

## New record of *Rhinolophus chiewkweeae* (Chiroptera: Rhinolophidae) from the east coast of Peninsular Malaysia with new information on their echolocation calls, genetics and their taxonomy

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**Abstract.** We present a range extension for a little known species, *Rhinolophus chiewkweeae*, in the east coast of Peninsular Malaysia. The new locality record for this species in the east coast was at Sungai Buweh, Tasik Kenyir, Terengganu. This represents the seventh known locality in Malaysia for this distinctive species. The echolocation call of the species was recorded for the first time from four individuals with constant frequency ranging from 53.6 to 54.7 kHz (n = 4). This study provides the first genetic information (cytochrome oxidase I) of the species in order to discriminate between members of the *pearsoni* species group (*R. pearsoni*, *R. chiewkweeae* and *R. yunanensis*), which are morphologically similar. We provide evidence that *R. pearsoni* is a sister taxon of *R. chiewkweeae* with a genetic divergence of 12% whereas no information was available from *R. yunanensis* for comparison. The information on the species taxonomy based on morphological and ecological data are provided. This discovery indicates that this species are more widely spread than previously thought. Additionally, it provides information that can be used to predict the potential distribution of this little known species elsewhere, especially in Peninsular Malaysia, which can consequently assist in its conservation.

**Key words.** conservation, distribution, echolocation, systematics, Terengganu,

### INTRODUCTION

The genus *Rhinolophus* or also known as horseshoe bats are distributed throughout the tropics, subtropics and temperate zones of the Old World (Corbet & Hill, 1992; Findley, 1993; Francis, 2001). Their common name, ‘horseshoe’, was coined after their complex noseleaf that represents a horseshoe. Their noseleaf, the raised segment (called sella), palatal, fifth and fourth digit metacarpal length are the most important morphological features to discriminate between members of the genus *Rhinolophus* (Payne et al., 1985; Corbet & Hill, 1992; Francis, 2008; Szalai et al., 2008). There are about 96 *Rhinolophus* species in the world, of which 23 are known to be within Malaysian distribution (Payne et al., 1985, Francis, 2008, Soisook et al., 2015, Volleth et al., 2015). Out of this, possibly two species, *Rhinolophus chiewkweeae* and *R. convexus*, are known to be endemic

to Malaysia. However, information on the taxonomic and phylogenetic relationship of *Rhinolophus* is still lacking in Malaysia (Szalai et al., 2011).

*Rhinolophus chiewkweeae* (Fig. 1) was only known from the west coast of Peninsular Malaysia (Yoshiyuki & Lim, 2005). The holotype of *R. chiewkweeae* was collected at Gunung Ledang, Johor, Peninsular Malaysia, on 13 September 1998. The species was described as a member of the *pearsoni* species group which consists of *R. pearsoni* and *R. yunanensis* (Yoshiyuki & Lim, 2005). It showed adequate coefficients of differences in its external and osteological characters that mark it as a separate species from either *R. pearsoni* or *R. yunanensis*. *Rhinolophus chiewkweeae* have intermediate dimensional characters existing between *R. pearsoni* and *R. yunanensis*, except for its short ears and tail (Yoshiyuki & Lim, 2005). Furthermore, the dorsal fur of *R. chiewkweeae* is orange brown whereas it is dark brown for both *R. pearsoni* and *R. yunanensis*.

*Rhinolophus chiewkweeae* was only known from the dipterocarp forests of the west coast of Peninsular Malaysia and an island nearby. The six known localities for this species include Gunung Ledang and Labis Forest Reserve in Johor; Asahan Forest Reserve, Melaka; Lubok Semilan, Langkawi Island and Ulu Muda Forest Reserve in Kedah; and Wang Kelian State Park, Perlis (Fig. 2; Yoshiyuki & Lim, 2005; Jayaraj et al., 2013).

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Fig. 1. Latero-ventral view of *Rhinolophus chiewkweeae*, from Sungai Buweh, Lawit

Inadequate sampling in the east coast of Peninsular Malaysia has hampered most of the understanding of the bat species diversity of that region. East coast is separated from the west coast by the Titiwangsa range in the middle of Peninsular Malaysia that runs from the southern Thailand towards southeast that ends near Jelebu, Negeri Sembilan (Hassan et al., 2012). Extensive field surveys have therefore been carried out to start a more precise documentation of the diversity of the region. These surveys resulted in the discovery of the Peninsular Malaysia's endemic *Rhinolophus chiewkweeae* in Terengganu that represents the seventh known locality in Peninsular Malaysia and the first in the east coast for this particular species.

**MATERIAL AND METHODS**

**Field work.** This study was carried out at Sungai Buweh, Terengganu (N 05°38.362', E 102°35.276'). The forest is a dipterocarp forest with a river stream and a waterfall. Eight mist nets and three four-bank harp traps were used in this study. All individuals of *R. chiewkweeae* were caught in the mist nets. Trapping was done from 1800 hours until 2200 hours. The species identification and sex determination were

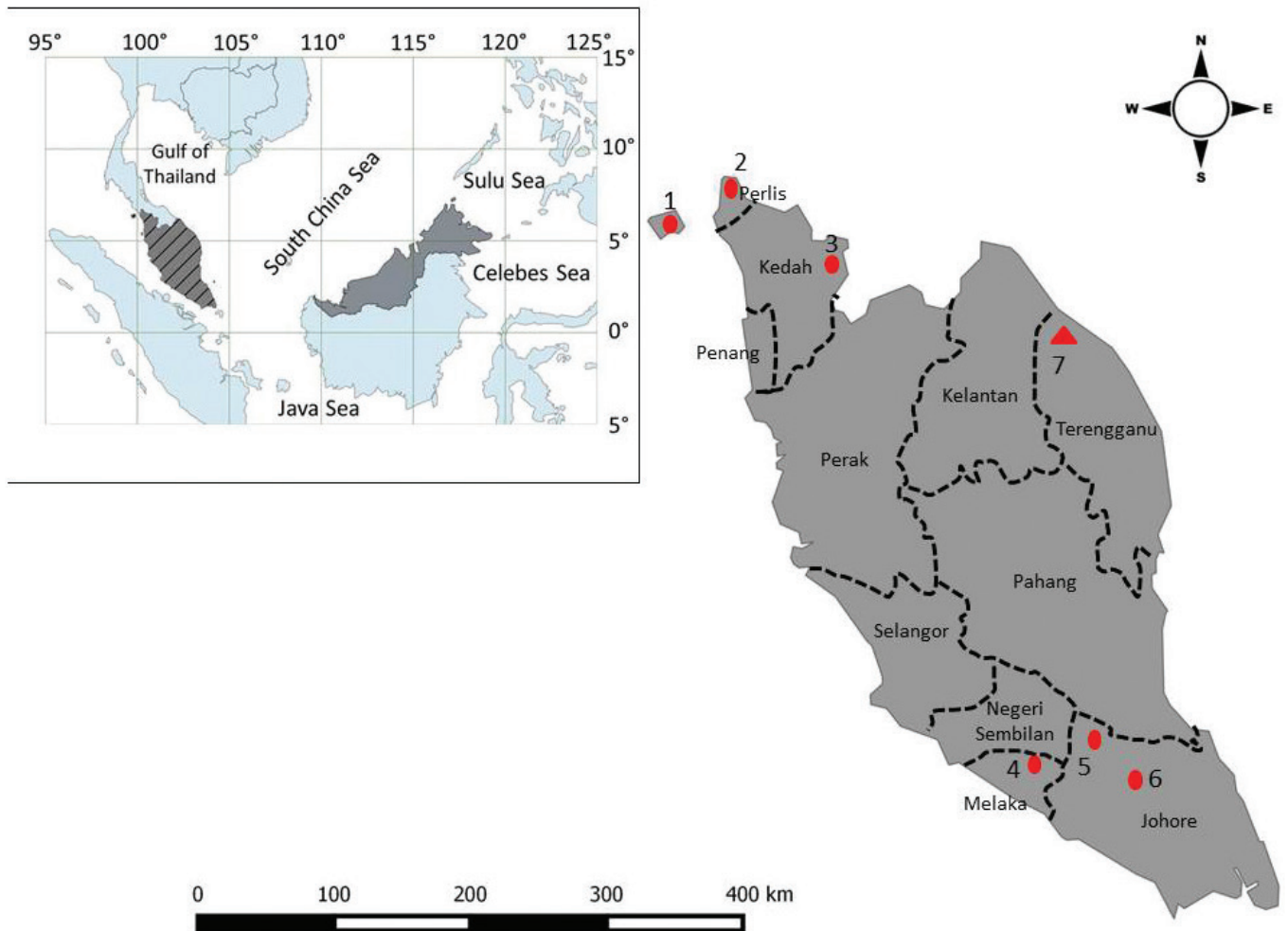


Fig. 2. Inset map highlighted in grey shows the Malaysian country boundary. Area coloured in grey with vertical diagonal lines highlight Peninsular Malaysia whereas area coloured in grey without the lines highlight Malaysian Borneo. Larger map of Peninsular Malaysia on the right shows the collection sites of *Rhinolophus chiewkweeae*. 1=Lubok Semilan, Kedah; 2=Wang Kelian State Park, Perlis; 3=Weng Subcatchment Area, Kedah; 4=Asahan Forest Reserve, Melaka; 5=Gunung Ledang, Johor; 6=Labis Forest Reserve, Johor; 7=Sungai Buweh, Terengganu (This study).

carried out directly in the field. All animals were handled following the guidelines for animal care and use established by the American Society of Mammalogists (Sikes et al., 2011). All works (capture, euthanasia, and dissection) were carried out with authorisation from the respective wildlife authorities in Malaysia that manage all the protected areas listed above: Department of Wildlife and National Parks (JPHL&TN(IP): 100-34/1.24(12)).

**Sound records and analysis.** The echolocation calls were recorded using bat detector Echo Meter EM3+ and recording was taken directly by holding the bats in the hand approximately 30 cm away from the bat detector. Calls were analysed using the Kaleidoscope software (Brand: Wildlife Acoustics). For each call, six parameters were measured (Douangboubpha et al., 2014). These were: PD: pulse duration (ms) – measured from the beginning to the end of call pulse; PI: pulse interval (ms) – measured from the end of one pulse to the beginning of the next pulse; MinF: minimum frequency (kHz); MaxF: maximum frequency (kHz); MaxEF: maximum energy frequency (kHz); and MidF: middle frequency. Measurements of all echolocation parameters were computed automatically in the Kaleidoscope software by selecting five calls with good wavelength quality (without noises and does not clip out from the analysed frame) from each individuals.

**Measurements.** Measurements were recorded using Mitutoyo digital callipers. External measurements include: forearm length (FA), ear length (E), tibia length (TB), hind foot length (HF) and tail to ventral length (TV). Skulls of individuals were extracted according to Nagorsen & Peterson (1980). Cranial and dental characters that were measured include condylo-canine length (CCL), interorbital constriction (IOC), zygomatic width (ZW), width of braincase (WOB), mastoid width (MW), width from left upper canine to right upper canine (WUC1), width from left upper last molar to right upper last molar (WUM<sup>3</sup>), length from upper canine to upper last molar (CM<sup>3</sup>), mandible (MDB), and lower canine to lower last molar length (CM<sub>3</sub>) (Yoshiyuki & Lim, 2005; Velazco & Petterson, 2014).

**Genetic analyses.** Genetic sampling was done using muscle and liver samples that were stored in absolute ethanol. DNA extraction, gene amplification, and nucleotide sequencing were performed on three samples (LHT-15-017, LHT-15-043, LHT-15-044) following protocol described in Francis et al. (2010), Khan et al. (2010) and Ivanova et al. (2012) for mammalian DNA barcode analyses. Cytochrome oxidase I gene sequence analysis was done using Neighbor- Joining tree by implementing Kimura-2-parameter model (K2P) with the inclusion of other *Rhinolophus* species sequences that are available in the GenBank through BLAST program that matched above 85% identity for comparison. None of the *R. yunanesis* GenBank sequences were available for our sequenced region and therefore were not included in our analysis (Appendix 1: Sequences used in analyses). To test for the phylogenetic relationship we ran 1000 bootstraps and included *Hipposideros bicolor*\_ JF443885 and *Coelops frithii*\_HQ918409 as the outgroup. Finally, we calculated

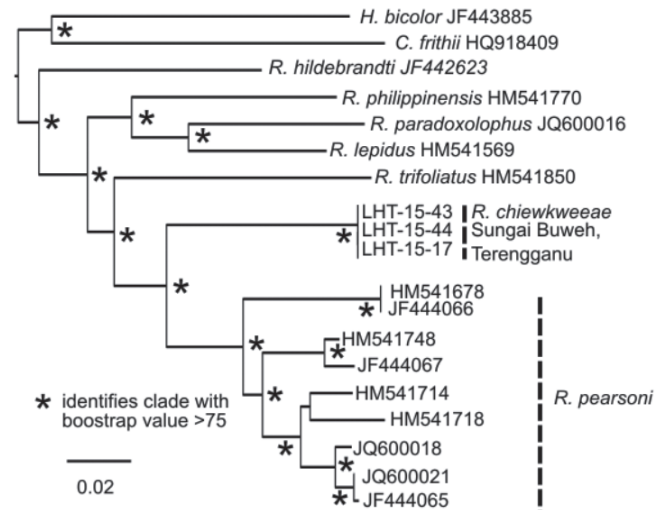


Fig. 3. Phylogenetic tree of selected *Rhinolophus*, *Hipposideros* and *Coelops* (Kimura-2-parameter model)

genetic distance using K2P model that is commonly used for mammalian species to allow comparison with other similar studies.

## SYSTEMATIC DESCRIPTION

### *Rhinolophus chiewkweeae*, Yoshiyuki & Lim, 2005 Chiewkwee's horseshoe bats

*Rhinolophus chiewkweeae*, Yoshiyuki & Lim, 2005; Gunung Ledang, Tangkak, Muar, Johor, Peninsular Malaysia (2°84'N, 102°57'E, 1276 m a.s.l.).

**New material.** LHT-15-002, male, LHT-15-017, male, LHT-15-043, female, and LHT-15-044, male from Sungai Buweh, Lawit, Tasik Kenyir, Hulu Terengganu, Terengganu, Malaysia (N 05°38.362', E 102°35.276', 155 m a.s.l.).

## RESULTS

**Genetic data.** Only single haplotype was observed from the four individuals analysed here (Figure 3). Neighbour-joining analyses showed that *R. chiewkweeae* is sister to *R. pearsoni* (Bootstrap >95). The lowest genetic divergence for *R. chiewkweeae* to other *Rhinolophus* species studied here was observed between *R. chiewkweeae* and *R. pearsoni* (K2P=12%) and the highest was between *R. chiewkweeae* and *R. hildebrandti* (K2P=16.4%) (Table 1).

**External characters.** The specimens from Sungai Buweh, Terengganu had forearm length ranging from 51.9–55.9 mm for male and 55.3 mm for female individuals (Table 2). All four specimens had unique morphological characteristics as recorded in *R. chiewkweeae* (Yoshiyuki & Lim, 2005). The horseshoe was large with wide anterior and emarginated deeply at the central portion with sparse hair spikes on the surface. It had a broad triangular lancet with three pairs of deep cells of lancet. The connecting process originated from the tip of the hairless sella and formed a low, rounded profile. The sella was broadly rounded at the apex with wide base and constricted in the middle (described as small basal

Table 1. Pairwise distance test of selected *Rhinolophus*, *Hipposideros* and *Coelops* (Kimura-2-parameter model)

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]
[1] <i>R. chiewkweeae</i> (N=3)									
[2] <i>R. pearsoni</i> (N=9)	12.0								
[3] <i>R. trifoliatus</i>	16.0	15.8							
[4] <i>R. hildebranti</i>	16.4	16.9	17.4						
[5] <i>R. paradoxolophus</i>	15.9	17.2	19.2	17.9					
[6] <i>R. lepidus</i>	16.3	16.2	16.4	14.8	9.8				
[7] <i>R. philippinensis</i>	15.4	16.6	16.4	17.8	13.4	12.8			
[8] <i>Hipposideros bicolor</i>	20.8	20.5	23.0	19.1	21.8	20.7	19.4		
[9] <i>Coelops frithii</i>	22.2	23.2	19.3	17.9	20.5	19.6	20.2	19.7	

Table 2. External and cranio-dental measurements (mm) of four specimens of *Rhinolophus chiewkweeae* from Sungai Buweh, Lawit, Tasik Kenyir, Hulu Terengganu, Terengganu, together with measurements of specimens from Yoshiyuki & Lim (2005).

Character	This study (n = 4)	Yoshiyuki & Lim, 2005 (n = 7)
Forearm	54.2 ± 1.727 (51.9 – 55.7)	56.1 ± 0.81
Head and body	60.9 ± 6.366 (51.5 – 65.0)	64.0 ± 0.93
Tail	17.0 ± 1.696 (14.7 – 18.4)	18.9 ± 0.99
Hind foot cum unguis	13.7 ± 1.153 (12.0 – 14.4)	14.0 ± 0.926
Tibia	27.6 ± 0.783 (26.7 – 28.3)	26.4 ± 0.903
Ear length	23.3 ± 2.196 (20.1 – 25.0)	25.0 ± 0.76
Condylar-canine length	22.5 ± 0.688 (21.9 – 23.4)	22.3 ± 0.404
Interorbital constriction	2.8 ± 0.112 (2.8 – 3.0)	2.8 ± 0.186
Zygomatic width	12.8 ± 0.609 (12.0 – 13.2)	13.2 ± 0.106
Width of braincase	10.2 ± 0.300 (9.9 – 10.5)	10.3 ± 0.186
Mastoid width	11.4 ± 0.429 (10.9 – 11.9)	11.5 ± 0.202
Width from left upper canine to right upper canine	6.8 ± 0.121 (6.7 – 6.9)	6.7 ± 0.213
Width from left upper last molar to right upper last molar	9.4 ± 0.308 (9.1 – 9.8)	9.5 ± 0.215
Length from upper canine to upper last molar	10.3 ± 0.481 (10.0 – 11.1)	10.1 ± 0.122
Mandible	18.2 ± 0.588 (17.8 – 19.1)	17.2 ± 0.307
Length from lower canine to lower last molar	11.0 ± 0.563 (10.4 – 11.7)	10.8 ± 0.203



Fig. 4. Lateral, dorsal and ventral views of *Rhinolophus chiewkweeae* skull from Sungai Buweh, Lawit. Scale bar = 5mm

lappets in Yoshiyuki & Lim, 2005). The basal one-third of the sella was wide, and after a slight constriction, the following two-third of the sella was parallel-sided. The lower lips had single groove. The fur was dense, long and woolly in texture. The dorsal colour was orange brown, whereas the ventral surface was lighter and the membrane brownish.

**Cranial and dental characters.** The specimens from this study had a condylo-canine length ranging from 21.91–23.41 mm for male and 22.0 mm for female (Table 2). The skulls of these specimens had large braincase. Comparison between the zygomatic and mastoid width showed that zygomatic width were always greater than mastoid width. This character is consistent with the description in Yoshiyuki & Lim (2005). The anterior upper pre-molar was found to be separated from the canine and also the posterior premolar. It had large crown areas that were pentagonal in shape. The lower first, second and third premolar were similar in characters, except that the crown area for the first lower premolar was slightly smaller and its height was shorter than the other two lower premolars. The upper first, second and third molar were almost the same in height, crown area and characters. The size of the third upper molar crown area was only two-third of the crown area size of the second and first molars. The lower molars were similar to each other (Fig. 4).

**Echolocation calls.** The echolocation pulses consisted of four harmonics ( $H_1$ ,  $H_2$ ,  $H_3$  and  $H_4$ ) where  $H_1$  represent the fundamental pulse. Analyses of the four pulses clearly indicated that the second harmonic ( $H_2$ ) contained the highest energy and later was further analysed and the minimum frequency was found to range between 38.2 to 48.7 kHz. The start frequency ranged between 59.6 to 60.7 kHz, the maximum energy frequency ranged between 53.6 to 54.7 kHz, the middle frequency ranged between 53.6 to 54.7 kHz, the pulse duration ranged between 28.4 to 63.26 ms and pulse interval ranged between 32 to 189 ms (Table 3; Fig. 5).

**Ecological notes.** All four individuals from this study were caught at the sub-canopy level (7–15 m from forest floor) of secondary dipterocarp forest with the elevation of 155 m a.s.l. and coexisting species at the same habitat are *Rhinolophus lepidus*, *R. luctus*, *R. affinis*, *Hipposideros bicolor*, *H. larvatus*, *H. cervinus*, *H. dyacorum*, *H. diadema*, *Kerivoula pellucida*, *Megaerops ecaudatus*, *Penthetor lucasi*, *Cynopterus brachyotis* and *Macroglossus minimus*.

This species was caught in area surrounded by big rocks along a waterfall at Sungai Buweh that runs into Kenyir Lake. This waterfall is accessible to public via the main road (Jalan Pengkalan Utama). There were almost no canopy around this waterfall area. Based on the weather

Table 3. Call parameters of *Rhinolophus chiewkweeae*. The included parameters are minimum frequency (MinF), maximum frequency (MaxF), maximum energy frequency (MaxEF), middle frequency (MidF), pulse duration (PD) and pulse interval (PI).

Parameters	LHT-15-002	LHT-15-017	LHT-15-043	LHT-15-044
MinF (kHz)	48	47.6	38.2	48.7
MaxF (kHz)	59.6	59.6	60.3	60.7
MaxEF (kHz)	53.6	53.6	54.3	54.7
MidF (kHz)	53.6	53.7	54.4	54.7
PD (ms)	28.4	63.3	39.6	42.9
PI (ms)	32	189	83.6	81.3

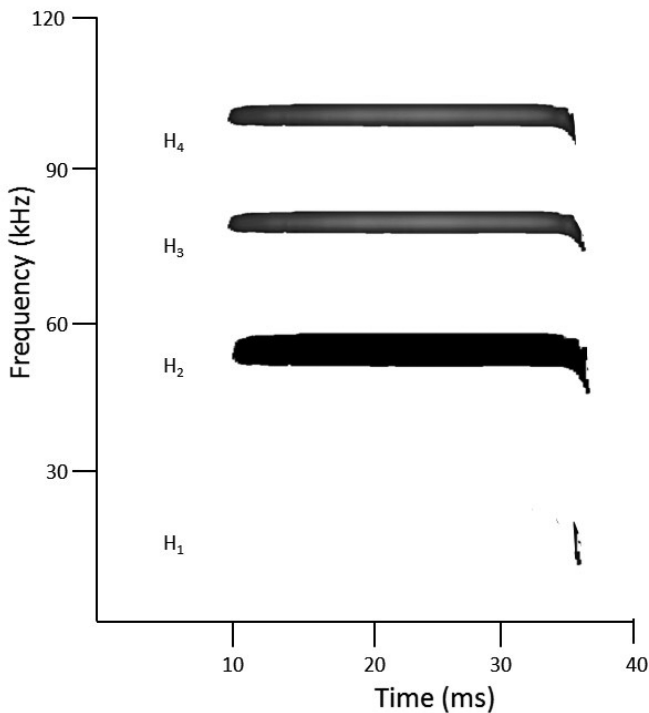


Fig. 5. The echolocation call of *Rhinolophus chiewkweeae*. Legend: H<sub>1</sub> (first harmonic), H<sub>2</sub> (second harmonic), H<sub>3</sub> (third harmonic) and H<sub>4</sub> (fourth harmonic).

and climatic database, this area has an average temperature of 28°C, with a relative humidity of 84%, and receives 266 mm precipitation of rainfall annually (World Weather & Climate Information, 2015).

## DISCUSSION

The discovery of *R. chiewkweeae* in a new locality about 300 km away from the type locality across Titiwangsa range indicates that this species is more widely spread than previously thought. However, population density for this species can be considered low as the capture rate was only 8.7% throughout the five sampling nights at Sungai Buweh, Terengganu (4 out of 46 individuals). Our finding is similar to previous record of this species in the Northern Peninsular Malaysia (Perlis State Park) where only one individual was recorded throughout four sampling sessions that comprised of 69 mist-net and harp trap nights (Jayaraj et al., 2013).

The results represent the first nucleotide sequence for this species. Morphologically, *R. chiewkweeae* is described as the intermediate form (overlap) between the larger-sized *R. yunanensis* and smaller-sized *R. pearsoni*. This interpretation corroborates with the genetic findings of *R. chiewkweeae* examined here, which shows that it appears to be a sister clade to *R. pearsoni* that is known primarily from the Indian subregion and Indochinese region. However, this relationship needs to be treated with caution because the genetic data of the nucleotide region studied here for *R. yunanensis* was not available for comparison. Genetic divergence (12%) observed between the two sister clades (*R. chiewkweeae* vs. *R. pearsoni*) further supports the current recognition of these two clades as separate species.

We also found some variance in the external and cranial measurements and morphological features based on the four individuals recorded in this study, compared to those by Yoshiyuki & Lim (2005). Two of the adult male individuals recorded here (FA: 51.9–55.9 mm) were smaller in size compared to the previous measurements (FA: 55.29–56.91 mm) reported in Yoshiyuki & Lim (2005) (Table 2). Similarly, condylo-basal length of a male specimen (LHT15-002) was slightly larger than previously reported (Table 2; Yoshiyuki & Lim, 2005). Both findings suggest that this species may have larger morphological variation than previously recorded. However we were not able to determine the existence of sexual dimorphism in this species due to small sample size. We also refined previous characters provided in Yoshiyuki & Lim (2005) which may be unclear. This includes the existence of small lappet that actually refers to the widened base of sella that was constricted in the middle. We also clarified the original description that mentioned only three deep cells of lancet which supposedly refers to three pairs of deep cells lancet.

We also found that the maximum energy frequency (MaxEF) for *R. chiewkweeae* (53.6–54.7 kHz) is very unique as it does not overlap with any other species from the genus *Rhinolophus* known from Peninsular Malaysia. However, when comparisons were made between members of *pearsoni* species group (*R. chiewkweeae*, *R. pearsoni* and *R. yunanensis*) known from outside Peninsular Malaysia, the echolocation call of *R. chiewkweeae* overlaps with *R. pearsoni* from Vietnam and Thailand (53.7–60.04 kHz) but not with *R. yunanensis* from Thailand (49–50.95 kHz) (Francis, 2008; Hughes et al., 2010; Thong, 2014). This

finding highlights the possibilities of the existence of similar call frequency range between two different genetic lineages but not in sympatry. This further strengthens the hypothesis that non-overlapping echolocation call frequency is necessary for mate recognition and thus play an important role in bat speciation (Kingston et al., 2001).

It is possible that *R. chiewkweeae* is more commonly distributed in areas with similar abiotic conditions described herein. However this information should be interpreted with caution as our data and previous study on this species had only described them from Peninsular Malaysia, whereas the counterpart for this species, *R. pearsoni* and *R. yunanensis* are known to be dispersed within the Indochinese and Indian sub regions with a very different abiotic conditions. *Rhinolophus chiewkweeae* is a cryptic species which may be previously misidentified as either *R. pearsoni* or *R. yunanensis* thus the distribution maybe much more widely dispersed than previously thought, towards mainland Asia. Such distribution of these species lineages in this region explains the importance of geological and climatic changes over time that has shaped the faunal diversity in this region.

This species was first described in 2005 and very little is known. Its conservation status remains unknown as it is still unlisted in IUCN Red List (IUCN, 2016). To date, the species is only known from seven localities in Peninsular Malaysia (Figure 2). This study provided new information on the member of *pearsoni* group within the genus *Rhinolophus* that is known to be taxonomically challenging due to their cryptic morphology and lack of specimens for comparisons. We hope future studies on this group should incorporate multiple datasets as presented here including echolocation, genetics, and morphology to clarify their taxonomic complexity.

#### ACKNOWLEDGEMENTS

We are extremely grateful to the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak for helping with logistics and transportation. Many thanks to the team from the Kenyir Research Institute, Universiti Malaysia Terengganu especially to Director of Kenyir Research Institute, Faizah Shahrom, for their help and support. We also would like to extend our gratitude to all colleagues from FAAK's Lab, for their continuous support during sampling and in completion of this manuscript. Thanks to Ernisa Marzuki from Center for Language Studies UNIMAS for editing the manuscript. We also would like to thank the Ministry of Higher Education for providing the research grants: RACE/g(2)/1107/2013 (15), GGP/68007/2014/127 and NRGS-UMT 53131 and Department of Wildlife and National Parks for granting us the research permit.

#### LITERATURE CITED

Corbet GB & Hill JE (1992) The Mammals of The Indomalayan Region: A Systematic Review. Oxford University Press, Oxford, 496 pp.  
 Douangboubpha B, Bumrungsri S, Soisook P, Karapan S, & Bates PJJ (2014) The discovery of *Kerivoula krauensis* (Chiroptera: Vespertilionidae) in southern peninsular Thailand provides new

information on the distribution and conservation status of this data deficient species. Songklanakarin Journal of Science and Technology, 36(5): 577–582.  
 Findley JS (1993) Bats: A Community Perspective. Cambridge University Press, Cambridge, 167 pp.  
 Francis CM, Borisenko AV, Ivanova NV, Eger JL, Lim BK, Servent AG, Kruskop SV, Mackie L & Hebert PDN (2010) The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. PLoS ONE, 5(9): e12575.  
 Francis CM (2008) A Field Guide To The Mammals of South-East Asia: Thailand, Peninsular Malaysia, Singapore, Myanmar, Laos, Vietnam and Cambodia. New Holland (Publishers) Ltd, London, 392 pp.  
 Francis CM (2001) A Photographic Guide To Mammals of South-East Asia Including Thailand, Malaysia, Singapore, Myanmar, Laos, Cambodia, Vietnam, Java, Sumatra, Bali and Borneo. New Holland (Publishers) Ltd, London, 128 pp.  
 Hassan NH, Khan FAA, Senawi J, Ketol B, Sait I & Abdullah MT (2012) A report on bats survey at Air Panas-Gua Musang, Kelantan, Malaysia. Journal of Tropical Biology and Conservation, 9(2): 156–162.  
 Hughes AC, Satasook C, Bates PJJ, Soisook P, Sritongchuay T, Jones G & Bumrungsri S (2010) Echolocation call analysis and presence-only modelling as conservation monitoring tools for rhinolophoid bats in Thailand. Acta Chiropterologica, 12(2): 311–327.  
 Ivanova NV, Clare EL & Borisenko AV (2012) DNA Barcoding in Mammals. In: Kress WJ & Erickson DL (eds.) DNA Barcodes: Methods and Protocols, Methods in Molecular Biology. Humana Press New York, New York. Pp 153–182.  
 Jayaraj VK, Daud SHM, Azhar MI, Sah SAM, Mokhtar SI & Abdullah MT (2013) Diversity and conservation status of mammals in Wang Kelian State Park, Perlis, Malaysia. Check List, 9(6): 1439–1448.  
 Khan FAA, Solari S, Swier VJ, Larsen PA, Abdullah MT & Baker RJ (2010) Systematics of Malaysian woolly bats (Vespertilionidae: Kerivoula) inferred from mitochondrial, nuclear, karyotypic and morphological data. Journal of Mammalogy, 91(5): 1058–1072.  
 Kingston T, Lara MC, Jones G, Akbar Z, Kunz TH & Schneider CJ (2001) Acoustic divergence in two cryptic *Hipposideros* species: a role for social selection? Proceedings of the Royal Society of London, 268: 1381–1386.  
 Nagorsen DW & Peterson RL (1980) Mammal Collector's Manual, Life Sciences Miscellaneous Publication, Royal Ontario Museum, Toronto, 79 pp.  
 Payne J, Francis CM & Phillipps K (1985) A Field Guide To The Mammals of Borneo. The Sabah Society and World Wildlife Fund Malaysia, Kota Kinabalu, 332 pp.  
 Sazali SN, Besar K & Abdullah MT (2011) Phylogenetic analysis of the Malaysian *Rhinolophus* and *Hipposideros* inferred from partial mitochondrial DNA Cytochrome *b* gene sequences. Pertanika Journal of Tropical Agricultural Science, 32(2), 281–294.  
 Sazali SN, Hasan NH, Laman CJ & Abdullah MT (2008) A morphometric analysis of Malaysian *Rhinolophus* species. Malaysian Journal of Mathematical Sciences, 2(1): 83–95.  
 Sikes RS, Gannon WL & the Animal Care and Use Committee of the American Society of Mammalogist (2011) Guidelines of the American Society of Mammalogist for the use of wild mammals research. Journal of Mammalogy, 92: 235–253.  
 Soisook P, Struebig MJ, Noerfahmy S, Bernard H, Maryanto I, Chen S-F, Rossiter SJ, Kuo H-C, Deshpande K, Bates PJJ, Sykes D & Miguez RP (2015) Description of a new species of the *Rhinolophus trifoliatus*-group (Chiroptera: Rhinolophidae) from Southeast Asia. Acta Chiropterologica, 17: 21–36.  
 IUCN (2016) The IUCN Red List of Threatened Species. Version 2015-4. <http://www.iucnredlist.org> (Accessed on 23 April 2016).

- Thong VD (2014) Acoustic identification and taxonomic remarks of Hipposiderids and Rhinolophids (Chiroptera: Hipposideridae, Rhinolophidae) in Tam Dao National Park, Northeastern Vietnam. *Tap Chi Sinh Hoc Journal of Biology*, 36(4): 487–493.
- Velazco PM & Patterson BD (2014) Two new species of yellow-shouldered bats, genus *Sturnira* Gray, 1842 (Chiroptera, Phyllostomidae) from Costa Rica, Panama and western Ecuador. *ZooKeys*, 402: 43–66.
- Volleth M, Loidl J, Mayer F, Yong HS, Muller S & Heller KL (2015) Surprising genetic diversity in *Rhinolophus luctus* (Chiroptera: Rhinolophidae) from Peninsular Malaysia: description of a new species based on genetic and morphological characters. *Acta Chiropterologica*, 17(1): 1–20.
- World Weather & Climate Information (2015) <https://weather-and-climate.com/average-monthly-Rainfall-Temperature-Sunshine,Kuala-Trenganu,%20Malaysia> (Accessed on 1 July 2015).
- Yoshiyuki M & Lim BL (2005) A new horseshoe bat, *Rhinolophus chiewkweeae* (Chiroptera, Rhinolophidae), from Malaysia. *Bulletin of the National Science Museum Tokyo, Series A*, 31(1): 29–36.

## APPENDIX

Appendix 1. Sequenced used in analyses (NA = Not Available).

Species	GenBank ID	Field ID
<i>Rhinolophus chiewkweeae</i>	NA	LHT-15-17
<i>Rhinolophus chiewkweeae</i>	NA	LHT-15-43
<i>Rhinolophus chiewkweeae</i>	NA	LHT-15-44
<i>Rhinolophus pearsoni</i>	JQ600021	ROM118512
<i>Rhinolophus pearsoni</i>	HM541748	ROM112448
<i>Rhinolophus pearsoni</i>	JQ600018	ROM118500
<i>Rhinolophus pearsoni</i>	HM541714	ROM116121
<i>Rhinolophus pearsoni</i>	HM541718	ROM112484
<i>Rhinolophus pearsoni</i>	JF444067	ROM112334
<i>Rhinolophus pearsoni</i>	JF444066	ROM116415
<i>Rhinolophus pearsoni</i>	HM541678	ROM116435
<i>Rhinolophus pearsoni</i>	JF444065	ROM118499
<i>Rhinolophus trifoliatus</i>	HM541850	ROM113101
<i>Rhinolophus philippinensis</i>	HM541770	SMF83403
<i>Rhinolophus paradoxolophus</i>	JQ600016	ROM118487
<i>Rhinolophus lepidus</i>	HM541569	HZM31.36495
<i>Rhinolophus hildebrandti</i>	JF442623	CDC-IK-377
<i>Hipposideros bicolor</i>	JF443885	ROM113119
<i>Coelops frithii</i>	HQ918409	ZMMU-S-184638