Indian Journal of Entomology Online published Ref. No. e23633 DoI. No.: 10.55446/IJE.2023.633

mtCO1 ANALYSIS OF THE BUTTERFLY GENUS *PAPILIO*, FROM INDONESIA

Roni Koneri[1], Syamsul Bachry[2]* and Beivy Jonathan Kolondam[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences,
Sam Ratulangi University. Jl. Kampus Bahu, Manado 95115, North Sulawesi, Indonesia
[2]Department of Biology, Faculty of Life Sciences, Pahlawan University, Tuanku Tambusai No.23,
Bangkinang, Kec. Bangkinang Kampar, Riau 28412, Indonesia
*Email: syamsulbachry89@gmail.com (corresponding author): ORCID ID:

ABSTRACT

The genus *Papilio* is a butterfly that has many subspecies with similar phenotypes. So until now, researchers are still studying the genus *Papilio* based on morphology and genetics. Partial sequences of mitochondrial cytochrome oxidase-I (mtCOI) genes from individuals from 13 species of Papilionidae from North Sulawesi: Indonesia, Papua New Guinea, Malaysia: Negeri Sembilan, India: Assam, South Korea, China, Taiwan: Nantou, and Thailand: Fang, Chiang Mai have been compared in this study. Nucleotide divergences showed that the mean genetic distance between species was 0.023 (2.3%) to 0.723 (72.32%). Phylogenetic analysis revealed that groups of Papilionidae from North Sulawesi: Indonesia formed their own group; Species from Papua New Guinea are closely related to other Papilionidae species from other regions. There are 50 distinct sites or single nucleotide polymorphism (SNP) in the mtCO1 sequence of eleven species. These mtCO1 sequences can reveal the genetic differentiation of closely related species in the genus *Papilio*.

Key words: Butterfly *Papilio* spp., cytochrome C oxidase-I, North Sulawesi, sequences nucleotide,mtCO1 variations, comparisons, phylogenetic analyses, NcB accessions

Butterflies are a group of insects that can be found in tropical and subtropical areas. Butterfly species are insects that have various morphological and colour characteristics that may vary due to environmental habits and geographic location (Simonsen et al., 2012). Due to these butterflies can be used as bioindicators of changes in environmental quality (Basset et al., 2012). The presence of butterfly populations in a habitat ecosystem has begun to decline due to various threats such as uncontrolled exploitation and hunting, as well as land clearing and forest conversion (Basset et al., 2012). Koneri and Maabuat (2016) state that butterfly diversity is very influential in their habitat. Taxonomically, butterflies are included in the order Lepidoptera. Butterflies have an important role in the ecosystem, namely as pollinators of flowers or pollinators (Duara et al., 2014). The classification of insect species is very important for basic and applied research. Classification based on morphological characteristics creates problems for many insect groups due to their small size, and morphological shapes that change over time (Akhilesh and Sebastian, 2014; Tsao and Yeh, 2008). The Papilionidae family, commonly known as swallowtail butterflies, is large and has a variety of colours. This species can be found in tropical regions and can be found on almost every continent. There are an estimated 550 species in this family. In the 1990s, studies of this type of butterfly began to focus on morphological and molecular systematics (Simonsen et al., 2011). Some morphological evidence in the subfamily Baroniinae, Parnassinae and Papilionidae shows no specific differences. (Vane-Wright, 2003). Based on the cladistic method, Kristensen (1976) found that the Papilionidae family has close ties with several family groups such as Pieridae, Nymphalidae Lycaenidae, and Riodinidae.

This genus *Papilio* contains many cryptic species where phenotypic and evolutionary variability has led to misidentification. Apart from that the dimorphism also causes confusion (von-Maria et al., 2011). Taxon identification based on DNA sequences has begun to be used to facilitate identification and even discover new species (Blaxter, 2005; Hebert et al., 2003a, b). DNA sequences from the mitochondrial cytochrome oxidase 1 (mtCO1) gene can serve to identify and analyze the phylogeny (Hebert et al., 2003a, 2004b; Ward et al., 2005; Huang and Ke, 2015), especially the cryptic or similar species in the tropics (Wilcox et al., 1997; Berkov, 2002; Hebert et al. 2004b; Monaghan et al., 2005; Hajibabaei et al. 2006; Bachry et al. 2019) and insects in different places and at various growth stages (Janzen et al., 2005). In Indonesia, substantial studies

on butterfly systematics, morphology, and ecology have been carried out (Tallei et al., 2015; Koneri and Maabuat, 2016; Koneri et al., 2019; Koneri and Nangoy, 2019; Koneri et al., 2020). However, the study of the composition and genetic differences of *Papilio* spp. is still underreported. This study analyzes the mtCO1 sequence composition of the *Papilio* spp. from various regions.

MATERIALS AND METHODS

Three sequences were found in this study, namely *P. gigon* from the *Talaud archipelago*, *P. rumanzovia*, and *P. polytes* from the Sangihe Islands, Indonesia (Table 1; Fig. 1). Each of these samples was analyzed at the Genetics Laboratory, Department of Biology, FMIPA, Sam Ratulangi University. Furthermore, ten sequences of *Papilio* spp. from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov) were also used for comparison (Table 1). Extraction of DNA from tissue samples was performed using a commercial kit from Dneasy® Blood and Tissue Kit no 69504 (50) (Qiagen, Germany) following the manufacturer instructions with some modification (the sample was first crushed, then dried). The target gene was amplified using the polymerase chain reaction using universal primer set LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACC AAAAAATCA-3') (Folmer et al. 1994), producing a 658 bp partial sequence. The total volume of PCR reaction was 25 µl, consisting of 10.8 µl of ddH2O, 4 µl of Q5 buffer, 5 µl of Q5 enhancer, 1 µl of dNTP, 1 µl of forward primer, 1 µl of reverse primer, 2 µl of DNA template, and 0.2 µ of Taq Hot Start Q5. CO1 amplification began with an initial pre-denaturation at 94°C for 5 min, followed by denaturation for 35 cycles at 94°C for 30 s, annealing at 54°C for 45 s, elongation at 72°C for 30 s, and a final cycle of extension at 72°C for 5 min. PCR products were purified by electrophoresis through a 1.2% agarose gel using a 1 × TBE (Tris-borate-EDTA acid) buffer. A single band of PCR product was sequenced by a sequencing service (1st BASE www.base-asia.com) Malaysia. Sequence

Table 1. Papilionide specimens collected and Genbank accession numbers

Species	Locality (for specimens sequenced in this study)	Specimen ID	Code access NCBI
Papilio gigon	Indonesia; Island Talaud	RK3	MW167784
Papilio rumanzovia	Indonesia; Island Sangihe	RK4	MW167785
Papilio polytes	Indonesia; Island Sangihe	RK5	MW168164
Papilio ulysses	Papua New Guinea	USNM:ENT:00645977	HQ570589
Papilio ambrax	Papua New Guinea	USNM:ENT:00645984	HQ570594
Papilio aegeus	Papua New Guinea	USNM:ENT:00645996	HQ570605
Papilio euchenor	Papua New Guinea	USNM:ENT:00645995	HQ570604
Papilio demoleus malayanus	Malaysia: Negeri Sembilan	UMKL:JJW0037	KF226558
Papilio bianor gladiator	India: Assam	CBGPFLC_00008	JQ982045
Papilio macilentus	South Korea	SWC-09-5007	GU696025
Papilio noblei	China	FD22	JF747532
Papilio hopponis	Taiwan: Nantou	CBGPFLC_00049	JQ982076
Papilio arcturus	Thailand: Fang, Chiang Mai	CBGPFLC_00003	JQ982040

Fig. 1. Map of the study area in Sangihe Islands and Talaud Islands, North Sulawesi, Indonesia (Google Maps, 2021)

divergence among species and genera was calculated using the Kimura two-parameter distance model in MEGA 7.0 (Kimura et al., 2016). The Neighbor-Joining method (Saitou and Nei, 1987) was used to reconstruct the phylogenetic tree based on K2P from MEGA7.0 (Kimura et al., 2016). Statistical support for the internodes in the phylogenetic tree was tested by bootstrap % with 1000 replicates (Felsenstein, 1985). Sequence analysis and sample identification were done by inputting the trimmed sequence in NCBI's BLAST tool. A phylogenetic tree was then constructed using MEGA7 software (Kimura et al., 2016).

RESULTS AND DISCUSSION

The result of mtCO1 gene analyses revealed sequence length of 658 bp found at 274 bp (41.64%) conservative sites, 384 bp (58.35%) varied sites, 334 bp (50.75%) parsimony sites, and 50 bp (7.59%) singleton sites (Table 2). The nucleotide composition of the from 13 species showed a higher % of Thymine (T) (38.7%)followed by adenine (A) (32.0%), cytosine (C) (14.9%), and the lowest was guanine (G) (14.4 %). The composition of nucleotides A + T was 70.7% and G + C was 29.3%. Total 50 single nucleotide polymorphism (SNP) for mtCO1 gene sequences were found from 13 species (two species without SNP) of *Papilio* (Table 3). *P. euchenor* had the most SNP (10), compared to other species and *P. Bianor gladiator (*2 SNP); *P. gigon*, *P. polytes*, and *P. Rumanzovia* (6, 8, and 9 SNP, respectively); *P. ulysses*, *P. ambrax*, *P. demoleus malayanus*, and *P. noblei* (3 SNP) *P. aegeus* and *P. macilentus* (4 SNP). Based on genetic distance analysis with a 2-parameter Kimura model of the interspecies *Papilio* spp. shows the mean value of the genetic distance from 0.023% to 0.723% (Table 4). The difference in genetic distance between *Papilio* spp. is high enough. Based on the phylogenetic tree using the neighbor-joining (NJ) method, it shows three clusters. Black show group I consists of *P. gigon* (MW167784), *P. rumanzovia* (MW167785), and P. *polytes* (MW168164) from North Sulawesi, Indonesia. Red show group II consists of *P. macilentus* (GU696025) from South Korea, *P. ambrax* (HQ570594), and *P. aegeus* (HQ570605) from Papua New Guinea. Green show group III consists of *P. hopponis* (JQ982076) from Taiwan: Nantou, *P. arcturus* (JQ982040) from Thailand: Fang, Chiang Mai, *P. bianor gladiator* (JQ982045) from India: Assam, *P. ulysses* (HQ570589), *P. euchenor* (HQ570604) from Papua New Guinea, *P. demoleus malayanus* (KF226558) from Malaysia: Negeri Sembilan, and *P. noblei* (JF747532) from China.

Papilio spp. from northern Indonesia, showing a single monophyletic group without overlapping with *Papilio* spp. from the Asia Pacific region (Fig. 2). Sekimura et al, (2017) revealed that the phylogenetic relationship of the genus *Papilio* from Japan and the genus *Papilio* from Africa did not show a close relationship. The mtCO1 gene is highly effective in

Table 2. Characteristics of the CO1 gene nucleotides of *Papilio* spp.

No.	Species/ access code	Conserve sites	Variables		Total of variable sites-	Base composition (%)			
			Pi	S		T	C	A	G
1	*Papilio gigon* (MW167784)	274	334	50	384	32.1	13.1	38.1	16.7
2	*P. rumanzovia* (MW167785)	274	334	50	384	31.9	13.4	37.8	16.9
3	*P. polytes* (MW168164)	274	334	50	384	31.9	13.5	38.6	16.0
4	*P. ulysses* (HQ570589)	274	334	50	384	40.3	14.9	31.0	13.8
5	*P. ambrax* (HQ570594)	274	334	50	384	41.2	14.7	30.2	13.8
6	*P. aegeus* (HQ570605)	274	334	50	384	39.7	16.1	30.7	13.5
7	*P. euchenor* (HQ570604)	274	334	50	384	41.5	15.7	28.9	14.0
8	*P. demoleus malayanus* (KF226558)	274	334	50	384	40.4	15.8	30.2	13.5
9	*P. bianor gladiator* (JQ982045)	274	334	50	384	41.0	14.9	30.2	13.8
10	*P. macilentus* (GU696025)	274	334	50	384	40.3	15.5	30.4	13.8
11	*P. noblei* (JF747532)	274	334	50	384	41.1	15.4	29.8	13.7
12	*P. hopponis* (JQ982076)	274	334	50	384	41.0	15.0	30.2	13.7
13	*P. arcturus* (JQ982040)	274	334	50	384	40.4	15.5	30.2	13.8
	Avg.					38.7	14.9	32.0	14.4

Notes: Avg = Average; Pi= parsimony informative site; s= singleton site; A= Adenine; T= Thymine; G= Guanine; C= Cytosine.

Tabel 3. Singel nucleotide polymorphisme (SNPn)

Species /code access	Nucleotide base site																	
	1	4 0	6 7	7 9	9 5	1 1 4	1 1 6	1 1 9	1 3 9	1 5 4	1 6 6	2 1 7	2 2 9	2 4 1	2 6 1	2 6 5	2 8 7
Papilio gigon (MW167784)	A	A	A	A	A	A	A	T	T	T	A	A	A	A	G	A	T
P. rumanzovia (MW167785)	.	.	.	G	G	.	.	A	.	G	.	.	.	.	T	.	.
P. polytes (MW168164)	.	.	G	.	.	.	G	A	.	.	.	.	T	.	.	.	.
P. ulysses (HQ570589)	.	.	.	.	.	.	.	A	.	.	.	G	.	.	.	.	.
P. ambrax (HQ570594)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
P. aegeus (HQ570605)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.
P. euchenor (HQ570604)	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.
P. demoleus malayanus (KF226558)	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
P. bianor gladiator (JQ982045)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C
P. macilentus (GU696025)	.	G	.	.	.	.	.	A	C	.	G	.	.	.	.	.	.
P. noblei (JF747532)	C	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.
P. hopponis (JQ982076)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
P. arcturus (JQ982040)	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.

Continue…

Species /code access	Nucleotide base site																
	3 1 3	3 1 7	3 2 3	3 3 9	3 5 5	3 6 2	3 9 2	4 2 7	4 3 3	4 3 6	4 4 2	4 5 1	4 5 4	4 6 3	4 6 7	4 7 2	4 8 7
Papilio gigon (MW167784)	T	G	A	A	T	G	A	T	A	G	T	A	A	T	C	T	T
P. rumanzovia (MW167785)	.	.	G	.	.	.	C	.	.	A	G	.	.	.	.	.	.
P. polytes (MW168164)	A	.	.	.	.	.	T	.	.	A	A	T	T	.	.	.	.
P. ulysses (HQ570589)	.	.	.	.	A	.	.	.	.	A	.	.	.	.	.	.	.
P. ambrax (HQ570594)	.	.	.	.	.	.	.	.	T	A	.	.	.	.	.	.	C
P. aegeus (HQ570605)	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.
P. euchenor (HQ570604)	.	.	.	.	.	.	.	C	G	T	.	.	.	.	.	C	.
P. demoleus malayanus (KF226558)	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.
P. bianor gladiator (JQ982045)	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.
P. macilentus (GU696025)	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	.	.
P. noblei (JF747532)	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.
P. hopponis (JQ982076)	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.
P. arcturus (JQ982040)	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.

Continue…

Species /code access	Nucleotide base site																
	4 9 6	5 0 6	5 1 9	5 2 0	5 2 9	5 3 1	5 4 2	5 5 0	5 6 5	5 7 7	5 8 3	6 0 1	6 1 9	6 3 2	6 4 0	6 4 1
Papilio gigon (MW167784)	G	G	G	A	T	A	C	A	A	G	G	T	T	G	T	T
P. rumanzovia (MW167785)	A	.	T	.	.	T	.	.	.	A	A	.	.	A	.	.
P. polytes (MW168164)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
P. ulysses (HQ570589)	A	.	.	.	.	T	.	.	T	A	A	.	.	.	.	.
P. ambrax (HQ570594)	A	.	.	T	.	T	.	.	.	A	A	.	.	.	.	.
P. aegeus (HQ570605)	A	.	.	.	.	T	.	.	.	A	A	C	C	.	.	.
P. euchenor (HQ570604)	A	A	.	.	C	T	T	T	.	A	A	.	.	.	.	C
P. demoleus malayanus (KF226558)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	C	.
P. bianor gladiator (JQ982045)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
P. macilentus (GU696025)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.

Continue…

Species /code access	Nucleotide base site																
	4 9 6	5 0 6	5 1 9	5 2 0	5 2 9	5 3 1	5 4 2	5 5 0	5 6 5	5 7 7	5 8 3	6 0 1	6 1 9	6 3 2	6 4 0	6 4 1
P. noblei (JF747532)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
P. hopponis (JQ982076)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.
P. arcturus (JQ982040)	A	.	.	.	.	T	.	.	.	A	A	.	.	.	.	.

Table 4. Pairwise distance between of *Papilio* spp.

Sample code	1	2	3	4	5	6	7	8	9	10	11	12	13
Papilio gigon (MW167784)													
P. rumanzovia (MW167785)	0.096												
P. polytes (MW168164)	0.081	0.074											
P. ulysses (HQ570589)	0.713	0.740	0.724										
P. ambrax (HQ570594)	0.701	0.741	0.732	0.079									
P. aegeus (HQ570605)	0.702	0.733	0.721	0.086	0.064								
P. euchenor (HQ570604)	0.723	0.754	0.754	0.096	0.110	0.114							
P. demoleus malayanus (KF226558)	0.702	0.734	0.729	0.103	0.098	0.098	0.105						
P. bianor gladiator (JQ982045)	0.699	0.713	0.717	0.062	0.079	0.077	0.086	0.098					
P. macilentus (GU696025)	0.681	0.716	0.704	0.077	0.059	0.064	0.125	0.107	0.084				
P. noblei (JF747532)	0.706	0.738	0.737	0.101	0.100	0.094	0.101	0.031	0.091	0.103			
P. hopponis (JQ982076)	0.678	0.697	0.701	0.057	0.075	0.077	0.092	0.084	0.031	0.070	0.082		
P. arcturus (JQ982040)	0.695	0.725	0.713	0.064	0.074	0.072	0.092	0.093	0.041	0.079	0.089	0.023	

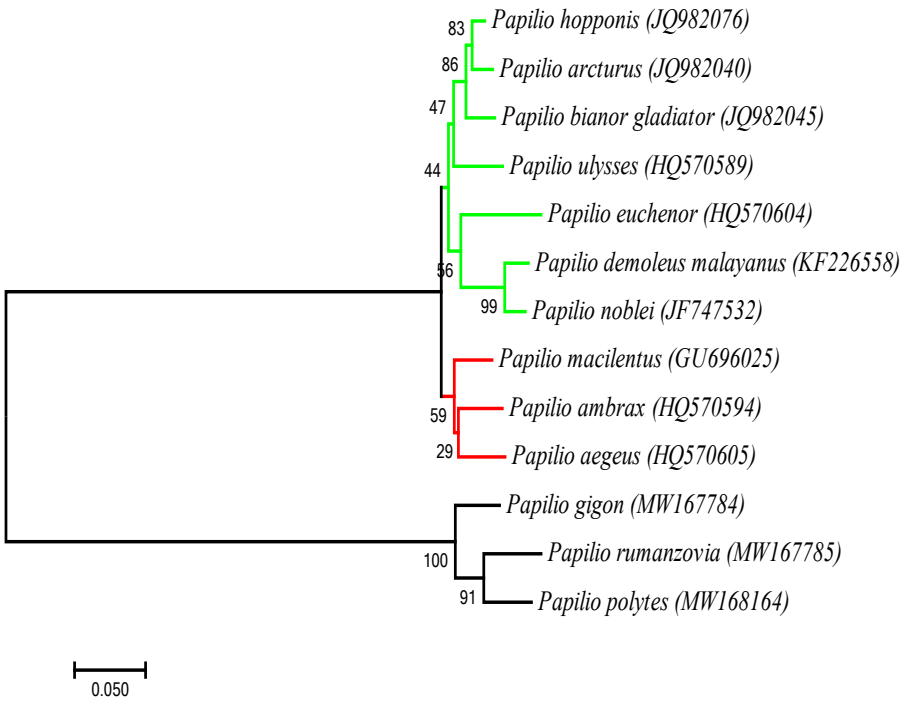

Fig. 2. Phylogeny tree construction of *Papilio* spp. based on a 2-parameter Kimura model with a 1000-loop bootstrap. Black show group I, Red show group II, and Green show group III

distinguishing closely related and morphologically similar species (Hebert et al., 2004b). In addition, phylogenetic results also prove that *Papilio* spp. from Talaud Island reveal that the origin of the island of Sangihe is closely related to form a subgroup. Akhilesh and Sebastian (2016) reported that between and intraspecies *P. polytes* from the Calicut region, Kerala, India, have shown genetic divergence based on the mtCO1 gene. *Papilio* spp. from the territory of Indonesia formed a monophyletic group. *P. euchenor* (HQ570604) had the highest SNP site, and the lowest SNP site was *P. bianor gladiator* (JQ982045). Meanwhile, three types of species originating from the territory of Indonesia showed a relatively high genetic variation. The divergences between the *Papilio* subspecies from Taiwan, Hong Kong, and China based on the mtCO1 sequence were low (Tsao and Yeh. 2008). These nucleotide variations provide evidence that groups of species originating from different regions have specific nucleotide site markers. SNPs of the mtCO1 gene found in *Papilio* spp. can be used to differentiate species. Hebert et al. (2004a) revealed that the mtDNA CO1 gene sequence variation in the 648 bp area could be a barcode for animal species. Waugh (2007) stated that each species has a specific nucleotide sequence for the mtCO1 gene. Vandewoestijne et al. (2004) revealed no differences in mtCO1 in the subspecies *Aglais urticae* (Lepidoptera: Nymphalidae) from the entire Palaearctic region.

The results of the analysis of the genetic distance in *Papilio* spp. ranged from 0.023 (2.3%) to 0.723 (72.32%) (Table 4). Differences in the genetic distance in *Papilio* spp. from northern Indonesia with *Papilio* spp. in other Asia Pacific regions showed a relatively significant difference. Tsao & Yeh (2008) found differences in genetic distance between swallowtail butterfly subspecies of 1.7% to 11.6% and between genera within the same family from 6.7% to 17%. Geographical distance affects the genetic distance between species. Where gene flow in isolated species will change along with environmental conditions. The factor of geographical area differences causes these species to adapt to the environment, such as habitat, temperature, and type of feed. *Papilio* spp.widespread in Africa and Madagascar show divergences ranging from 0% -1.2% (Zakharov et al., 2004). Brower and Jeansonne (2004) reported that the difference in mtCO1 between Nymphalidae populations in the *Danaus plexippus* species from North and South America was <0.8%. *Papilio* spp. group from North Sulawesi, Indonesia shows its own group. The interspecies genetic distance is quite high. There is a single nucleotide polymorphism which is a feature of the *Papilio* spp. from each region. The nucleotide composition shows a higher % of A + T than G + C.

ACKNOWLEDGEMENTS

This research was supported by a Directorate of Research and Community Service Directorate General of Research and Development Strengthening Ministry of Research, Technology and Higher Education, Indonesian In accordance with the Research Contract for the 2018 Fiscal Year Number: 087/ SP2H/ LT/ DRPM/ 2018.

FINANCIAL SUPPORT

The author is thankful to the Indian Journal of Entomology for publishing our article

AUTHOR CONTRIBUTION STATEMENT

All authors, RoniKoneri, Syamsul Bachry, Beivy Jonathan Kolondam: our article is original from research results

CONFLICT OF INTEREST

The authors declare that they have no conflicts.

REFERENCES

Akhilesh V P, Sebastian C D. 2014. Cytochrome oxidase subunit I gene based phylogenetic description of common mormon butterfly *Papilio polytes* (Lepidoptera: Papilionidae). IJSR 5(3): 977-980.

Bachry S, Solihin D D, Rudhy G, Kadarwan S, Butet N. 2019. Genetic diversity of the *Haliotis diversificolor squamata* from Southern Coastal Java (Banten, Pangandaran and Alas Purwo) and Bali based on mitochondrial CO1 sequences. TLSR 30(3): 83-93. DOI:10.21315/tlsr2019.30.3.6

Blaxter M, Mann J, Chapman T, Thomas F, Whitton C, Floyd R. Abebe E. 2005. Defining operational taxonomic units using DNA barcode data. Philosophical Transactions of the Royal Society B: Biological Sciences 360: 1935-1943.

Basset Y, Eastwood R, Sam L, Lohman D J, Novotny V, Treuer T, Miller S E, Weiblen G D, Pierce N E, Bunyavejchewin S, Sakchoowong W, Kongnoo P, Osorio-arenas M A. 2012. Cross-continental comparisons of butterfly assemblages in tropical rainforests: implications for biological monitoring. Insect Conserver Divers 6(3): 1-10.

Brower A V Z, DeSalle R. 1998. Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of wingless as a source of characters for phylogenetic inference. Insect Molecular Biology 7(1): 73-82.

Brower A V Z, Jeansonne M M. 2004. Geographical populations and "subspecies" of new world monarch butterflies (Nymphalidae) share a recent origin and are not phylogenetically distinct. Annals Entomological Society America. 97(3): 519-523.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrate. Molecular Biology Biotechnology 3: 294-299.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PD. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences 103: 968-971.

Hebert P D, Cywinska A, Ball S L, deWaard J R. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B Biological Sciences 270: 313-321.

Hebert P D, Penton E H, Burns J M, Janzen D H, Hallwachs W. 2004b. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences 101: 14812-14817.

Hebert P D, Ratnasingham S, deWaard J R. 2003b. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B Biological Sciences 270 (Suppl 1): S96-S99.

Hebert P D, Stoeckle M Y, Zemlak T S, Francis C M. 2004a. Identification of birds through DNA barcodes. PLoS Biology 2 (10): e312.

Huang Z H, Ke D H. 2015. DNA barcoding and phylogenetic relationships in Timaliidae. Genetic and Molecular Research 14: 5943- 5949.

Janzen D H, Hajibabaei, Burns J M, Hallwachs W, Remigio E, Hebert P D. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. Philosphical Transaction of the Royal Society of London B Biological Sciences 360: 1835-1845.

Koneri R, Maabuat PV. 2016. Diversity of Butterflies (Lepidoptera) in Manembo-Nembo Wildlife Reserve, North Sulawesi, Indonesia. PJBS 19(5): 202-210.

Koneri R, Nangoy M J, Siahaan P. 2019. The abundance and diversity of butterflies (Lepidoptera: Rhopalocera) in Talaud Islands, North Sulawesi, Indonesia. Biodiversitas 20 (11): 3275-3283.

Koneri R, Nangoy M J. 2019. Butterfly community structure and diversity in Sangihe Islands, North Sulawesi, Indonesia. Applied Ecology and Environmental Research 17(2): 2501-2517.

Koneri R, Maabuat PV, Nangoy MJ. 2020. The distribution and diversity of butterflies (Lepidoptera: Rhopalocera) in various urban forests in North Minahasa Regency, North Sulawesi Province, Indonesia. Applied Ecology and Environmental Research 18(2): 2295-2314.

Sekimura T, Matsubara A, Zhi-Hui S. 2017. Molecular phylogenetic relationship of African swallowtail butterflies, *Papilio dardanus, P. phorcas, P. nireus*, and *P. demodocus*, to Japanese papilionid butterflies analyzed by DNA sequences of mitochondrial ND5, COI, and COII genes. Lepidoptera Science 68(2): 46-52.

Kristensen N P. 1976. Remarks on the family-level phylogeny of butterflies, (Insecta, Lepidoptera, Rhopalocera). Journal of Zoological and Systematics and Evolution Research 14(1): 25-33.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Moleculer Evolutionary Genetics Analysis version 7.0 for bigger databasets. Molecular Biology and Evolution 33(7): 1870-1874.

Saitou N, Nei M. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Simonsen T J, Zakharov E V, Djernaes M, Cotton A, Vane-Wright R I, Sperling F A H. 2011. Phylogeny, host plant associations and divergence time of Papilioninae (Lepidoptera: Papilionidae) inferred from morphology and seven genes with special focus on the enigmatic genera Teinopalpus and Meandrusa. Cladistics 27: 113-137.

Simonsen T J, de Jong R, Heikkila M, Kaila L. 2012. Butterfly morphology in a molecular age e Does it still matter in butterfly systematics?. Arthropod Structure and Development 41: 307-322.

Tallei T E, Nanggoy M J, Koneri R, Saroyo. 2015. Biodiversity Assessment of Mt. Tumpa Forest Park, North Sulawesi, Indonesia. Asian Journal of Biodiversity 6 (2):1-21.

Tang J, Pruess K, Cupp E W, Unnasch T R. 1996. Molecular phylogeny and typing of blackflies (Diptera: Simuliidae) that serve as vectors of human or bovine onchocerciasis. Medical and Veterinary Entomology 10(3): 228-234.

Tsao W C, Yeh W B. 2008. DNA-based discrimination of subspecies of swallowtail butterflies (Lepidoptera: Papilioninae) from Taiwan. Zoological Studies 47(5): 633-643.

Vandewoestijne S, Baguette M, Brakefield P M, Saccheri I J. 2004. Phylogeography of Aglais urticae (Lepidoptera) based on DNA sequences of the mitochondrial CO1 gene and control region. Molecular Phylogenetics and Evolution 31(2): 630-646.

Von-Maria C, Helena K, Maria P, Jouko R. 2011. DNA barcoding: a tool for improved taxon identification and detection of species diversity. Biodiversity Conservation 20 (2): 373-389.

Ward R D, Zemlak T S, Innes B H, Last P R, Hebert P D. 2005. DNA barcoding Australia's fish species. Philosphical Transaction of the Royal Society B Biological Sciences 360(1426): 1847-1857.

Waugh J. 2007. DNA barcoding in animal species: progress, potential and pitfalls. BioEssays 29(2): 188-197.

Wilcox T P, Lugg H, Zeh J A, Zeh D W. 1997. Mitochondrial DNA sequencing reveals extreme genetic differentiation in a cryptic species complex of Neotropical pseudoscorpions. Molecular Phylogenetics and Evolution 7(2): 208-216.

Xiang B, Kochar T D. 1991. Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera). Genome 34: 306-311.

Zakharov E V, Caterino M S, Sperling F A H. 2004. Molecular Phylogeny, Historical Biogeography, and Divergence Time Estimates for Swallowtail Butterflies of the Genus *Papilio* Lepidoptera: Papilionidae). Systamatic Biology 53: 193-215.

(Manuscript Received: July, 2022; Revised: August, 2022;
Accepted: March, 2023; Online Published: April, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23633