Tropical Natural History 20(2): 182–190, August 2020

Molecular Phylogenetic Support to the Resurrection of *Pareas macularius* from the Synonymy of *Pareas margaritophorus* (Squamata: Pareidae)

SUNUTCHA SUNTRARACHUN[1*], LAWAN CHANHOME[1], SJON HAUSER[2], MONTRI SUMONTHA[3] AND KIRATI KANYA[4]

[1]*Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok 10330, THAILAND*
[2]*71 Wiang Phing Road, Mueang, Chiang Mai 50100, THAILAND*
[3]*Ranong Marine Fisheries Station, Ranong 85000, THAILAND*
[4]*Nakhon Ratchasima Zoo, Nakhon Ratchasima 30000, THAILAND*
[*] Corresponding author. Sunutcha Suntrarachun (sunutcha@yahoo.com)
Received: 6 March 2019; Accepted: 17 June 2020

ABSTRACT.– The taxonomy of the Asian snail-eating snakes in the genus *Pareas* Wagler, 1830 has been an ongoing controversy**.** Recently suggested taxonomic changes included the synonymization of *Pareas macularius* Theobaldi, 1868 with *Pareas margaritophorus* **(**Jan *in* Bocourt, 1866**),** and subsequent resurrection of *P. macularius* as a valid species on the basis of morphological data for more than 60 specimens from northern Thailand and the holotypes of both species**.** To confirm the validity of *P. macularius*, we analyzed mitochondrial DNA sequences data for 14 bicolored and spotted snail-eaters from Thailand (morphologically identified as either *P. macularius* or *P. margaritophorus*)**.** The results strongly supported the validity of *P. macularius* as a species distinct from *P. margaritophorus***.**

KEY WORDS: *Pareas margaritophorus*, *Pareas macularius*, DNA sequencing

INTRODUCTION

The mollusc-eating snakes of the family Pareidae (see Savage (2015) for the correct spelling of the family name) are small-sized nocturnal snakes, characterized by blunt snout, lack of mental groove that separates the paired chin shields in most other snakes, and specialized feeding-apparatus including more or less asymmetric dentition to extract snails from their asymmetrically coiled shells (Danaisawadi et al., 2015; Hoso, 2017). The pareid snakes are endemic to the tropical and subtropical areas of the Oriental Realm (Smith, 1943; Guo et al., 2011; Wallach et al., 2014).

Three genera are generally recognized, of which the genus *Pareas* Wagler, 1830 is the most speciose. It differs from the other pareid genera by the number of dorsal scale rows (15), the presence of pre- and suboculars (in most species), and the characteristic chin shields (Grossman and Tillack, 2003). At present, about 12 species are recognized for the genus (Wallach et al., 2014). There have been a number of revisions, of which Smith (1943) synonymized the bicolored-spotted *Amblycephalus moellendorffi* (Bötger, 1885) with *P. margaritophorus* (Jan in Bocourt, 1866) and the bicolored-spotted *A. tamdaoensis* Bourret, 1935 with *P. macularius* Theobald, 1868. These accounts were widely accepted in the following decades. In the early 2000s, many other *Pareas* species were synonymized in Chinese studies (e.g. Huang, 2004, see below).

By then, four species in the genus *Pareas*, including two bicolored-spotted *P.*

margaritophorus and *P. macularius*, were recognized from Thailand (Taylor, 1965; Cox et al., 2012). With the shared features in appearance, such as grey ground color and ornamentation of numerous black-and-white spots of one scale's size, the two species showed a strong resemblance. However, they could be easily discriminated by a good number of characters (Boulenger, 1896; Smith, 1943; Taylor, 1965; Cox et al., 2012). Nevertheless, *P. macularius* was synonymized with *P. margaritophorus* (Huang, 2004). The study was solely based on data from Chinese materials and interpretations of data in Smith's (1943) work. The types of materials, collected from southern Myanmar and Thailand, respectively, were not examined. In spite of these shortcomings, and reservations in a few subsequent works by experts (Vogel, 2010; Cox et al. 2012), the synonymization was widely accepted, leading to removal of *P. macularius* from various databases including the reputed Reptile Database (Uetz and Hozek, 2018), and to changes of Latin names for Museum materials, originally deposited as *P. macularius*, to *P. margaritophorus*. However, Huang's (2004) claim was challenged by Hauser (2017), who argued for the validity of *P. macularius* based on co-variation in morphological characters in more than 60 fresh road-killed specimens of bicolored-spotted snail-eaters from northern Thailand.

In this study, we adopt DNA sequence analysis to further verify the validity of *Pareas macularius* as a species distinct from *P. margaritophorus*. The method is known to be useful in delimiting the species boundary or boundaries within a morphologically confusing assemblage of individuals (You et al., 2015, Mulcahy et al., 2017, Zaher et al., 2019).

MATERIALS AND METHODS

Fourteen live and freshly road-killed specimens of bicolored-spotted snail-eaters were collected during the rainy seasons from northern (Chiang Mai, Nan, and Tak), northeastern (Nakhon Ratchasima) and western Thailand (Kanchanaburi). Each specimen was photographed at the collecting site and was examined for the presence or absence of keeled dorsal scale rows by carefully screening the dorsal surface with a magnifying glass. The specimens and their photographs were subsequently examined again for other characters, such as the total length, sex, form, and color of the nuchal collar; type I: entire or tripartite, orange, pink, yellow or white without speckles; type II: W- or butterfly - shaped with tiny brown speckles, the presence or absence of an intensive black blotch (IBB) on the 7th supralabial scale (Hauser, 2017), and the number of ventral shields following Dowling (1951).

Only freshly road-killed specimens were fixed in 10% formalin for one week, subsequently rinsed in water for 24 hours and preserved in 70% ethanol for several years. Other specimens were skinned: their skins were sealed in plastic. The tails were either discarded or preserved in 95% ethanol. These materials were deposited together with their sampling data in the Snake Farm, Queen Saovabha Memorial Institute (QSMI), The Thai Red Cross Society, Bangkok. From one live specimen, fresh blood was taken and DNA extraction was performed using Genomic DNA extraction kit (Blood/Bacteria/Cultured Cells) (RBC Bioscience, Taipei, Taiwan). Also, the dried skin of one specimen and 1-2 cm pieces of skeletal muscle tissue from 12 formalin-fixed ethanol-preserved (FFEP) specimens, deposited in the Snake Farm,

TABLE 1. Oligonucleotide primers of cytochrome b and 12S and 16S ribosomal RNA were designed based on NCBI GenBank nucleotide sequence database. GenBank Accession Number: *Naja naja* (NC_010225.1), *Naja atra* (NC_011389.1), *Ophiophagus hannah* (NC_011394.1), *Bungarus fasciatus* (NC_011393.1), *Bungarus multicinctus* (NC_011392.1), and *Daboia russellii* (NC_011391.1)

Primers	Nucleotides (5'→ 3')	Product size (bps)
Cytochrome1 - Forward	GCCTGAAAAACCACCGTTGT	342
Cytochrome1 - Reverse	CTTTATTTAGGTAAGATCCAT	
Cytochrome2 - Forward	AGGACTCTACTATGGATTTTA	311
Cytochrome2 - Reverse	CCTTCGTTATGAAGTAGAATGAT	
Cytochrome3 - Forward	ATCCTCAATCCATATCATCCT	314
Cytochrome3 - Reverse	ATATAAGTAGGGCTAGTGTT	
Cytochrome4 - Forward	AACTAGGAGGAACACTAGCCCT	333
Cytochrome4 – Reverse	CCGTCTTTGGTTTACAAGAAC	
122 - Forward	CAAGGTCTTGGTCTTAAACCT	495
122 - Reverse	ACTCGTAGTTATTTGGCGAACA	
122S - Forward	AACTCGTGAAAGCAAGGACACAA	478
122S - Reverse	CCATGTTACGACTTGCCCTG	
12SWalk - Forward	GCAATGAAGTGGCGCACACA	518
16SWalk – Reverse	CTAAAGGTTATGTTTTTGTT	
16S - Forward	AAAGGCAACGCCTGCCCAGT	517
16S – Reverse	CGGTCTGAACTCAGATCACGT	

were used. Genomic DNA extraction mini kit (Tissue) (RBC Bioscience, Taipei, Taiwan) was used for DNA extraction from the dried skin. Samples of FFEP muscle tissue were cut into portions of 3-5 mm and were soaked in phosphate buffer saline (PBS) pH7.2 for 3 days, during which the buffer was changed every 8 hours. Then, the samples were completely chopped and dehydrated overnight. The extraction steps were performed according to the user guide of a commercial DNA isolation kit (DNeasy Blood and Tissue Kit). Oligonucleotide primers of cytochrome b, and 12S and 16S ribosomal RNAs were designed based on the NCBI GenBank database (Table 1). DNA amplification, using the Polymerase Chain Reaction (PCR), was carried out with 50 µl reaction buffer containing 10xbuffer, 100mM of each dNTP, 25 mM $MgCl_2$, 50 pmol/µl of sense and antisense primers, Taq DNA polymerase, and 10 µl DNA template. The amplification was performed by using a thermocycler (MWG Biotech, USA) at 94°C for 3 minutes, followed by 40 cycles at 94°C/56°C/72°C for one minute each with a final extension of 72°c for 7 minutes. The final products were electrophoresed on a 1.5% agarose gel containing ethidium bromide in 1xTAE buffer along with appropriate molecular size markers. The gel fragment containing the amplified product was excised and extracted using Gel/PCR DNA fragments extraction kit (RBC Bioscience, USA). DNA sequencing was carried out using the same primers as used

FIGURE 1. The differences between *Pareas macularius* and *Pareas margaritophorus* based on the type of nuchal collar and 7th supralabial scale. **Left:** Head of a bicolored-spotted *Pareas* spec. of which the whitish nuchal collar is finely speckled with brown and a large intense black blotch (IBB) covering much of the rudder-shaped 7th supralabial. These characteristics, together with the presence of rows of *keeled* dorsal scales, identify the specimen as *Pareas macularius* (Hauser, 2017). **Right:** Head of a bicolored-spotted *Pareas* spec. of which the (entire) nuchal collar is orange without any tiny brown speckles. A large part of the rudder-shaped, 7th supralabial is mottled grey, but there is no IBB. These characteristics, together with the absence of any *keeled* dorsal scales, identify the specimen as *Pareas margaritophorus* (Hauser, 2017). (Photo by Sjon Hauser)

in the PCR by 1st BASE sequencing (Malaysia-http://www.base-asia.com). Nucleotide sequences were aligned and compared to find regions of identity using NCBI Nucleotide BLAST (www.ncbi.nlm.nih.gov). The pairwise matrix of sequence divergence in the *p*-distance was calculated by Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA7: www.megasoftware.net). The phylogenetic reconstruction was performed also using MEGA7. Genetic relationships between *P. margaritophorus* and *P. macularius* were inferred based on a maximum likelihood method with 1000 bootstrap iterations. Representative of another pareid genera, *Aplopeltura boa*, was used as an outgroup.

RESULTS

Morphological analysis

Examination of the specimens included determining the presence or absence of keeled dorsal-scale rows, the type of nuchal collar color. Following the descriptions in Boulenger (1894), Smith (1943), and Hauser (2017) , the specimens were identified as either *Pareas margaritophorus* or *P. macularius*. Seven specimens were identified as *Pareas margaritophorus* (Fig. 1; Right), because all dorsal scale rows were smooth, IBBs were absent on both 7th supralabials and the nuchal collar was entire or tripartite, pink, yellow or white without speckles (type I). All other characters, such as the number of ventral shields and the total length, were in the range of the species in Thailand as given by Hauser (2017) . These specimens were assigned the code names PMAR 01 to PMAR 07.

The other seven specimens were identified as *Pareas macularius* (Fig. 1; Left), because all had a good number of keeled dorsal scale rows, distinct IBBs were present on both 7th supralabials and their nuchal collar was W- or butterfly-shaped with tiny brown speckles (type II). All other characters, such as the number of ventral shields and the total length, were in the

TABLE 2. The range of mitochondrial DNA sequencing from specimens representing *P. margaritophorus* (PMAR) and *P. macularius* (PMAC) in this study

Code Number	Type of Sample	Range of Cytochrome b Sequence (1,200 bp)	Range of 12S and 16S ribosomal RNA Sequence (2,367 bp)
PMAR 01	Blood	1,123	1,416
PMAR 02	FFEP Tissue	1,090	1,410
PMAR 03	FFEP Tissue	1,087	1,413
PMAR 04	FFEP Tissue	NA	NA
PMAR 05	FFEP Tissue	1,103	1,414
PMAR 06	FFEP Tissue	NA	NA
PMAR 07	FFEP Tissue	NA	NA
PMAC 01	FFEP Tissue	627	NA
PMAC 02	FFEP Tissue	NA	1,229
PMAC 03	FFEP Tissue	625	1,213
PMAC 04	FFEP Tissue	NA	NA
PMAC 05	FFEP Tissue	625	1,229
PMAC 06	95% ethanol preserved Tissue	620	1,216
PMAC 07	Dry Skin Tissue	1,118	1,212

NA = Not available (DNA could not be retrieved from the specimens and led to failure of PCR amplification)
FFEP = Formalin-Fixed Ethanol-Preserved

range of the species in Thailand as given by Hauser (2017). These specimens were assigned the code names PMAC 01 to PMAC 07.

Molecular phylogenetic analysis

The sizes of mitochondrial DNA fragments which could be successfully amplified and sequenced are shown in Table 2. The results of nucleotide sequences of cytochrome b, 12S and 16S ribosomal RNAs from *Pareas margaritophorus* and *P. macularius* in Thailand were deposited in the NCBI GenBank under accession numbers MK557848, MK557846, MK511794, MK557847, MK511796, and MK511795, respectively. The complete DNA sequence was identified and compared using the Basic Local Alignment Search Tool (BLAST) provided by NCBI. The DNA sequence-based phylogenetic tree using MEGA7 is shown in Fig. 2. The DNA sequences of cytochrome b were chosen from the NCBI database for phylogenetic analysis including JF827675 (*P. margaritophorus*; China), KJ642197 (*P. margaritophorus*; Vietnam/Hong Kong),

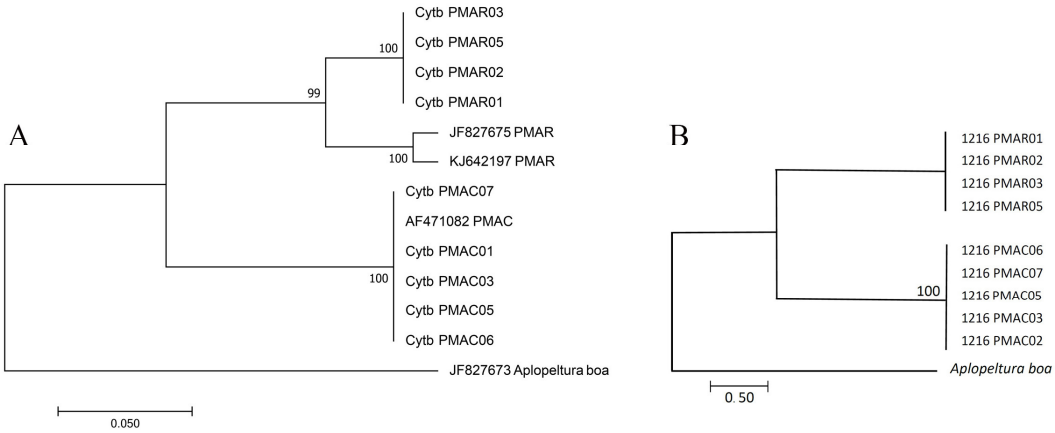

FIGURE 2. Maximum Likelihood trees of *Pareas margaritophorus, P. macularius*, and related species based on cytochrome b gene **(A)** and 12S and 16S ribosomal RNA genes **(B)**. JF827675 is *Pareas margaritophorus* isolate hainan3 cytochrome b gene, partial sequence. KJ642197 is *Pareas margaritophorus* haplotype Mo3 cytochrome b gene, partial sequence. AF471082 is *Pareas macularius* cytochrome b gene, complete sequence. *Aplopeltura boa* was used as outgroup. The scale bars represent 0.05 (cytochrome b) and 0.50 (12S and 16S rRNA) substitutions per nucleotide position.

and AF471082 (*P. macularius*: Myanmar). *P. margaritophorus* samples from Thailand were clustered in the same clade of *P. margaritophorus* from Hainan, China, and Vietnam/Hong Kong. Moreover, *P. macularius* samples were also in the same clade of *P. macularius* deposited in GenBank from Bago Division, Myanmar. Based on a pairwise matrix of sequence divergences with the *p*-distance model in MEGA7 (Table 3), the nucleotide differences in mitochondrial cytochrome b gene between PMAR and PMAC from Thailand were 0.151 (15.1%). PMAR and PMAC in Thailand showed 0.230 (23.0%) and 0.238 (23.8%) nucleotide differences when compared with *Aplopeltura boa* as an outgroup, respectively. Finally, the genetic distance of mitochondrial ribosomal RNAs between PMAR and PMAC in Thailand was 0.163 (16.3%) (data not shown).

DISCUSSION

The present study has confirmed Hauser's (2017) argument that *Pareas macularius* is a valid species distinct from *P. margaritophorus* and that the characters useful to discriminate the former from the latter include body size (total length), the number of ventral shields, the presence or absence of keeled dorsal scales, the shape, color and speckling of the nuchal collar, and the presence or absence of an intense black blotch on the 7th supralabial scale. Additional indirect support for the distinctiveness of the two species came from the localities where the specimens were collected. *P. margaritophorus* was commonly found at elevations below 900 m, whereas *P. macularius* at elevations from 900 to 1700 m. Our results were correlated with the recent study to confirm the validity of *P. macularius* as a distinct species from *P.*

TABLE 3. The sequence divergence in mitochondrial cytochrome b sequences using the pairwise method with *p*-distance model between *P. margaritophorus* and *P. macularius* in Thailand

Specimens	1	2	3	4	5	6	7	8	9	10
1. JF827673_Aplopeltura_boa										
2. CytbPMAR01	0.230									
3. CytbPMAR02	0.230	0.000								
4. CytbPMAR03	0.230	0.000	0.000							
5. CytbPMAR05	0.230	0.000	0.000	0.000						
6. CytbPMAC01	0.238	0.151	0.151	0.151	0.151					
7. CytbPMAC03	0.238	0.151	0.151	0.151	0.151	0.000				
8. CytbPMAC05	0.238	0.151	0.151	0.151	0.151	0.000	0.000			
9. CytbPMAC06	0.238	0.151	0.151	0.151	0.151	0.000	0.000	0.000		
10. CytbPMAC07	0.238	0.151	0.151	0.151	0.151	0.000	0.000	0.000	0.000	

margaritophorus based on mitochondrial and nuclear DNA, including morphological data (Wang et al., 2020).

Molecular phylogenetic analysis conducted in the present study was limited to sequences in the three domains of mitochondrial DNA (i.e., cytochrome b gene and two ribosomal RNA genes). The similarities between Thailand samples of the species in problem were 84.9% in cytochrome b gene and 83.7% in ribosomal RNA genes. Besides, the percent identity matrix was also calculated by ClustalW from cytochrome b and ribosomal RNA sequences, the sequence identity between the two species in Thailand that showed 84.94% and 98.62%, respectively. These may suggest that the mitochondrial cytochrome b is more useful in resolving phylogenetic relationships within and between closely related species. Meanwhile, 12S and 16S ribosomal RNA sequence analysis might be more suitable in examining phylogenetic relationships above specific levels rather than within the species (Lee et al., 1999). In comparative with previous publications, phylogenetic relationships within Pareidae using mitochondrial and nuclear genes indicated that *P. formosensis* was the sister species of *P. hamptoni* and *P. chinensis* was the sister of *P. boulengeri* (Guo et al., 2011). They also found large divergences among sampled specimens of *P. margaritophorus*. *P. macularius* was not valid in this publication and still recognized as a junior synonymized with *P. margaritophorus.* However, they finally mentioned to pay attention to the variation within *P. margaritophorus* in future studies (Guo et al., 2011).

Compared to fresh blood and dry skins from freshly killed specimens, tissue from formalin-fixed specimens often causes difficulties in DNA amplification. For example, cytochrome b DNA extracted from the blood of the living specimen PMAR01 (1,123 bp) and the dried skin of the freshly killed PMAC07 (1,118 bp) resulted in long-range sequences compared with the short-range sequences (620 to 1,087 bp) extracted from FFEP tissue. Long-read sequencing

provided the long-range information and increased the accuracy. Due to the degrading of the DNA extracted from FFEP tissue, sets of primers were designed bracketing overlapping 150 to 500 bp segments, within 1,200 bp and 2,367 bp sections of cytochrome b and 12 and 16 ribosomal RNA genes, respectively. Bibi et al. (2015) reported that formalin-fixation of samples damaged DNA in many ways, including irreversible denaturation, fragmentation, base modification, and cross-linkage within the DNA itself or between DNA and proteins. It is believed that formalin can inhibit primer annealing and suppress the replication process during the PCR reaction which leads to a reduction in available sequence length of amplified products. In several studies (Bibi et al., 2015; Suntrarachun and Chanhome, 2017) it was concluded that DNA structure is less affected when specimens are stored for short periods, less than 24 hours, in formalin which leads to easy extraction and amplification of mitochondrial DNA up to the size of 400 bp. It seems likely that a reduced exposure of samples to formalin fixation would reduce the degradation of DNA, leaving DNA extraction from these samples easy. According to high DNA damage rates by formalin, working with small DNA fragments, especially less than 500 bp, are crucial steps for consistent amplification and sequencing (Suntrarachun and Chanhome, 2017). For DNA sequencing study samples of fresh blood or dry skin are preferred above FFEP tissue. When only FFEP tissue is available, amplification of small DNA fragments, in particular of less than 500 bp with overlapping within the gene, is recommended to increase a success rate of amplification and sequencing.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of the Snake Farm, Queen Saovabha Memorial Institute (QSMI), The Thai Red Cross Society, for collecting snake samples. This work was supported by a Grant Number QSMI - 6002 from Thai Red Cross Society, Bangkok, Thailand. The authors have no conflict of interest to declare in this study.

LITERATURE CITED

Bibi, S. S., Rehman, A., Minhas, R. A. and Janjua, S. Evaluation of DNA extraction method from formalin preserved skin samples of *Panthera pardus* molecular genetic assessment. Journal of Animal and Plant Sciences, 25: 1196-1199.

Boulenger, G. 1896. Catalogue of the Snakes in the British Museum (Natural History), British Museum, London, 3: 727.

Cox, M. J., Hoover, M. F., Chanhome, L. and Thirakhupt, K. 2012. The snakes of Thailand. 1st ed. Bangkok: Sirabutr Printing.

Danaisawadi, P., Asami, T., Ota, H., Sutcharit, C. and Panha, S. 2015. Subtle asymmetries in the snail-eating snake *Pareas carinatus* (Reptilia: Pareatidae). Journal of Ethology, 33: 243-246.

Dowling, H. G. 1951. A proposed standard system of counting ventrals in snakes. British Journal of Herpetology, 1(5): 97-99.

Figueroa, A., McKeloy, A. D., Grismer, L. L., Bell, C. D. and Lailvaux, S. P. 2016. A species-level phylogeny of extent snakes with description of a new colubrid subfamily and genus. PLoS ONE 2016: 11 (9): e 016107 doi: 10.1371/journal.pone. 016107

Grossmann, W. and Tillack, F. 2003. On the taxonomic status of *Asthenodipsas tropidonotus* (Van Lidth de Jeude, 1923) and *Pareas vertebralis* (Boulenger, 1900) (Serpentes: Colubridae: Pareatinae). Russian Journal of Herpetology, 10(3): 175-190.

Guo, Y., Wu, Y., He, S. and Zhao, E. 2011. Systematics and molecular phylogenetics of Asian snail-eating snakes (Pareatidae). Zootaxa, 3001: 57-64.

Hauser, S. 2017. On the validity of *Pareas macularius* Theobald, 1868 (Squamata: Pareidae) as a species

distinct from *Pareas margaritophorus* (Jan *in* Bocourt, 1866). Tropical Natural History, 17(1): 147-174.

Hoso, M. 2017. Asymmetry of mandibular dentition is associated with dietary specialization in snail-eating snakes. Peer Journal, 5: e3011; DOI 10.7717/peerj.3011

Huang, Q. Y. 2004. *Pareas macularius* Theobald, 1868 Should be a Junior Synonym of *Pareas margaritophorus* (Jan, 1866). Sichuan Journal of Zoology, 23(3): 207-208. (in Chinese)

Jan, G. 1866. In Bocourt, F., Note sur les reptiles, les batraciens et les poisons recueillis pendant un voyage dans le royaume de Siam. Nouvelles Archives Museum Naturale Histoire, 2(2): 4-20.

Lawson, R., Slowinski, J. B., Crother, B. I. and Burbrink, F. T. 2005. Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution, 37: 581-601.

Lee, J. E., Yang, D. E., Kim, Y. R., Lee, H., Lee, H. I., Yang, S. Y. and Lee, H. Y. 1999. Genetic relationships of Korean treefrogs (Amphibia; Hylidae) based on mitochondrial cytochrome b and 12S rRNA genes. Korean Journal of Biological Sciences, 3: 295-301.

Mulcahy, D. G., Lee, J. L., Miller, A. H., and Zug, G. R. 2017. Troublesome trimes: potential cryptic speciation of the *Trimeresurus (Popeia) popeiorum* complex (Serpentes: Crotalidae) around the Isthmus of Kra (Myanmar and Thailand). Zootaxa, 4347(2): 301-315.

Pyron, R. A., Burbrink, F. T., Colli, G. R., de Oca, A. N., Vitt, L. J., Kuczynski, C. A. and Wiens, J. J. 2011. The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. Molecular Phylogenetics and Evolution, 58: 329-342.

Savage, J. M. 2015. What are the correct family names for the taxa that include the snake genera *Xenodermus*, *Pareas*, and *Calamaria*? Herpetological Review, 46: 664-665.

Smith, M. A. 1943. The Fauna of British India, Ceylon and Burma, including the whole of the Indo-Chinese sub-region. Reptilia and Amphibia. Vol. III. Serpentes, Taylor and Francis, London.

Suntrarachun, S. and Chanhome, L. 2017. Molecular genetic assessment of formalin-fixed ethanol-preserved King Cobra: A comparative study on pretreatment protocols and DNA extraction methods. Asian Journal of Conservation Biology, 6(1): 14-20.

Taylor, E. H. 1965. The serpents of Thailand and adjacent waters. The University of Kansas Science Bulletin, 45 (9): 609-1079.

Theobald, W., 1868. Catalogue of the reptiles of British Burma embracing the provinces of Pegu, Martaban and Tenassarim; with descriptions of new or little-known species. Journal of the Linnean Society (Zoology), 10: 4-67.

Uetz P. and Hošek, J. 2018. The Reptile Database. Available from http://www.reptile-database.org Accessed on 20-12-2018.

Vogel, G. 2010. On the distribution of *Pareas hamptoni* (Boulenger, 1905) in Thailand (Serpentes: Pareatinae). Russian Journal of Herpetology, 17(3): 2019-222.

Vogel, G. 2015. A new montane species of the genus *Pareas* Wagner, 1830 (Squamata: Pareatidae) from northern Myanmar. Taprobanica, 7(1): 1-10.

Wallach, V., Williams, K. L. and Boundy, J. 2014. Snakes of the World. A Catalogue of Living and Extinct Species. CRC Press, Boca Raton (Florida). 1227.

Wang, P., Che, J., Liu, Q., Li, K., Jin, J. Q., Jiang, K., Shi, L., and Guo, P. 2020. A revised taxonomy of Asian snail-eating snakes *Pareas* (Squamata, Pareidae): evidence from morphological comparison and molecular phylogeny. ZooKeys, 939: 45-64.

You, C. W., Poyarkov Jr, N. A. and Lin, S. M. 2015. Diversity of the snail-eating snakes *Pareas* (Serpentes, Pareatidae) from Taiwan. Zoologica Scripta. 1-13.

Zaher, H., Murphy, R. W., Arredondo, J. C., Grabski, R., Machado-Filho, P. R., Mahlow, K., Montingelli, G. G., Quadros, A. B., Orlov, N. L., Wilkinson, M., Zhang, Y. P., and Grazziotin, F. G. 2019. Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes). PLoS One, 14(5): e021 6148; DOI: 10.1371/journal.pone.0216148