) (Turner, 2006). In total, there are six subspecies of Barn Swallow: *H. r. savignii*, which breed in North Africa; *H. r. transitiva*, which breeds in the Middle East; *H. r. rustica*, which breeds in Europe; *H. r. tytleri*, which breeds in Siberia and Mongolia; *H. r. erythrogaster* breeds in North America (Ismail et al., 2020); and *H. r. gutturalis*, which pass through or overwinter in the Thai-Malay Peninsula (Mansor et al., 2020) and breed from the eastern Himalayas to northeast Russia (Siberia), China, the Korean Peninsula, and Japan (Dor et al., 2010). In addition, the wintering population of *H. rustica* included the subspecies *saturata* Ridgeway and/or *mandschurica* Meise among a majority of *gutturalis* Scopoli. However, the recovery site of one Malayan-ringed bird at Krashyi Chikoi (its presumed breeding location) confirmed that the subspecies *tytleri* Jerdon was also represented among the population wintering in towns in this part of Pahang State in Peninsular Malaysia (Wells, 2007).

Variability in the degree of sexual dimorphism among bird species is due to differences in their social mating mechanisms (Lande & Arnold, 1985; Owens & Hartley, 1998). Such information could help researchers understand the evolutionary features of a population, such as behavioral adaptation (Kissner et al., 2003; Mansor et al., 2018), gender differential distribution (Cristol et al., 1999), sexual selection (Andersson & Iwasa, 1996), and survival problems linked to unfavorable population dynamics arising from a skewed sex ratio (Donald, 2007; Saino & Møller, 1996). Notably, Barn Swallow sexual dimorphism has previously been measured via many sizes and colour-related characteristics (Kose & Møller, 1999; Perrier, 2002; Safran & McGraw, 2004). One method used to identify the sex of adult swallows involves measuring the length and width of white patches on the five outermost tail feathers (Kose & Møller, 1999). This method also provides a total area estimation of the white patches, resulting in sex identification. Hermosell et al. (2007) suggested that sex determination can be analyzed by a discriminant analysis that uses three morphometric variables of Barn Swallows: the length of the outermost tail feathers, the length of the inner tail feathers and the length of the keel). Wells (2007) also has previously reported that, among 1150 wintering birds previously handled when netted at this or neighboring winter roosts, during several years, two peaks in wing-length measurement, at 114-115 mm and 110-112 mm, and two peaks in tail length at 90-95 mm and 80-85 mm, "are likely to have been adult male and adult female modes." Additionally, various additional characteristics, such as tail streamer length and plumage ornaments of various colors (including colouration on the throat, forehead, and ventral regions), are also important parameters in determining Barn Swallow sex (Taylor et al., 2011; Vortman et al., 2011), however, these have not been observed in most studies. Most ecological studies of *H. rustica* have focused on their breeding grounds, while relatively few studies related to their migratory routes and wintering grounds have been performed. Notably, studies on Barn Swallows in Peninsular Malaysia have primarily focused on their population dynamics (Mansor et al., 2020; Medway, 1973), behavior (Ismail et al., 2020), and diet (Mansor et al., 2020). The degree to which genetic and sexual selection influences remains disputed and no relevant studies have been conducted on the East Asian subspecies in this regard. Hence, the present study aims to provide new insights into the morphological sex determination during the migration period in non-breeding and stopover areas, particularly in Peninsular Malaysia.

# MATERIALS AND METHODS

## **Location of Study Area**

The study was conducted in Bentong, Pahang (389835 N, 823163 E), central Peninsular Malaysia (Figure 1). Bentong is located opposite the Titiwangsa Range, approximately 80 km northeast of Kuala Lumpur, the capital of Malaysia. Bentong District is approximately 1831 km<sup>2</sup> and borders the states of Negeri Sembilan and Selangor at its southern and western edges, respectively.



*Figure 1*. The location of Bentong town is in the central region of Peninsular Malaysia. The black dot indicates the sampling site of the present study

# Sample Collection

Between December 2019 and February 2020, swallows were captured using a modified scoop net attached to a telescopic pole. The capture sessions were performed in the late evening (from 2200 h onwards) when swallows are said to sleep (based on the behavior of tucking their heads into the feathers of their ventral sides) (Medway, 1973). Parameters such as wing and tail length were measured following Nam et al. (2018), while forehead patch color length was measured following Borras et al. (2000), and the length of the white patch on the outermost tail feather was measured according to Taylor et al. (2011) (Figure 2). All individuals were kept safely in boxes for release in the morning. We also collected feathers from the tertiary section of their wings to extract deoxyribonucleic acid (DNA) from quill roots for individual sexing and subspecies determination based on the protocols of Griffiths et al. (1998) and Lijtmaer et al. (2012), respectively. A commercial extraction kit (G-spin<sup>TM</sup> Total DNA Extraction Mini Kit, South Korea) was used to aid the DNA extraction process. The primers used for sex determination were the P2 and P8 primers, which amplified two chromobox-helicase-DNA-binding genes (CHD-W and CHD98 Z) (Griffiths et al., 1998). For subspecies identification, we used the ProgND5F and ProgCBR primers 99 (Dor et al., 2010).



*Figure 2*. Morphological measurements taken from *H. rustica*: a) bill; b) wings; c) outermost tail feather, T6; d) second outermost tail feather, T5; e) innermost tail feather, T1; f) forehead patch; g) white tail patch

#### **Statistical Analysis**

We checked data distributions for normality using the Shapiro-Wilks test before performing the parametric statistical analyses. The one-sample *t*-test was used to compare the gender differences for each variable, except for two parameters—male wing length and female bill length—that did not fit a normal distribution. Thus, the Mann-Whitney U test was performed. Thereafter, the sexual dimorphism index (SDI) was calculated as Log<sub>10</sub> (mean male size) - Log<sub>10</sub> (mean female size) for each variable (Møller, 1994). The stepwise discriminant function analysis (DFA) chose the best parameters for separating the sexes. The discriminant score of each individual was calculated by a canonical discriminant analysis (Bavoux et al., 2006; Dmitrenok et al., 2007). Jackknife cross-validation methods were used to estimate the proportion of correctly classified individuals based on the quadratic DFA (Dechaume-Moncharmont et al., 2011). All computational analyses were conducted using the JMP Pro 14.0.0 software.

## RESULTS

The molecular sexing method successfully confirmed the subspecies of *H. rustica*. It differentiated male and female individuals by presenting a typical pattern of bands, with females showing two bands and males showing one band. A total of 46 individuals of *H. rustica* (19 females and 27 males) were captured in the study area. Males tend to be consistently larger than females for most measured parameters, except bill size, which did not display any significant differences among genders. The SDI value for tail streamer length (0.2246) was highest, followed by tail fork depth (0.1814) and outermost tail feather length (0.1105). However, the tail streamer length variable was excluded as an optimal predictor for sexing since it has a lower correct classification rate for males and females (Table 1). Outermost tail feather length (T6) and tail fork depth (T6-T1) were selected as the best predictors for determining Barn Swallow sex (Figure 3). When parameters were entered singly at a time into the stepwise DFA, high correct classification rates for the outermost tail feather length (female: 78.95%, male: 96.30%) and tail fork depth (female: 84.21%, male: 92.60%) were observed.

However, when combining both parameters simultaneously, the DFA results showed higher correct classification rates of 89.47% for females and 96.3% for males. The computed discriminant function shows the result: D = 0.1182\*outermost tail, T6-137 0.0007\*tail fork depth, T6-T1. Low Wilks' lambda values (outermost tail, T6=0.4491; tail fork depth, 138 T6-T1= 0.4888) indicate that the Wilks' lambda has a great discriminatory ability to separate cases into groups (Table 2). Seven morphological characters can aid in sexing *H. rustica*. However, one of these characters (bill length) was excluded due to an insignificant difference between genders, leaving only six characters. Since female swallows were generally found to have slightly shorter tails (T6, T6-T1, T6- 143 T5) when compared to males, these characters were selected as the best predictors for sex determination.

			Female			Male		t (U <sup>§</sup> )	Sexual dimorphism	
	I	z	Mean ± standard deviation (SD)	Range	z	Mean ± standard deviation (SD)	Range		index (SDI)	
Wing		19	$106.72 \pm 4.2167$	96.48 - 116	27	$109.87 \pm 4.2837$	95.97 - 116.82	$131.00^{***}$	0.01262	
Bill		19	$7.75 \pm 0.6683$	6.64 - 8.94	27	$7.55 \pm 0.6644$	6.13 - 9.00	224.00 <sup>§NS</sup>	-0.01111	
Outermo (T6)	st tail	19	$64.55 \pm 0.2821$	43.32 - 77.67	27	$83.25 \pm 8.6506$	65.83 - 100.03	7.3468***	0.110509	
Tail stre	umer	19	$19.22 \pm 5.7149$	7.11 - 28.74	27	$32.24 \pm 7.7885$	16.41 - 49.34	6.1992***	0.224644	
Tail fork (T6-T1)	depth	19	$30.97 \pm 7.0671$	12.75 - 40.62	27	$47.02 \pm 8.4333$	29.21 - 65.42	6.7892***	0.181403	
Forehead	l patch	19	$5.01 \pm 1.4952$	2.46 - 7.60	27	$6.10\pm0.7230$	4.93 - 7.56	3.3089**	0.085958	
Tail whit	e patch	19	$15.13 \pm 3.5927$	5.67 - 22.36	27	$19.20 \pm 4.9693$	9 - 27.85	3.0361**	0.102954	
<i>Note.</i> **:	$v \leq 0.01, **$	*: <i>p</i> ≤ condu	146 0.001. NS: not si cted using the Mann-	ignificant; §: Male Whitney U statisti	wing le	ingth in males and fema	le bill length in fema	lles did not fit a n	ormal distribution,	
Table 2 <i>Two impo</i> .	rtant variabı	les fro	m seven morphologic	al measurements c	of the H.	rustica based on stepw	ise discriminant func	ction analysis		
Step	Entered	l varis	ıble	F	p>F	Wilks' lambe	la Canonica	il correlation (C	c) p>CC	
1	Outermo	ost tail	(T6)	53.97	<0.000	0.4491		0.7422	<0.0001	
7	Tail fork	< deptl	n (T6-T1)	46.02	<0.000	0.4888		0.7150	<0.0001	

Table 1 The difference in morphological measurements of H. rustica by gender

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*Figure 3*. The relationship between tail fork depth length (T6-T1) and outermost tail feather length (T6) for adult male and female Barn Swallows

## DISCUSSION

We found that the rate of correct sex classification in male H. r. gutturalis was higher than females, contrary to the trend observed for European Barn Swallow H. r. rustica breeding populations (Hermosell et al., 2007). We also found that the tail fork depth (T6-T1) parameter is best paired to the outermost tail feather (T6) for better sex predictors; in contrast, Nam et al. (2018) reported that tail fork depth and tail streamer are better predictors due to their higher F-values, SDI scores, and effect sizes. According to Neuman et al. (2007), males possess more variation in streamer length when compared to females, although some findings reported considerable overlap in this parameter between the sexes (Turner, 2006). Furthermore, Taylor et al. (2011) suggested that swallows cannot be correctly sexed based on tail streamer length alone. Additionally, several other studies combined T6 measurements with other parameters, such as the length of the innermost tail feather and white tail patch length (Hermosell et al., 2007; Taylor et al., 2011). Tail morphology evolution might be revealed through the comparative trials of *H. r. gutturalis*, which possess shorter tail feathers than H. r. rustica (Romano et al., 2017; Scordato & Safran, 2014). Several reports of *H. rustica* populations have identified the white tail patch as a sexual accessory that functions as a handicap rather than a signal enhancement (Kose et al., 1999; Kose & Møller, 1999); however, with shorter tail feathers than other subspecies, no significant reproductive benefits were identified in correlational datasets for *H. rustica* (Hasegawa et al., 2010; Hasegawa & Arai, 2013). While the present study revealed that white tail patch length can be used as a criterion for sex determination, it has a lower power of discrimination than T6-T1 and T6.

Moreover, the white tail patch measurement may not be necessary for fieldwork since the measurement of fork depth (T6-T1) alone also predicted sex at a similar level of accuracy. However, Taylor et al. (2011) suggested that white patches should be measured wherever possible since the patch is an easily measurable and meaningful supporting parameter for sex determination and can be measured even in severe cases involving abraded or damaged tails. On the other hand, Taylor et al. (2011) suggested that wing length was the most useful variable for discriminating sex and subspecies. However, according to Nam et al. (2018), wing length shows considerable overlap between the sexes. This indicates that the East Asian population's wing length is not recommended for sex determination in the field. According to Hasegawa and Arai (2013), in Japanese Barn Swallows populations, the length of dark-red plumage patches on the throat is also known as a sexually selected trait. Unfortunately, the present study did not take this into account due to its low discriminant power and some failures in measurement repeatability.

# CONCLUSION

We revealed that external morphometrics seems to be a successful tool for sex determination in *H. rustica*. Moreover, using external morphometries to classify birds is less costly and invasive than gathering laparotomy and blood samples (Montalti et al., 2012). The morphometric approach may also provide valuable knowledge for taxonomic, biological, behavioral, physiological, and evolutionary studies (Mansor & Ramli, 2017). Additionally, DFA proved to be effective for sexing the Barn Swallow and other bird species, such as the Black-crowned night heron (*Nyxticorax nyxticorax*; Piro et al., 2018) and Chilean flamingo (*Phoenicopterus chilensis*; Montalti et al., 2012). Furthermore, using DFA also proved sufficient for live birds (Montalti et al., 2012). Therefore, the present work could be useful for sexing other bird species. To fully understand the vital role of DFA in sexual dimorphism, sex determination, and its implications on sexually selected traits, future studies on the color and size of plumage—including that of the throat and forehead—are required.

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